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Introduction to sterilization: definitions and challenges

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Abstract: Sterilization is an important step in manufacturing medical devices, as well as in the reprocessing of reusable ones in healthcare centers. This chapter will present the main definitions related to sterilization and the classification of sterilization technologies into industrial/clinical and into traditional/non-traditional methods. The main challenges related to sterilization will also be discussed, such as prion and endotoxin deactivation, the growing use of small and delicate materials, as well as new economic and ecological constraints related to the reduction of costs and the protection of the population/environment, respectively. Finally, a brief overview of the book content will be presented.

Key words: definitions, challenges, security assurance level, disinfection, classification of sterilization techniques, validation and monitoring, prions.

1.1 Introduction

Sterilization is an important step of manufacturing of implants or medical devices (MDs) to prevent the spread of infection. It is also a major step when reusing MDs in clinical centers. Failures in adequate sterilization result in significant institutional costs related to patient nosocomial infections and mortality/morbidity concerns. In developed countries, from 5% to 10% of patients admitted to acute care hospitals acquire an infection which was not present or incubating on admission. This rate exceeds 25% in developing countries (Wenzel *et al.*, 2008). Thanks to much progress in the methods for device sterilization, most nosocomial infections nowadays are not related to this issue but rather to direct contact, ventilation, water and autologous infection along urinary catheters (called hospital acquired urinary tract infections, accounting for about 40% of the total number of all nosocomial infections). However, it is important to consider sterilization issues and requirements at the earliest stages of development of any new MD, to ensure that the final product can be sterilized effectively and safely, with the most cost-effective and environment-friendly procedures. This book aims to help industrial and healthcare workers to choose a

sterilization method and better understand regulations and hazards related to the sterilization of MDs.

In the present chapter, the main concepts of sterilization will be defined. We will differentiate between sterilization and disinfection, between industrial and clinical sterilization, as well as between sterilization efficiency and safety, which are two of the most important aspects to consider when choosing a biomaterial, designing the device and choosing the packaging and sterilization technique. This book will focus on medical devices. Methods to reduce microorganisms in food, water or air in healthcare settings will not be discussed. We will present the main challenges facing sterilization and briefly discuss the criteria of an ideal sterilization technique. Finally, a brief overview of the various sterilization technologies available will be given. These will be further detailed in the next chapters.

1.2 Definitions of sterilization in the context of biomaterials

The main concepts of sterilization to be discussed in this section are sterilization efficiency, the difference between real sterilization and disinfection as well as between industrial and clinical sterilization.

1.2.1 Sterilization efficiency

Sterilization efficiency is defined as the ability to remove or destroy all forms of microbial life, including viruses, bacteria and fungi, under vegetative forms or spores (Crow, 1993). Since absolute sterility cannot be verified, the statistical definition of sterility is used in practice, by using the security assurance level (SAL), defined as 'the probability of a single viable micro organism occurring in or on a product after sterilization'. The worldwide accepted definition of sterility of medical devices is defined as the chance of finding a viable organism in or on a medical device to be at most 1 in 1 000 000 or an SAL of at most 10^{-6} (Block, 2000). However, in the case of sterile devices intended only for contact with intact skin, the American Food and Drug Administration (FDA) recommends a SAL of 10^{-3}. Except for the rare instances when sterilization can take place where the sterile products are to be used, MD must be packaged to preserve their sterility during storage, handling and transport. The majority of sterile MDs are terminally sterilized – that is, they are sterilized already packaged. In principle, sterilization should mean the destruction of all forms of pathogens. However, as we will see at the end of this chapter, prions and endotoxins are not completely

removed or inactivated by the current sterilization methods and still represent a challenge.

1.2.2 Sterilization versus disinfection

It is important to distinguish sterilization from disinfection, which does not ensure the same security level and does not necessarily inactivate all forms of microorganisms – bacterial spores, for instance. Low, intermediate and high levels of disinfection can be obtained depending on the efficacy of the sterilant, duration of the process and ability to prevent deposition of new pathogens on the product after processing. Methods where samples are not wrapped to keep the sterility post-procedure should also rather be called high-level disinfection.

The choice between sterilization and disinfection must be made according to the risk of spreading infection. The classification originally proposed by Earle H. Spaulding in 1957 has been retained, refined and is still used to determine which devices should be sterilized and which disinfected. Sterilization is required for all critical medical devices – that is, those intended to be used in contact with sterile tissues – and recommended for 'semi-critical devices' – for example, those intended to be in contact with mucous tissues or nonintact skin. A high level of disinfection can still be acceptable for these (Spaulding, 1972; McDonnell and Burke, 2011). This category includes respiratory therapy and anesthesia equipment, some endoscopes, laryngoscope blades, esophageal manometry probes, etc. Flexible endoscopes are particularly challenging due to their fragility and their long and narrow lumens; they do not easily withstand sterilization techniques. Moreover, they are difficult to clean.

Laparoscopes and arthroscopes entering sterile tissue ideally should be sterilized between patients. The American Dental Association also recommends surgical instruments that penetrate soft tissue or bone (e.g. extraction forceps, scalpel blades, bone chisels, periodontal scalers and surgical burs) to be classified as critical devices and be sterilized after each use. Proper cleaning and high-level disinfection is, however, currently performed rather than real sterilization.

Sterilization should also not be confused with cleaning, which is defined as the removal of foreign material (soil, dust and organic debris). Thorough cleaning of devices is an important step before high-level disinfection and sterilization, especially in healthcare centers, since it has been demonstrated that it is more difficult to sterilize devices where microorganisms hide behind proteineous or grassy matter. Cleaning becomes a challenge when cavities and long lumens are present, such as in surgical tools for minimally invasive surgical procedures (Alfa and Nemes, 2004). Chemicals, minerals and

water can also limit the efficiency of sterilization or induce damage on the MDs, thus rinsing with distilled or demineralized water, followed by complete drying of the instrument, is generally required, as will be discussed in chapters related to the sterilization techniques.

Cleaning is also important in industrial settings where it decreases the bioburden (living organisms) before sterilization, but also to eliminate contaminants originating from manufacturing processes, such as cutting or polishing fluids and particles, mold release agents, polymer processing aids, airborne contamination, etc. These can negatively impact on device biocompatibility and further processing such as coating adhesion or bonding between two surfaces, corrosion resistance, etc. Finally, the term decontamination refers to the action of reducing the number of microorganisms from objects so they are safe to handle, use or discard.

1.2.3 Industrial versus clinical sterilization

Sterilization is the last step in manufacturing biomedical devices intended for use in contact with sterile tissues, severely damaged skin or mucous and sometimes with intact skin (newborns, etc.).

Industrial sterilization can take place either in-house or as contract sterilization. In-house sterilizers produce goods requiring sterilization and sterilize them as part of their production process. Contract sterilizers are companies that specialize in offering sterilization services to clients, but generally do not produce any of the goods being sterilized. The trend towards the use of contract sterilizers continued throughout the 1990s, as more and more companies focused on their core business and contracted out other services that they needed. Radiation sterilization (described in Chapter 3), in particular, is a technique limited to industrial sterilization and typically used on a contract basis, since this technology requires costly and high-risk radioactive sources.

Clinical sterilization takes place at healthcare centers and faces somewhat different challenges. Indeed, many devices are reusable and must sustain several cycles of cleaning and sterilization in clinical settings. They are then contaminated by a larger amount and variety of pathogens than those present at the end of the manufacturing process. Moreover, presence of biological tissues, blood or soils may prevent the efficiency of the process. In particular, bacteria within biofilms (found on numerous medical devices (e.g. contact lenses, pacemakers, hemodialysis systems, urinary catheters, central venous catheters, endoscopes) have been shown to be up to 1000 times more resistant to antimicrobials than are the same bacteria in suspension (Vickery *et al.*, 2004). Finally, safety issues and duration of the sterilization cycle in clinical settings have more impact than in industrial sterilization. Therefore,

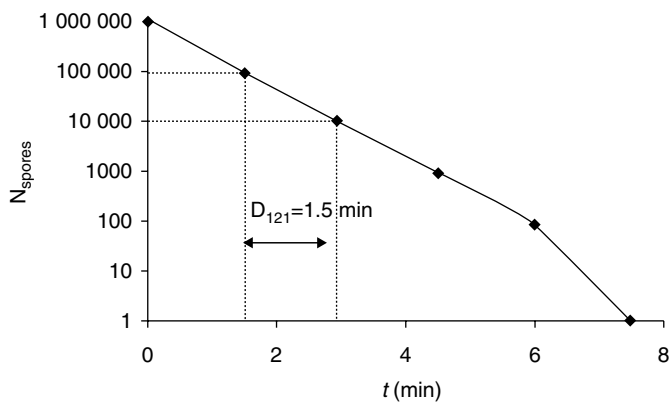
as we will discuss throughout this book, the most common sterilization technologies are not the same for industrial as for clinical sterilization.

1.3 Validation, monitoring and safety of sterilization methods

1.3.1 Validation studies of sterilization methods

Sterilization validation studies must document that the product attains the required SAL after exposure to the proposed process. In the industry, sterilization validation is generally evaluated by, first, determining the qualitative and quantitative bioburden (the type and number of viable microorganisms present on the device just prior to sterilization) after the manufacturing process, then, studying the rate of killing using fractional-run sterilization studies and, finally, deducing the time required to achieve a 10^{-6} SAL. In a fractional sterilization run, product samples (in packages) are exposed to various fractions of the desired sterilization process or dose. The number of surviving microorganisms is reported graphically on a semi-logarithmic scale to extrapolate the exposure time or dose required to achieve a 10^{-6} SAL (Fig. 1.1).

As seen in Fig. 1.1, the dynamics of microbial inactivation is not linear. Microorganisms are destroyed in a logarithmic or first order rate. This means that, under specific conditions (e.g. here during autoclaving at 121°C), the



1.1 Typical deactivation curve of bacterial spores during steam sterilization, which allows the calculation of the D-value (D_{121}), as the time required to decrease the number of spores by 90% at the temperature of 121°C. (Source: Previously published in Chapter 13 by Lerouge, in *'Metals for biological devices'*. Edited by M. Niinomi (Woodhead Publishing ©).)

same exposure time is required to decrease the number of microorganisms from 10^6 to 10^5 as from 10^2 to 10^1. This exposure time (or dose, in the case of radiation sterilization) required to reduce the surviving population by 90% or 1 $\log_{10}$ is called the D-value (the decimal reduction time) and is very useful to calculate the exposure time required to achieve SAL from a known bioburden. This method enables the limitation of the dose, thus preventing damage to the device. It should, however, be kept in mind that all sterilization process do not have a perfect logarithmic inactivation curve. It should also be realized that the microbial death observed is really a failure of the microbe to reproduce when placed under favorable environmental and optimal recovery medium and that microorganisms' inactivation is different from physical destruction. This means that effective inactivation of bacteria does not mean their physical removal, as discussed in Section 1.4.2 under the challenge of endotoxins.

It is important to differentiate industrial sterilization from resterilization of reusable devices as achieved in healthcare centers. In this latter case, the bioburden cannot be easily determined since it can be influenced by several factors (previous patient treated, efficiency of the decontamination and cleaning step, etc.) and a more drastic sterilization called 'overcharge' sterilization is generally achieved, considering an initial microbial charge of 10^6 per device. Moreover, clinical centers apply safety factors, thus multiplying time required by a safety factor that depends on the risks associated with the device (generally higher for permanent implants than for surgical tools, for instance).

Factors that affect the efficacy of sterilization are numerous: cleaning, bioburden, pathogen type (prions being the most resistant and most virus less resistant), presence of protein and salts, biofilms accumulation, lumen length and diameter, restricted flow and device design and construction.

Validation studies must be done on product samples prepared under actual manufacturing conditions and exposed to the sterilization process under its final packaging configuration. Device geometry (small lumen or cavities) can be of concern when sterilization agents have limited penetrability. More details about sterilization process validation can be found in the specific ISO documents (ISO11134, 1994; ISO11135, 1994; ISO11137, 1993). Moreover, cleanliness is another important parameter for reusable devices, and this must be taken into account when designing the device, to avoid unreachable cavities.

1.3.2 Monitoring sterilization methods

Once the process has been validated, its efficiency must be verified regularly on real sterilization loads. One must distinguish between (a) physical

monitoring (any deviation from the expected physical parameters (e.g. temperature, pressure, time) should alert the operator to potential problems); (b) chemical indicators, which change color when sterilization parameters (e.g. steam-time, temperature and/or saturated steam; ethylene oxide (EO)-time, temperature, relative humidity and/or EO concentration) have been reached, or dosimeter (for radiation dose); and (c) biological indicators which confirm complete inactivation of spores that are particularly resistant to the specific process (*Bacillus stearothermophilus* for autoclave, *Bacillus subtilis var*. niger for EO, etc.). Biological monitoring is the most important check on sterilizer function in hospital centers. Depending on the location, it is used for each sterilization load (especially when implantable devices are processed) or at least weekly. If a sterilizer is used frequently (e.g. several loads per day), daily use of biological indicators (BI) allows earlier discovery of equipment malfunctions or procedural errors and thus minimizes the extent of patient surveillance and product recall needed in the event of a positive biological indicator.

In industrial sterilization, when a fully validated terminal sterilization method by steam, dry heat or ionizing radiation is used, parametric release – that is, release of a batch of sterilized items based on process data rather than on the basis of submitting a sample of the items to sterility testing – may be carried out, subject to approval of the competent authority. This eliminates the requirement for a finish product sterility test as a condition to batch release. For example, as discussed in the next chapters, monitoring the delivered radiation dose may be sufficient to allow product release after gamma sterilization. In contrast, EO process control is more complex since several parameters such as EO concentration, humidity rate, time, temperature and vacuum/pressure influence process efficiency. The verification of sterilization efficiency then requires adding BI-containing bacterial spores in the sterilization load and verifying their inactivation. This method generally limits the availability of processed devices since conventional BI requires 24–48 h spore incubation before results can be read and sterilized products released. However, progress has been made recently to shorten these times and a new generation of BI using spore-associated enzymes is now able to detect sterilization failure in a few hours only (Rutala *et al*., 1996; Rutala and Weber, 2001; Leventon, 2002; McCormick *et al*., 2003). Thus, a rapid-readout biological indicator which detects the presence of enzymes of *B. stearothermophilus* by reading a fluorescent product produced by the enzymatic breakdown of a nonfluorescent substrate has been marketed for more than ten years. This field is rapidly progressing and a new rapid-readout biological indicator for EO has also been recently cleared by the FDA.

Finally, an expiration date must be labeled on sterilized products given that packaging materials cannot indefinitely prevent recontamination with microorganisms. Commonly used wrapping materials are 140 threadcount

muslin, Kraft paper, nonwoven wraps and paper/plastic peeldown packages. Items to be sterilized are generally wrapped in two thicknesses of paper or nonwoven fabric to avoid contamination from the exterior surface upon opening. The time allowed between sterilization and clinical use mostly depends on the type of packaging material used. The term 'shelf-life' is defined as the period during which sterility can be maintained. In healthcare centers, this time is typically about six months. The delay can be higher in industry. For costly single-use devices, such as implants, manufacturers should take this matter into account to avoid withdrawal of unused and expired sterilized products from the market before sale.

1.3.3 Sterilization safety

'Safety of sterilization' is a broad term including concerns for patients, sterilization personnel and environment. The first concern with the choice of a sterilization process is the demonstration that the product is compatible with the sterilization process; the integrity of the product and of the packaging, which maintains its sterility after the process, must be demonstrated since every type of material can be degraded to some extent by one or more sterilization process. This could lead to a significant loss of functionality or biocompatibility of the device. Sterilization can also leave toxic residues or by-products formed during sterilization. It is then essential to ensure that patient exposure to these residuals stays below safe limits. For these reasons, functional and biocompatibility testing for FDA or European Community (EC) approval must be performed on the final packaged and sterilized products.

Safety issues greatly depend on the sterilization technique and type of material. Metallic biomaterials are generally well resistant to sterilization processes, including steam and dry heat which reach temperatures of 121°C or 134°C (steam) or even higher (162°C) in the case of dry heat. However, steam autoclaving can cause corrosion of some metallic devices, in particular high carbon steels used for surgical and dental instruments and cause unprotected cutting edges to dull. Moisture also can adversely affect electronics. To avoid this, it is of utmost importance to clean and thoroughly dry the instruments before sterilizing by autoclave. One way to reduce progressive corrosion of carbon steel instruments is to dip them in an anticorrosive solution prior to autoclaving (Holmlund, 1965; Stach *et al.*, 1995). In surgical trays, contact between instruments of dissimilar metals should be avoided to prevent galvanic corrosion.

To avoid metallic corrosion, *dry heat sterilization* can be used as an alternative, but it is less efficient than wet heat and requires longer times and/or higher temperatures. Thus temperature needs to be increased to 140°C for 3 h or 160–170°C (320–338°F) for 1 or 2 h (Block, 2000; Kowalski and Morrissey, 2004). Metals are also resistant to radiation and do not absorb

EO, so they are also compatible with these low-temperature techniques. New sterilization technologies such as Sterrad® or ozone can cause surface oxidation and corrosion.

Bioinert ceramics (alumina, zirconia) are generally also resistant to the conventional sterilization methods (gamma rays, steam and EO), but with some limitations (Tsai *et al*., 2007; Nam *et al*., 2009). Steam sterilization has been reported to be associated with surface roughening of zirconia ceramic femoral head components of total hip prostheses, due to a phase transformation in the crystal structure of the zirconia material under exposure to steam and elevated temperatures, with consequences on wear resistance and premature failure. EO is thus preferred for zirconia (Burlington, 2007). Very little data is available regarding the resistance of bioactive ceramics (hydroxyapatite, calcium phosphate, etc.) that may be more prone to degradation during sterilization. Careful literature review or testing should be undertaken before choosing a method.

Polymer is the class of biomaterials which is the most sensitive to sterilization procedures, with large variation, however, among polymers in regards to their sensitivity to radiation, heat and humidity. Damage to polymers ranges from some oxidation to cross-linking, complete distortion and melting. Moreover, polymers absorb EO, so that EO can leave a significant amount of toxic residues. Polymer compatibility with sterilization technique is an important topic, since packaging materials are often polymers and most new devices now incorporate polymeric compounds or coatings which are sensitive to sterilization conditions, such as heat and radiation. Biological tissues, cells or molecules are also increasingly used, especially in tissue engineering approaches. These issues will be specifically addressed when discussing each sterilization process (Chapters 2–5), as well as chapters on polymers and allografts (Chapters 7 and 8). One should also be aware of the possible direct harm of the sterilization process towards manufacturing personnel and the environment, as will be discussed in Chapter 4 on EO sterilization.

1.4 Challenges and constraints of sterilization methods

In addition to the above-mentioned points, sterilization nowadays faces several challenges related to technological progress, new regulations and diseases, as well as human, environmental and socio-economic factors. These tend to complicate the task of the sterilization staff in clinical settings.

1.4.1 Prions

Healthcare resterilization is challenged by the risk of transmission of prions (short for proteinaceous infectious particles). As found by Stanley Pruisner

(Nobel Prize in 1997 for physiology or medicine for his theory), a prion is an abnormal conformational isoform of a normal cellular protein, the prion protein (PrP). Although it contains no DNA or RNA, it is able to self-replicate and to be the agent of infection in a variety of degenerative brain diseases, such as Creutzfeldt-Jakob disease (CJD), fatal familial insomnia, an unusual form of hereditary dementia known as Gertsmann-Straeussler-Scheinker disease and possibly some cases of Alzheimer's disease (Prusiner, 1995; Rosenberg, 1997). Prions have been shown to be transmissible via several routes, including transplantation and contaminated medical products. They are notoriously resistant to sterilization processes and disinfecting agents known to inactivate bacteria, spores and viruses (Steelman, 1999; Zobeley *et al.*, 1999; Taylor, 2000). In contrast to all other known infectious agents (bacteria, virus, fungus, parasite), which all contain nucleic acids (DNA, RNA or both), prions contain no genetic material. Their inactivation requires the denaturation of the protein to a state where the molecule is no longer able to induce the abnormal folding of normal proteins. Prions are generally quite resistant to proteases, heat, radiation and formalin treatments, although their infectivity can be reduced by such treatments. None of the conventional sterilization procedures has been shown to be completely efficient. Since these diseases are progressive and cannot be diagnosed efficiently, preventing their transmission is a real challenge. To prevent accidental transmission of CJD, various decontamination procedures have been adopted for reusable medical devices in contact with high-risk tissues such as instruments for brain, spinal cord and eye surgeries. For patients with known or suspected CJD, it is even recommended to discard instruments used for surgery since no proven technique is available. In terms of practical application, autoclaving at 134°C for 18 min or 121°C for 30 min, and 1 N sodium hydroxide for 15 min strongly reduced infectivity but did not completely eradicate it (Collins *et al.*, 2004).

The World Health Organization recommends any of the following three procedures for the sterilization of all heat-resistant surgical instruments to ensure that they are not contaminated with prions (Sutton *et al.*, 2006):

1. Immerse in a pan containing 1 N NaOH and heat in a gravity-displacement autoclave at 121°C for 30 min; clean; rinse in water; and then perform routine sterilization processes.
2. Immerse in 1 N NaOH or sodium hypochlorite (20 000 parts per million available chlorine) for 1 h; transfer instruments to water; heat in a gravity-displacement autoclave at 121°C for 1 h; clean; and then perform routine sterilization processes.
3. Immerse in 1 N NaOH or sodium hypochlorite (20 000 parts per million available chlorine) for 1 h; remove and rinse in water; then transfer to an open pan and heat in a gravity-displacement (121°C) or in a porous-load

(134°C) autoclave for 1 h; clean; and then perform routine sterilization processes.

Moreover, most materials do not sustain such aggressive conditions. Recently, promising results showed elimination of detectable levels of infectivity after using alkaline cleaner followed by autoclave (Fichet *et al.*, 2004; Lawson *et al.*, 2007; Lemmer *et al.*, 2008) or when using enzymatic cleaning preparation in conjunction with gaseous hydrogen peroxide (Fichet *et al.*, 2007). In contrast to the gas form, liquid peroxide was not effective. Other novel sterilization methods have shown promising preliminary results and are under assay, such as ozone and plasma processes (Baxter *et al.*, 2005).

1.4.2 Endotoxins

Endotoxins are another challenge for sterilization. Endotoxin, a lipopolysaccharide found in the cell wall of Gram-negative bacteria, is a pyrogen which induces inflammation and fever as an immune response in higher organisms. Pyrogens can lead to anaphylactic shock and death of patients. This risk has been illustrated in rare but serious clinical cases (Cookson *et al.*, 1997). Pyrogens can be present on 'sterile' biomedical devices, since sterilization processes do not remove microorganisms but simply deactivate them or destroy them partly. Endotoxins are highly resistant to sterilization processes. Presently, the only procedure recommended by the United States Pharmacopeia (USP) is dry heat at 250°C for 30 min or 180°C for 3 h, which is completely unsuitable for polymeric, and even metallic, materials (Nakata, 1993; Cookson *et al.*, 1997). Recent studies showed that plasma techniques have interesting potential for depyrogenization, with a fast (10 s to a few minutes) removal rate of the immune-stimulating competence of these molecules (Kylian *et al.*, 2006; Tessarolo *et al.*, 2006).

Endotoxins also represent a challenge for industrial sterilization. Indeed, legislations such as the FDA require documention that all blood-contacting devices, permanent implants, devices that contact cerebrospinal fluid and devices labeled pyrogen free or non-pyrogenic are, in fact, non-pyrogenic (FDA website).

1.4.3 Antimicrobial coatings

Despite device adequate sterilization and aseptic procedures, device infection can appear as a result of bacterial adhesion and growth and subsequent biofilm formation after implantation. This is a major clinical problem,

especially with urethral catheters, subcutaneous sensors and some implants. The development of biomaterials that resist bacterial adhesion – for example, by releasing antimicrobial agents such as antibiotics, silver ions, antibodies and nitric oxide – is in progress (Hetrick and Schoenfisch, 2006; Darouiche, 2007; Ramritu *et al*., 2008). This will be the subject of Chapter 9.

1.4.4 Miniaturization of surgeries and treatments

Sterilization must adapt to the growing use of small miniaturized devices developed for minimally invasive diagnostic and therapeutic procedures. Most of them include polymeric parts that are heat sensitive, small lumen or cavities which are difficult to reach by the sterilant. The increasing use of drugs or biological products with synthetic materials – for example, in coated stents or tissue engineering applications – is also challenging. Biodegradable and biological materials (cellulose, bone, collagen, chitosan, proteins, heparine, etc.) are increasingly used. Some of them are extremely sensitive to heat, radiation and oxidation (Nair, 1995; Gogolewski and Mainil-Varlet, 1996; Mitchell *et al*., 2004).

1.4.5 Ecological constraints

Health risks associated with pollution and degradation of air quality regularly lead to new legislation that has impact on sterilization methods. Thus, the interdiction of chlorofluorocarbons (CFCs) since 1996 (related to the Clean Air act (or 'Montreal Protocol on Substances that Deplete the Ozone Layer'), signed in September 1987) has placed hospitals and industry in a vulnerable position since it required the revision of the sterilization processes of several hundred products (Jorkasky, 1993). EO processes using hydrochlorofluorocarbon (HCFC), less aggressive towards the ozone layer, were proposed (Alfa *et al*., 1997), but they were only a temporary solution since HCFC, as CFC, must be eliminated before 2030 (Jorkasky, 1993). Sterilizers using pure EO or EO mixed with carbon dioxide (CO_2) have been developed to face this legislation. However, EO is recognized as a toxic waste by the Environmental Protection Agency (EPA) (Steelman, 1992) and its release in air is now severely restricted by regulations in many countries (requiring the installation of an emission control system) and could be banned. More generally, the release of toxic wastes will be the subject of increasing regulation and limitation in the future and should be taken into consideration when choosing alternative technologies. In this sense, hydrogen peroxide gas plasma and ozone (see Chapter 5) present some advantages. More generally, national or international regulations, which are created to protect patients, working

staff and the environment, are in constant evolution and differ among the countries, in particular between Western, emerging and Third World countries.

1.4.6 Economic constraints

Medical devices are getting more and more costly and their use grows year after year. For instance, the increasing use of endoscopes and catheters carries high costs for healthcare centers, often subjected to budget compression. Development of sterilization methods and devices that can withstand several sterilization cycles is a serious economic issue.

Presently, a vast majority of devices are sold as single-use. Economic constraints have led healthcare centers to reprocess single-use devices. Approximately 20–30% of US hospitals reported that they reuse at least one type of single-use device, although this is extremely controversial and is an evolving area of regulations. Thus, in the USA, the FDA considers the hospital that reprocesses a single-use device as the manufacturer of the device and regulates the hospital using the same standards by which it regulates the original equipment manufacturer. The options for hospitals are, thus, to stop reprocessing single-use devices, comply with the rule, or outsource to a third-party reprocessor.

Ethylene oxide is, on this basis, still the best available sterilization technique, compatible with most materials. However, using methods with short sterilization cycles is also important to help reduce the volume of material required to treat patients, since the material can rapidly be reused. This is one of the limitations of ethylene oxide, which involves not only several hours for sterilization itself but also up to 30 h of aeration before allowing batch release (Steelman, 1992; Page, 1993). This short example illustrates that no ideal sterilization technique exists and that a compromise is always to be found.

1.5 Ideal versus actual sterilization methods

1.5.1 Requirements for an ideal sterilization method

An ideal sterilization method should be efficient and highly reliable. This can be achieved when the sterilant is highly penetrating and can be homogeneously distributed within the load. It should be able to deactivate prions and endotoxins, in addition to virus, bacterials, fungi and endospores. It should withstand reasonable organic material challenge without loss of efficacy and have good penetrability to reach SAL on devices with all geometries, including cavities, narrow lumens and hinges, and enable

sterilization of large volumes at once to decrease costs. It should not damage any device, even when repeated sterilization cycles are carried out.

It should also be compatible with all materials. This implies working at low temperature (below 60°C). It should be safe also for patients and staff workers and pose no hazard to the environment – thus, not leaving any toxic residues or by-products on biomedical devices or in the atmosphere. It should be easy to monitor, by checking that physical or chemical conditions required were achieved within each package. It should also be quick, to enable rapid release to decrease the amount of material held by the healthcare institution. It should be easy, cheap and safe to operate and should be suitable for large or small (point-of-use) installations.

It is easy to understand that such ideal sterilization technology does not exist. This is why several methods are used and differ from industrial to clinical settings, as well as between countries, depending on regulations.

1.5.2 Overview of sterilization processes

Initially limited to processes working with moist heat (autoclave), hot water or dry heat, sterilization adapted to the introduction of plastic materials in the 1940s by the development of low-temperature techniques, such as gamma radiation (limited to industrial sterilization) or EO gas sterilization. Their characteristics, advantages and limitations are briefly summarized in the Table 1.1. These traditional methods are detailed in Chapters 2, 3 and 4.

Recently, clinical sterilization has evolved considerably. New low-temperature sterilization methods have emerged, such as hydrogen peroxide gas plasma (or Sterrad®), ozone, peracetic acid, vaporized hydrogen peroxide, microwave radiation, etc. Some of them tend to replace EO in clinical settings (Sterrad®, ozone, steam formaldehyde). Others are only rarely used or even experimental. These new technologies are presented in Chapter 5.

1.6 Conclusions

Sterilization is an important and problematic step that should be considered as early as possible in the design of any new medical device intended for use in contact with sterile tissues, mucous membranes or breached skin, in order to save money, time and trouble. There is no single sterilization method that is compatible with all healthcare products including drugs, polymers, devices and materials, because of the severity of a process to meet the sterilization criteria and definition. As discussed, metallic alloys are generally compatible with most sterilization processes. However, devices are getting smaller and more fragile, with complex geometries, and often include polymeric compounds (such as coatings or adhesives) that require low-heat sterilization

Table 1.1 Parameters, advantages and limits of the main sterilization processes

Technique	Main parameters	Advantages	Limits
Steam sterilization (autoclave)	121°C, 5–20 min Gravity High humidity level	Simple, safe, rapid and efficient Good penetrability Easy to monitor No toxic residues Can sterilize liquids Low cost	High temperature and moisture Incompatible with many thermosensitive polymers Metals may corrode Requires breathable packaging
	Options: Flash autoclave 134°C, 3–6 min in vacuum with steam pulses	Rapid	Incompatible with many materials due to very high temperature and moisture
	'Low-temperature' steam (110–115°C, 35–40 min)	Allows sterilization of more heat-sensitive materials	Longer exposure Efficiency subject to some controversy
Dry heat	160–170°C 1–2 h	Generally avoids metal corrosion	Longer exposure than steam
Gamma radiation	10–40 kGy (most common 25 kGy) Ambient temperature A few hours	Excellent penetrability,efficiency and reliability Dosimetric release Large product volumes Cost effective Compatible with many materials No residues Environmentally safe, except for the disposal of radioactive waste	Isotope containment requires costly facilities for safety reasons Only available in a few industrial centers Some polymer damage, increasing with increased doses Limited to single industrial sterilization

(*Continued*)

Table 1.1 Continued

Technique	Main parameters	Advantages	Limits
Electron-beam	11–40 kGy	Very rapid (seconds/minutes) More material compatibility than gamma rays	Lower penetrability than gamma rays
Ethylene oxide (EO)	25–65°C (generally 55°C) High humidity EO: 400–1500 mg/L 2–4 h + aeration	Compatible with most materials Efficiency, relatively good penetrability	Long cycle and safety risks EO toxicity and mutagenicity: safety risks for sterilization personnel Toxic EO residues and reaction products in polymers: requires aeration EO may be banned by regulations Difficult to monitor

processes. The increasing use of drugs or biological products with synthetic materials – for example, in coated stents or tissue engineering applications – is also challenging.

The growing importance of polymers, on the one hand, and the hazardous nature of EO on the other hand, has led to the development of novel sterilization methods. These, however, have their own limitations. Therefore, no ideal sterilization technique exists presently. The parameters and effects of different sterilization methods must, thus, be evaluated and reviewed before selecting the proper method. As discussed earlier, high variation of resistance to sterilization techniques is observed in biomaterials, particularly for polymers. It is recommended, if possible, to choose materials that are compatible with radiation. Manufacturers should take advantage of the new medical-grade materials compatible with radiation sterilization. In the case of reusable devices, their design should take into account that they must be cleaned before they can be resterilized and that sterilization methods other than radiation will be used. Materials that are compatible with steam sterilization should also be favored when possible. Devices should be designed to avoid regions sealed off from the cleaning or sterilization process or allow the device to be disassembled to expose all parts during cleaning and sterilization. The efficiency and safety of sterilization will have to be demonstrated before device approval. Due to more and more complex regulations and standards, an increasing number of industrial manufacturers are moving over to outside contract sterilization.

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2

Steam and dry heat sterilization of biomaterials and medical devices

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Abstract: An ounce of prevention is worth a pound of cure. Without sterilization, infectious disease could exist everywhere in hospitals or healthcare facilities, and it is debatable whether antibiotics could control such an extensive attack. Heat sterilization is typically divided into steam sterilization and dry heat. Steam sterilization and dry heat have many similarities, such as the ability to sterilize virtually all organisms with no toxic residues or waste, but they also have differences. Steam sterilization can distort, corrode or wet materials, whereas dry heat can degrade and melt many heat-sensitive materials and devices. Dry heat also has excellent penetration capabilities. Heat in general can improve and enhance the microbial effectiveness of other methods of sterilization. Determining which sterilization method is most appropriate to use in any given situation requires the identification and discussion of the sterilizing principles, qualities, uses and prospects of the different techniques. This chapter will provide a discussion of each method and describe how heat can improve sterilization.

Key words: sterilization, lethality, effectiveness, bioburden, biological indicator, validation, process variables, types of sterilizers (sterilization), recommended uses, enhancements and improvements, sterility assurance level (SAL).

2.1 Introduction

Heat is the oldest form of sterilization.[1,2] It is referred to in the Bible (probably dating to about 1200 BC). Heat sterilization of medical instruments is said to have been used in the Roman period, though the practice disappeared in the Dark Ages and was only rediscovered in the eighteenth and nineteenth centuries. Heat sterilization can be carried out using steam (moist heat) or dry heat. The two methods have many characteristics and qualities in common. They both use high temperatures to inactivate microbes and resistant spores, with the specific temperature required depending on exposure time.

To determine which sterilization method is appropriate depends on a number of factors including:

- type of microorganism(s) to be targeted,
- product materials,

- product design and packaging,
- volume and throughput of sterilized product required.

At lower sterilizing temperatures, more polymers and other materials can tolerate the heat required for sterilization.[3] Choosing the right method may involve feasibility studies to determine the compatibility of the product with the selected process,[4] and then preliminary validation studies to demonstrate both product compatibility and that the required sterility assurance level (SAL) can be obtained.

2.2 Steam sterilization

Steam sterilization in the form of saturated steam under pressure is one of the most effective methods of sterilization. With its latent heat of vaporization, steam provides at least 7 times as much heat on an equimolar basis as dry heat at the same 121°C temperature. On this basis, steam provides a heat-up period, of at least, 12 times faster than a typical dry heat process, from ambient to their equivalent sterilizing temperatures.[5] Steam sterilization is typically recommended for items which are not likely to suffer from heat or water damage. If suitably applied, steam sterilization is able to inactivate prions which some other methods cannot treat.[6]

2.2.1 Materials that can be steam sterilized

Steam can be used to sterilize glass, most metals, many heat stable polymers (including acetal, nylons, polycarbonate, polypropylene, polysulfones and polytetrafluoroethylene (PTFE)), celluloses (papers), liquids, fabrics, many drugs, pharmaceuticals and some medical devices and reusables[7] (Table 2.1). Some of these materials cannot be sterilized by other methods. If prudently applied and controlled, steam does not corrode metals.

In hospitals, where reusable materials are frequently and routinely sterilized, steam sterilization predominates. It can be used to sterilize and resterilize wraps, linens, papers, cotton and many surgical trays that cannot be sterilized using other methods. It is still the principal method for inactivating *Pyronema domesticatum* on cotton, which other techniques such as ethylene oxide and irradiation cannot sterilize easily. Steam sterilization can also be used to sterilize glass, acetals, liquids, natural polypropylene, most Teflons and many other reusable materials that cannot be sterilized using other techniques such as irradiation. It is also the preferred method for loose instruments, packs and other items that are not heat sensitive or moisture liable. It is widely used to decontaminate infectious waste materials.

There are many materials that can be damaged by high steam temperature, including ABS, acrylics, styrene, low density polyethylene (PE) and polyvinyl

Table 2.1 Some items, materials and polymers that are compatible with steam

Acetals – some
Butyl rubber (varies)
Canvas
Cellophane
Chinese cotton (with *Pyronema domesticatum* contamination)
Cotton
Crepe
Ethylene propylene diene monomer (EPDM) elastomers
Fluoropolymers (most Teflons)
Glass
Instruments – most reusable
Kraft paper
Liquid crystal polymer (LCP)
Metals (most) – instruments
Muslin
Nitrile elastomer
Nylon (questionable)
Paper (varies)
Polyethylene (some high density (HDPE), cross-linked (XLPE))
Polyamides (varies)
Polyesters
Polyallomer (or replaced by polypropylene copolymer (PPCO))
Polycarbonate (varies)
Polyester
Polyethylene (limited – high density)
Polyimides
Polyketones (e.g. polyetheretherketone)
Polypropylene
Polymethyl pentene (PMP or TPX*)
Polyurethane (limited)
Polyvinyl chloride (varies)
Polysulfones
Silica
Silicone (limited)
Syndiotactic polystyrene (SPS)
Textiles (many)
Wraps (some)

Note: Many of the above materials may be compatible at lower sterilizing temperatures, if they are used are used for sterilization processing (see AAMI TIR 17:2008 (ref. 8)).

* TPX is the Japanese trademark for PMP. It is a lightweight, yet hard, functional polymer with a unique combination of transparency, heat and chemical resistant properties.

chloride (PVC). Polyurethane may be hydrolytically attacked by steam, creating toxic 4,4′-methylenedianiline (MDA). However, since acetal, polypropylene and Teflon® can be damaged by other techniques such as radiation, they are possible candidates for steam sterilization under appropriate conditions. Selecting which polymers and materials are suitable for the steam sterilization temperature and process that is to be used is done by reviewing the glass transition temperatures of the polymers to be sterilized.[8] Polymers to be sterilized must be

compatible with conditions of relative humidity (RH) in excess of 95%. Some superheat (<100% RH) sterilization is tolerated at elevated temperatures.

If the temperature is reduced (e.g. 105–118°C) and exposure time is increased, steam can sterilize many heat-sensitive polymers, such as high-density polyethylene, PVC, non-plasticized PVC, polypropylene, natural and butyl rubber, and heat-resistant urethanes.[7,8] Under these conditions, polymers may be sterilized repeatedly. If there is no 'clinical' or risk concern with hemophilic spores (as there is with foods), steam sterilization can be applied at even lower temperatures (e.g. < 121°C). Sterilization of heat-sensitive drugs or solutions (e.g. dextrose, vitamins, amino acids, etc.) may also be conducted at lower temperatures than traditional steam methods (at 110–115°C rather than 121–134°C).

2.2.2 How steam sterilization affects microorganisms

The killing power of steam is principally due to the coagulation of proteins in microorganisms, which causes the denaturation of DNA and the breakdown of vital enzymes.[2] At sufficiently high moist heat temperatures, steam heat can inactivate all microbes, including the most resistant bacterial spores, mold (e.g. *Pyronema domesticatum)* and prions.

Typically, viruses are easier to sterilize than vegetative microbes. Pasteurization temperatures (e.g. 62–72°C) are required for non-spores (vegetative cells). Vegetative microbes such as *Staphylococcus, Streptococcus and Micrococcus* are easily inactivated after 10 min at 65°C. Microbial spores are more resistant.[9] Some are killed at temperatures slightly above boiling (e.g. 105°C), but others require higher temperatures (e.g. 121°C for 15 min and 1.03 Bar (15.03 psi)). Prions are even more resistant (e.g. 121°C for 1 h, 18 min at 134°C and 2.02 Bar (29.41 psig)).[6]

The bioburden is the presterilization population of viable microorganisms on an item or product, and affects the lethality of steam sterilization. A low bioburden is important. Unlike ethylene oxide sterilization, for example, steam can have a non-logarithmic inactivation death curve where heat activation of dormant spores can occur. Consequently, steam sterilization is best performed with presterilization and low spore bioburden levels, below 1000 cfu/ device. A number of factors impair the ability of steam to inactivate microbes (Table 2.2).

2.2.3 Mechanisms of steam sterilization

Sterilization occurs when available surfaces are heated with moisture. Steam sterilization requires saturated steam to be effective. The sterilizing power of steam is largely due to its latent heat of vaporization (Lv) (e.g. ~540 calories per gram) and temperature. As steam contacts a cooler surface, it condenses and raises the temperature, causing a huge decrease in volume of

Table 2.2 Examples that may impair microbiocidal effectiveness of steam

- Non-condensable gaseous moisture
- Grease
- Salt crystallization (occlusion of microbe) and organic encrustation
- Biofilm
- Processing residues (e.g. amines)
- Load mass and configuration
- Clumping of spores
- Artificial barriers or tortuous path to steam diffusion to microbial site (e.g. O rings – seals retard steam, remove rings, or extend exposure; upright empty containers are difficult to remove air from, with a gravity cycle, because air is heavier than steam and will remain in an upright empty container. Place empty containers upside down to allow heavier air to diffuse out and escape from the empty container and to remove any residual air with incoming lighter steam)
- Air pockets or immediate microenvironment barriers to microbes (e.g. keep lumens open, valves open, etc.)
- Change of immediate environment (e.g. pH, ionic, fluid chemicals, particulates)
- Micro-environment of microbes (heterogeneous population, environment favorable for formation of spores, formation of resistant spores with calcium and dipicolinic acid (DPA) complexing in cortex, slightly elevated incubation temperature, dessication or change in water activity)
- Steam quality – dryness, superheat, non-condensable, additives (amines, etc.)

Table 2.3 Factors influencing the ability of steam to kill microbes

Related to lethality of steam	Related to organisms to be killed
Intensity of heat transfer (condensation)	Types of microbes (e.g. spores, prions)
Exposure time	
Temperature	Number of microbes to be killed
Presence of air (conditions that impair inactivation)	Level of sterility assurance (probability)
Quality of steam	Previous history of microbes to be killed
Insufficient distribution of heat	
Less than saturation steam (non-condensing steam/air)	Places that harbor or protect organism

steam and setting up a negative pressure that draws in more steam. Steam condensation produces a high delivery of heat until temperature equilibrium is reached.

Temperature, steam pressure and length of exposure are the primary process variables. Saturated steam is effective at above 105°C. Moisture and heat must penetrate to all parts of a product or device to be effective. Since air can be a barrier to moisture contact, device design, construction, conductivity and packaging all influence the ability to kill microbes (Table 2.3).

Steam sterilizes at both high temperature and pressure. Saturated steam under pressure typically reaches high temperatures (e.g. 121°C at 15 psi.) As the temperature and saturated pressure increases, the time needed to complete sterilization decreases and conversely, as the temperature and pressure decreases, the time needed to sterilize increases. Lower temperatures can be used (e.g. 105–120°C) to prevent degradation or damage to material, nutrients, polymers and other substances. Higher temperatures (e.g. 132–138°C) are used for faster, flash sterilization.

2.2.4 Key steps in steam sterilization

Steam heats up the product or material to be sterilized with moist heat. As has been noted, the steam must penetrate all parts of a device to be effective. The typical phases or steps in a steam sterilization process are:

- preparation,
- loading,
- pre-evacuation to remove air prior to admission of steam,
- heat-up,
- exposure,
- cool-down,
- drying.

Preparation

Dense areas, mated surfaces and air are barriers to heating. Consequently, device design, construction, conductivity, packaging and loading are critically important in achieving adequate lethality and ultimate sterility. It is important to wash, clean and dry all instruments and other items to be sterilized. All jointed or mated-surface instruments should be opened or placed in an unlocked position, while instruments composed of more than one part or sliding parts should be disassembled. To help prevent dulling of sharp points and cutting edges, sharp edges and needle points should be wrapped in gauze before sterilizing.

Loading

The product must be placed and positioned carefully during loading so that the steam or moist heat can be easily dispersed and disseminated to reach all surfaces that need to be sterilized. Packs should be arranged in the chamber to allow free circulation and penetration of steam to all surfaces.[10] Instruments should not be held tightly together by rubber bands, clamps

or any other means that might prevent steam contact with all surfaces. For large loads, loading arrangements must be specified and validated for effective temperature distribution and/or penetration.

When using a steam sterilizer, it is best to wrap clean instruments or other clean items in a double thickness muslin or wrap for implantables. Unwrapped instruments must be used immediately after removal from the sterilizer, to minimize recontamination, unless kept in a covered, sterile container.

Pre-evacuation: air removal/displacement

Air is removed or displaced so that steam heat can effectively contact all surfaces and penetrate all areas to be sterilized. This may be performed by various methods, as discussed in section 2.3 on specific sterilization methods.

Heat-up/exposure

Heat-up is the period when the moist heat is brought up to the desired exposure temperature. During the heat-up phase, microbial inactivation may begin occurring above 105°C. A longer heat-up typically reduces exposure time, if heat lethality is integrated. Heat-up time enhances heating via condensation of steam on the material.

Exposure is the period of the cycle in which microbes and product are in contact with saturated steam at a set temperature for a certain period of time, calculated to be the time necessary to inactivate all microbes, typically with a probability of survivors of 10^{-6} or better. Typical relationships of time and temperature are shown in Table 2.4.

Saturated pressure under normal atmospheric pressure at 121°C is approximately 15.03 psi (1.03 Bar). The pressure needed will vary directly according to the temperature applied. A temperature of 131°C will have a saturated steam pressure of 29.41 psi (2.06 Bar). If steam pressure and temperature do not correlate according to the manufacturer's instructions or saturated steam table, sterilization may not be achieved.

Cool-down

Cool-down is the period or step after exposure when pressure, temperature and moisture are brought down to atmospheric conditions. Cooling reduces heat and eliminates moisture from the sterilizer. During this period, there is a risk that packaging or containers with entrained air may burst or become distorted with the change in internal pressure versus external pressure, which may require positive pressure overlay in the chamber. During cool-down phases, microbial inactivation may continue

Table 2.4 Time–temperature process relationships for steam sterilization

Temperature	Time (overkill)*
132/134°C (Prevac/flash)	3 min (unwrapped items)
132/134°C (Prevac)	4–20 min*
132/134°C (Prevac)	18 min (Prions)
121°C (gravity/flash)	10 min
121°C (gravity)	15 min (8 min†)–90 min
121°C (gravity)	60 min* (Prions)
115/116°C	30 min
111°C	150 min
105°C	300 min (5 h)

Notes: Flash sterilization may occur with only 3 min at 134°C, but not with packaging or steam barriers.

* The above exposures may vary depending upon sterilizer, test equipment, load, heat-up and cool-down time, and time to penetrate (e.g. lumens), and an overkill approach (typically with 10^6 spore population) versus a normal bioburden population. Shorter times may result based upon bioburden control and resistance, and integration of time/temperature during heat-up and cool-down steps.

† Reduced lethality (e.g. F_o) or exposure times may be the result of sterilization of approved less heat sensitive materials and conditions which use only non-thermophilic microbes or bioburden in sterilization.

above 105°C. A longer cool-down typically reduces exposure time, through integration of lethality during this period, and can help reduce overall exposure appropriately.

Drying

Drying is the period following exposure and cool-down where condensation is allowed to evaporate, and hydration effects are reversed. Drying removes residual moisture and polymer hydration. Post-sterilization heat drying may complete inactivation of a few microbes that were not killed during the heat-up, exposure and cool-down phases of the cycle. Post-heating drying can provide additional inactivation by not allowing damaged microbes to repair themselves through nucleic acid annealing, which might otherwise be observed as slow-grow sterility incubation phenomena. Drying with circulation and some heat can also help eliminate water marks and restore any temporary material distortion caused by heat and pressure.

2.3 Different methods of steam sterilization

The method of steam sterilization used is chosen to be compatible with the product or material to be sterilized.[11] Steam sterilization processes can be process controlled for 'just-in-time' (JIT) operations. Some products and

devices can be 'sterilized-in-place' (SIP) during assembly. Common steam sterilization processes are:

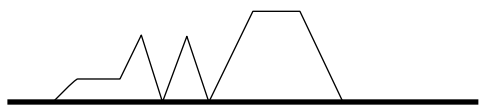
- gravity (downward displacement),
- dynamic air removal – prevacuum, high vacuum,
- pulsing (vacuum pulsing or pressure pulsing),
- flash sterilization or immediate-use steam sterilization,
- superheating and steam-air mixture.

2.3.1 Gravity air displacement

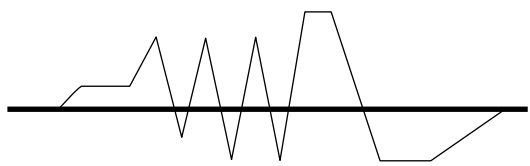
Air is displaced by a flow of steam from a vent in the top of the chamber. Under gravity, the air (which is heavier than steam) is exhausted through the floor of the chamber. Gravity displacement is typically used to sterilize liquids. The rate of exhaust is typically slowed to cool the product down without boiling over. The gravity method is simple and requires less equipment than evacuation. A dynamic gravity method (see Fig. 2.1) includes a steam purge and steam pulsing to improve sterilization efficacy through dense or troublesome product loads.

2.3.2 Vacuum or pressure pulsing and dynamic air removal

For the vacuum or pressure pulsing process, a vacuum is created in the chamber before allowing the steam to flow (Fig. 2.2). A series of vacuums



2.1 A dynamic gravity sterilization cycle. With steam purge and pressure pulses with heat-up phase, pressure and temperature hold period followed by a cool-down phase. A simple gravity method would have no steam purge or pulses.



2.2 A dynamic high evacuation pulsing steam sterilization cycle. With steam purge, pressure/evacuation pulses, heat-up with hold period followed by evacuation and drying period before back to atmospheric pressure. A simple high evacuation steam cycle would have no steam purge, or pressure/evacuation pulses.

and steam pressurizations are performed before the final steam pressure for exposure is reached, which drives out any residual or hidden air pockets in the load, and ensures the load is thoroughly heated. Pressure pulsing removes air from the sterilizer and also entrained air in the packed load. This method typically includes a steam purge followed by steam. A post-cycle hold is typically used to dry the load. Hold times may vary from 20 to 45 min or longer. In pressure autoclaving, air escaping from water heated at the bottom of vessel is displaced from a top vent.

Air removal can be checked by monitoring for leaks (where vacuum is held) or by a Bowie Dick test, which monitors the diffusion of moisture. Cycles with a vacuum are typically faster than the gravity method, but the product must be able to withstand vacuums and pressure/vacuum rates. Problems with this method include peel pouches bursting and covers from pipe ends blowing off. Package bursting is most likely during the post-vacuum phase of the sterilization cycle.

Dynamic air removal only requires that a prevacuum with a high vacuum be preformed without any pulsing, to remove air. This is a less effective means of removing air than typical vacuum-pressure pulsing cycles.

2.3.3 Flash sterilization and immediate-use steam sterilization

Flash sterilization[2,12,13] is a very rapid saturated steam sterilization process carried out at higher temperatures (e.g. 132–138°C) and higher pressures (e.g. 2.02 Bar or 29.41 psig), typically with prevacuum and without packaging or barriers to items being sterilized. Flash sterilization has recently been renamed as immediate-use steam sterilization because of the abuse of the former nomenclature. Facilities typically perform flash or immediate-use sterilization in the operating room, where the principal need exists.

Some disadvantages of this method include lack of timely biological indicators (BIs), the need to remove protective packaging and the risk of post-sterilization contamination. The immediate-use sterilization cycle describes the shortest possible time between a processed item's removal from the sterilizer and its aseptic transfer to the sterile field. Immediate-use implies that the sterilized item is used in a manner that minimizes its exposure to non-sterile air and other environmental contaminants. An immediate-sterilized item can not be stored for future use, nor held from one case to another, because of the potential for adventitious contamination. Only items sterilized and 'packaged' can be stored for sterility. 'Immediate use' implies to use immediately.

2.3.4 Superheated steam and other techniques

Superheated steam, with less saturation required, can be used for microbial de-activation at elevated temperatures, to minimize wetting or moisturizing. There are other more specialized methods used for particular types of product.

Water spray is a method for containers filled with heat-sensitive products or packaging, or for preventing high heat of condensation from occurring. Water spray can also be used in the cooling step of a steam process to bring down the product temperature more quickly and uniformly. Water spray tends to lead to more bursting of the product than a steam air overpressure cycle, but can maintain product configuration. Overpressure water spray cycles have been developed to prevent bursting.

The steam–air process involves mixing steam with air. It is less effective than saturated steam but is used to keep sealed packages with entrained air from bursting.[14,15] Recirculation fans are used to mix steam and air, because otherwise entrained air is a barrier to steam diffusion and penetration. Air cooling is often used during the post-exposure portion of the cycle. It can be efficient at drying the product at the end of the cycle, and keeping the product from bursting. This cycle does not require a final cooling water spray, unlike the over pressurized water spray cycle. Water immersion is another method to keep the product uniform and prevent packages and containers from distorting. Early water immersion methods included brine water, which reduced the boiling temperature and escape of water from evaporation.

2.3.5 Combining steam sterilization and other methods

Low steam sterilization processes (e.g. <100°C), combined with modification of pH or other conditions, may be used to sterilize polymers or metals that are otherwise too heat sensitive. An acidic or basic pH typically decreases the time and temperature needed to sterilize with steam at less than 100°C (e.g. 88°C). The low steam-formaldehyde process[16] is one example of a process using a chemical with steam at below 100°C (66–80°C).

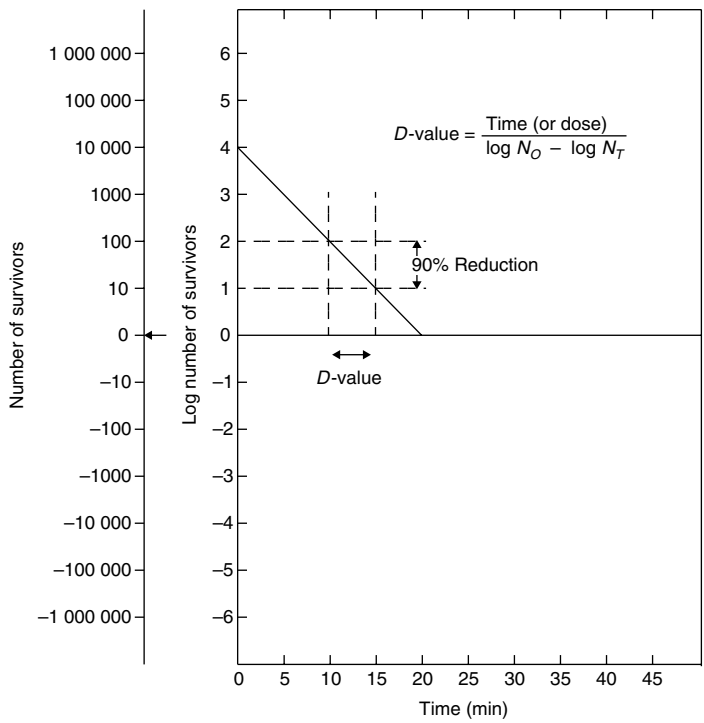
The next generation of steam sterilization processing may use novel sterilization processes. For example, propylene oxide could be applied with steam and eventually hydrolyzed to a non-toxic, preservative glycol. Propylene oxide is less explosive, less toxic and safer to use than ethylene oxide or formaldehyde, and more stable in water than ethylene oxide or formaldehyde under controlled conditions. Some glycols are known to have preservative effects. By increasing the temperature and adding glycols, the exposure time needed to sterilize with steam alone could be reduced.

2.3.6 D-values in steam sterilization

Colonies of microorganisms are characterized as colony-forming units (CFUs). When CFUs are unable to reproduce under suitable conditions post-sterilization (e.g. on growth plates), they are indicated as inactivated or killed. What is deemed favorable conditions for growth can vary. For example, *Geobacillus stearothermophilus* can be cultured to grow well at thermophile temperatures (e.g. >55°C), but not at normal mesophile temperatures (e.g. 28–37°C).

The dynamics of microbial inactivation reveal, in general, that microbes are destroyed in a logarithmic or first order rate (Fig. 2.3). The death value (or *D*-value)[17] is a measure of the dose required to achieve inactivation of 90% of a population (or one logarithm) of the test microorganism population under stated conditions (see ISO 11138):[18]

$$D\text{-value} = \frac{\text{exposure value}}{\log N_o - \log N_b} \qquad [2.1]$$



2.3 Microbial death rate curve – the decimal reduction value (*D*-value). *D*-value = number of minutes to reduce a bacterial or spore population by 90% or one logarithm.

where N_o is the initial microbial or spore population prior to exposure and N_b is the surviving microbial or spore population after time of exposure.

A temperature of 121°C with an exposure time of 12 min may be compared to a temperature of 111°C for 120 min, or 131°C for less than 2 min. Equivalent lethality can be extrapolated by integrating time and temperature during heat-up and cool-down as well as exposure (see equation [2.6]).

The simplified equation [2.1] for the D-value is the Stumbo equation. The *D*-value is the basis for measuring the effectiveness of steam, dry heat and other forms of sterilization. The *D*-value characterizes the resistance of a particular microbial population to a sterilization method. Sometimes it becomes difficult to determine a *D*-value because the microbial population is heterogeneous, the population and resistance are extremely low, or the indigenous population does not follow a perfect logarithmic order of death. To statistically determine the *D*-value, at least (four) fractional *D*-value test run results are needed (the new ISO standard currently in preparation may allow only two).

The more complex *D*-value is the Stumbo equation modified with the Halvorsen/Zieglar (most probable number) equation where:

$$D\text{-value} = \frac{\text{exposure time}}{\log N_o - \log(2.3 \log n/s)} \qquad [2.2]$$

where N_o is the initial spore population, n is number of spore carriers or BIs, s is number of sterile BIs or fractional negatives.

To determine the process time or (F_o) using the *D*-value, the following calculation[19] can be used:

$$F_o = D_{121°C}(\log N_o + \log R - \log N_s) \qquad [2.3]$$

$$N_s = \log - 1(\log N_o + \log R + \text{mean}\, F_o\, D_{121°C}) \qquad [2.4]$$

where R is the number of samples.

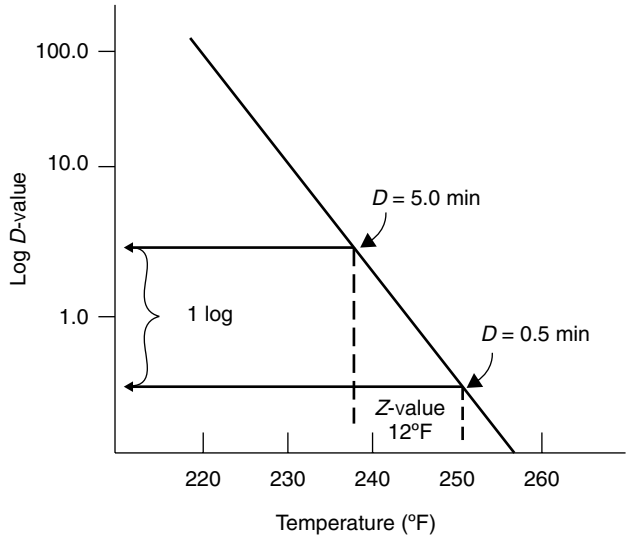
The z-value[17] may be derived from the following equation:

$$z = \frac{T_x - T_o}{\log D_o - \log D_x} \qquad [2.5]$$

where D_o is the *D*-value at the initial temperature T_o, D_x is the *D*-value at a later temperature T_x (see Fig. 2.4).

The application of the z-value, to determine a F_o value[17] is typically represented as follows:

$$F_o = \sum_{t_o}^{t_i} L(\mathrm{d}t) \qquad [2.6]$$



2.4 The *Z*-value. *Z*-value = the number of degrees required to exhibit a one log difference in *D*-value.

where F_o is the equivalent integrated time to sterilize at 121°C or 250°F, Σ is mathematical notation or symbol intended for summation of various intervals of a period or range of time.

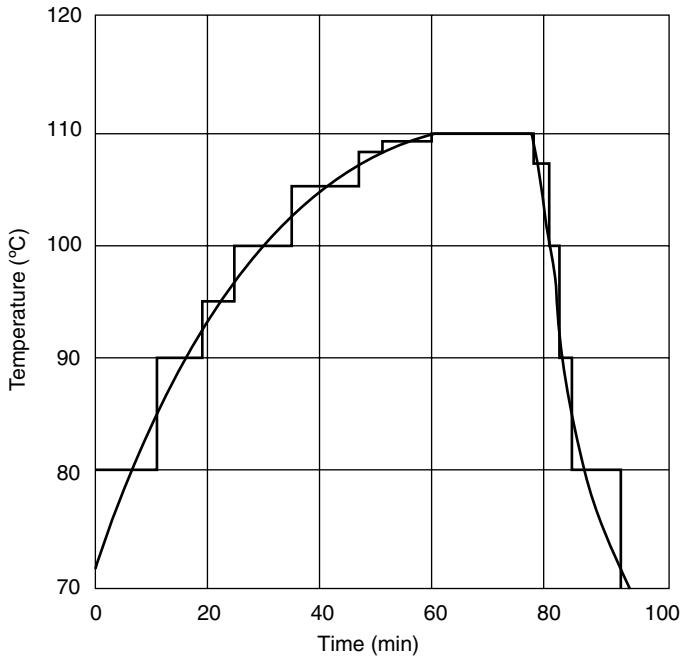
$$L = \text{is a measure of lethality} = 10^{\frac{T(t)-121°\text{C}}{z}} \quad [2.7]$$

F_o values typically integrate lethality (L) during the heat-up and cool-down phases of a heat cycle as well as the exposure phase (see Fig. 2.5 and Table 2.5).

2.4 Test methods for the effectiveness of steam sterilization

Demonstrating microbial effectiveness is based upon knowledge of factors such as product bioburden and microbial resistance (ISO-11737-1, 2).[20,21] The presence of some substances (bacteriostatic, fungistatic, or sporostatic) can inhibit the growth of viable organisms. To verify that such substances are not present (and would therefore produce a misleading result) requires a bacteriostatic or fungistatic (B/F) test of materials or devices before the bioburden or sterility test is performed.

The purpose of periodically performing bioburden tests is to ascertain that the presterilization bioburden count on products (sometimes referred to as the bioburden load) produced in a controlled environment is low



2.5 Time/temperature curve for integration of lethality (F_O). Approximate stepped temperature increments are drawn on the curve, giving the equivalent holding times and temperatures as shown in Table 2.5. The corresponding Table 2.5 shows how lethality values are calculated for each temperature step and then summed up for the total lethality.

Table 2.5 Integrating lethality

Temperature T (°C)	Time t (min)	(121 – T)	$10^{-(121-T)/10}$	$t \times 10^{-(121-T)/10}$
80	11	41	7.9×10^{-5}	0.00087
90	8	31	7.9×10^{-4}	0.0063
95	6	26	2.5×10^{-3}	0.015
100	10	21	7.9×10^{-3}	0.079
105	12	16	2.5×10^{-2}	0.30
108	6	13	5.0×10^{-2}	0.30
109	8	12	6.3×10^{-2}	0.50
110	17	11	7.9×10^{-2}	1.34
107	2	14	4.0×10^{-2}	0.08
100	2	21	7.9×10^{-3}	0.016
90	2	31	7.9×10^{-4}	0.0016
80	8	41	7.9×10^{-5}	0.0006
70	6	51	7.9×10^{-6}	0.00005
			Total lethality (F_o)	2.64

enough that using biological indicators or other validation processes continues to indicate successful sterilization. In general, there are three ways to test for sterility:[22]

- bioburden sampling and product sterility testing,
- BI testing,
- combined bioburden and BI sterility testing.

There are three microbiological validation approaches (Table 2.6).

2.4.1 Product sampling and sterility testing

Product sterility tests can be performed according to ISO 11737-2[21] or the appropriate pharmacopeia. Product sterility tests can be performed directly, with the product immersed in the sterility media, or indirectly/directly, by passing wash solutions through a filtration membrane.

Unless every sample from a load is tested, it is still possible that an unsterile unit remains, but it is virtually impossible to test every sterilized product without sacrificing the entire batch. Since most microbes die in a logarithmic manner, it is possible to predict sterilization without testing all units being sterilized, or parts of all units, by taking test samples after fractional cycles (Fig. 2.3) and evaluating results and outcomes by calculating the *D*-value.

However, evaluating survival of microbes under fractional conditions and with small sample sizes still means it is possible that non-sterile products

Table 2.6 Three methods of microbiological sterilization validation

Overkill method – applying only biological indicators or PCDs; in the overkill approach with typically no bioburden or minimal analysis necessarily required; typically half-cycle conditions are applied, in lieu of fractional cycles (< half-cycles) (see Fig. 2.5, half-point in time would be a half-cycle exposure for biological indicator and a 12 (12th)) or greater spore log reduction would be at full time for a 10^{-6} SAL), using a 10^{6} initial BI population
Bioburden approach – evaluating only bioburden and product sterility testing in the bioburden probability approach; no BI or PCD are used, but SAL or probability is determined by use of fractional or abbreviated sterilization cycles (see Fig. 2.5 at fractional time (the bioburden, presterilization count initially; added time would provide a bioburden SAL of 10^{-6}))
Combination of overkill BI and bioburden probability approach – evaluating presterilization bioburden/product sterility and biological indicator or PCD; fractional cycles are applied, in lieu of half-cycles
Notes: FDA (typical interpretation), no survivors at half-cycle.
SAL may be based on an overkill method (ISO 17665–1 Annex D), which is an easy method of using a more resistant spore (*Geobacillus stearothermophilus*) in a difficult-to-sterilize area of the product. (PCD, process control device; FDA, Food and Drug Administration).

Table 2.7 Product sterility testing of finished devices demonstrating statistical relationship between sample size and the probability of passing unsterile product

Sample size* (total units)	Probability of sample containing no non-sterile units (%)†		
	50 % tested	5 % tested	0.5 % tested
10	6.7 contaminated	25.9	41.1
20	3.4	13.9	33.3
30	2.3	9.5	16.2
40	1.7	7.2	12.4
60	1.1	4.9	8.5

Notes:
* United States Pharmacopeia Mack, Easton, PA.
† FDA Compliance Program Evaluation Report Fiscal Year (7324.04). Percentage of non-sterile units in a lot.
For example, if a lot contained 3.4% contaminated product and 20 units were sterility tested there is a 50% chance that no growth will occur and the lot will pass. If there was a 13.9% contamination, there is only a 5% chance that no growth will occur and the lot will pass.

may result after the full cycle. It is important to understand that there is a probability that some non-sterility will persist if all products are not tested. Table 2.7 shows the relationship between small sample size and the probability of passing a non-sterile sample from the results of a conventional sterility test.[23]

2.4.2 Biological indicator (BI) testing

A biological indicator is typically a solution or carrier consisting of a known concentration of spores (e.g. *Geobacillus stearothermophilus*) that are highly resistant to moist heat[18,24] sterilization. BIs generally consist of spores or spore enzymes of highly resistant microbes which are placed on or in the product load prior to sterilization. These indicators generally have a high microbial population (e.g. 10^6) in excess of what is naturally occurring on the product. The combination of high microbial population and high resistance to a specific sterilization process make these indicators a fairly reliable tool for determining process efficacy or product sterility.

BI testing for sterility is an indirect approach, and to be effective at predicting lethality to the presterilization bioburden, the BI must be more resistant than the product bioburden.[24] BI results typically take three to seven days at 50–55°C, but more rapid indicators may be available (e.g. 4 h). The types of spores used as BIs to monitor steam sterilization cycles are given in Table 2.8. BIs are used to check cycle parameters. Chemical indicators can

Table 2.8 Some conventional steam BIs

'Standard' steam biological indicator organism: *Geobacillus sterothermophilus* ATCC 7953 or ATCC 12980 *See* ISO 11138 for further description and details
Some 'other' steam sterilization BIs (at under lower temperature and conditions), typically in industrial and other uses: *Bacillus coagulans* FRR B666 or ATCC 51232 *Clostridium sporogenes* PA 3679 or ATCC 11437 *Bacillus subtilis* 5230 or ATCC 35021
The above spores have different *D*-values for different types of sterilization parameters and requirements; however, individual spores will have *D*-values that will vary from lot to lot.

Note: Use similar 'routine' BI *D*-values to those used during validation.

also be used to assess time and temperature parameters (autoclave tape, etc.), as well as adequate moisture levels (e.g. Bowie Dick tests).

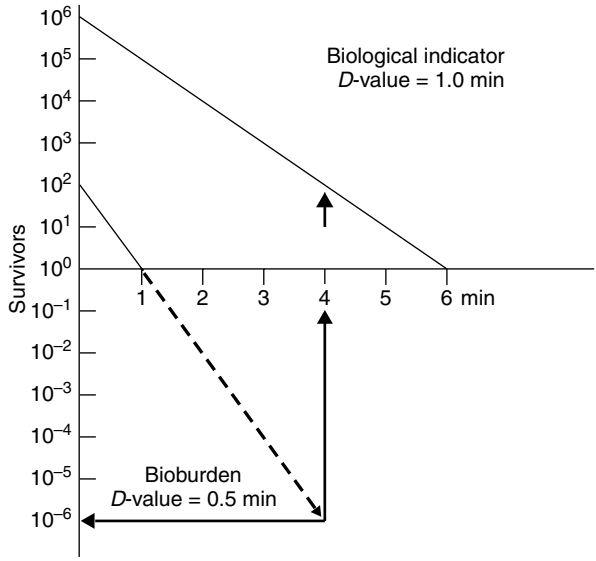
BI testing is used to assess the performance of the process using fractional (short) cycles to show lethality, or half-cycles to demonstrate total inactivation with a 10^{-6} probability of assurance under full or routine cycles. The overkill method typically[25] consists of three half-cycles, in which none of the (10^6) spores from the BI or PCD (product challenge device) survives. In a BI and bioburden approach, the resistances of BI and bioburden are compared and extrapolated to demonstrate a probability of 10^{-6} (Fig. 2.6). In a total bioburden approach, bioburden testing is used to determine inactivation and resistance to the sterilization process and demonstrate a 10^{-6} probability of survivors. Non-invasive medical devices may require only a 10^{-3} probability of assurance.

2.4.3 Combined bioburden and sterility testing

Combined bioburden and product sterility testing after fractional cycles[26,27] must be carried out according to ISO 11737-1 and -2,[20,21] or according to the appropriate pharmacopeia. Since steam sterilization methods destroy or eliminate microbes logarithmically, it is possible to measure the kill time logarithmically (*D*-value) from the measured bioburden and product sterility survivors from a fractional cycle and extrapolate the inactivation or sterility to a probability (e.g. SAL) for the entire process.

2.4.4 Validation of testing

The effectiveness of steam sterilization can be validated and tested to determine its SAL, which is denoted as typically 10^{-6} probability of survivors.



2.6 Microbiological kinetics inactivation and extrapolation. Microbial inactivation (*D*-values) comparison if bioburden and biological indication and extrapolation to a probability of one in a million (10^{-6}).

Requirements and guidance for this testing and validation come under ISO 17665-1:2006 *Sterilization of health care products – Moist heat – Part 1: Requirements for the development, validation and routine control of a sterilization process for medical devices*.[28] Validation of a sterilization process requires obtaining, demonstrating and documenting evidence that the equipment (and process), as installed and operated in accordance with operational procedures, consistently performs in accordance with predetermined protocols and thereby yields products meeting its specification.

2.4.5 Advantages and disadvantages of steam sterilization

There are a number of reasons why steam sterilization may succeed or fail (Tables 2.9 and 2.10). The major concern with steam sterilization is the damage, degradation or destruction of materials by heat or moisture.

Advantages[29] of steam sterilization include the following:

- It is relatively simple, compared to most chemical sterilization methods.
- There are no toxic residues or wastes.
- It requires minimal processing time. The higher the temperature, the shorter the processing exposure time.
- Steam sterilization is suitable for liquid materials and heat-resistant materials that can withstand moisture, hydration and high temperatures.

Table 2.9 Some further reasons why the steam sterilization method may fail

- Change in 'routine' sterilization parameters (e.g. vacuum, pressure, wet steam vs dry steam or less than saturation; changes in steam or vacuum rates or change in temperature, pressure and exposure)
- Steam additives (e.g. antioxidant inhibitors or contamination (e.g. pyrogens))
- Bioburden (increase population, new types, protection, dessication, etc.); sterility failure
- Biological or chemical indicator failure
- Long recovery (release protection, media, conditions)
- Environment changes (pre- and post-processing)
- Load, chamber size and change in configuration
- Long processing time implies inherent limitations that need to be overcome, and if there is change this could influence outcome
- Microenvironment (change conditions, interaction)
- Penetration, barriers (e.g. biofilm, oil, grease, etc.) diffusion, absorption (moisture), change in heat sink of the load
- Packaging – changes in size, material, penetration
- Inadequate statistical assurance of sterility
- Irrelevant validation, no validation, no revalidation
- Non-heat- and moisture-resistant materials
- Wetting of product
- Steam quality (e.g. consider effects of dry steam, superheated, non-condensing steam, etc.)

Table 2.10 Some reasons why steam (moist) heat sterilization may be successful

- Low bioburden
- Clean product
- Heat and moisture resistant materials and products
- No microbial biofilm, occlusion, clumping, or encrustation
- No barriers to the diffusion of steam to the microbial site
- Good load size, proper placement of items and load configuration
- Meets saturated steam or moisture conditions
- Short cycle times
- Adequate monitoring and review of time, pressure, steam quality, temperature conditions and cycle parameters
- Overcomes any barriers or diffusion limitations to steam or moist heat
- Validation of process and provide statistical assurance by overkill, bioburden or combination methods
- Reduced BI incubation or process control release
- Integrated lethality – F_o value release
- Good equipment
- Good maintenance
- Good packaging (when used)

- Steam is capable of destroying all viable forms of life, including prions.
- Steam is generally the most dependable sterilant for laboratory use.
- Steam can be used to decontaminate reusable (hospital) supplies and equipment.

- Steam can sterilize locations such as mated surfaces by wetting the surfaces (e.g. stoppers within glass containers and overlapping instruments), prior to exposure to saturated steam.
- Steam can be used to sterilize some devices in place (e.g. dialyzers).
- Steam sterilization can sterilize (inactivate) cotton mold *Pyronema domesticatum*, which is resistant to EO and radiation, without damaging the cotton fibers.

Disadvantages[29] of steam sterilization include the following:

- To use and operate steam sterilizers and sterilization properly requires special training in how to use the sterilizers and how to handle the items to be sterilized.
- The steam must reach a suitable pressure for condensation to occur.
- Boilers must be maintained and can corrode.
- Anticorrosive steam additives can be toxic.
- Superheated steam is less effective than saturated steam.
- Steam is damaging to heat- and moisture-sensitive instruments and materials. The high temperatures involved can be incompatible with heat- and moisture-sensitive polymers, such as ABS, acrylics, copper, low density PE, some standard styrene and some urethanes.
- Repeated resterilization can eventually lead to corrosion and blunting of instruments.
- Loading and packing configuration is critical to performance.
- Steam is not completely penetrable as EO, irradiation or dry heat, except for heating large volume liquids, or pre-wetting of surfaces not accessible to steam during processing.
- Steam cannot sterilize materials that are impermeable or non-hydroscopic to steam, such as silicone implants.
- Steam cannot be used to sterilize electronic components, which would be damaged by steam, moisture and wetness.
- The source of steam can be contaminated (with pyrogens), and requires a good quality water supply.
- The quality of the steam must be good; it must be condensable and free of non-condensable gases.
- Air, salts, organic matter, matched or mated surfaces, long tubing and enclosed spaces can be barriers to the diffusion of steam.
- Steam cannot be used to sterilize powders or oils, or moisture-sensitive materials.
- Steam cannot inactivate (depyrogenate) endotoxins, whereas dry heat can.
- Absorbable or hygroscopic materials, such as polyglycolic acid, can't be used with steam.

- The presence of fats, oils, grease, poorly soluble or insoluble salt crystals, biofilm, or organic matter, slows or prevents the penetration of saturated steam and increases the time needed. They may even prevent sterilization altogether.

2.5 Dry heat sterilization

Dry heat is an ancient form of preservation or inactivation.[30] It is used only for those materials that cannot be sterilized by steam or, in the case of certain glass containers, where it is undesirable to use steam. It has been used in the pharmaceutical industry (as part of aseptic processing), hospitals and space technology. It is not often used in the medical device industry, except for silicon prostheses. Typical uses of dry heat sterilization are shown in Table 2.11.

Sterilization by dry heat requires longer exposure times than steam at the same temperatures. It is relatively inefficient compared to steam, but it is used when removal of condensate or water is needed.[31] Unlike steam, dry heat sterilization occurs primarily by dehydration and oxidation, and at temperatures typically higher than steam, usually 160–190°C compared to 121–134°C in steam sterilization. The temperature for killing microbes must be in excess of 105°C. This limits the types of heat-sensitive materials and polymers that can be sterilized using this method. Temperature and heat must penetrate all parts of a device to be effective. Typically, heat flows into cooler areas. Dense areas and non-conductive materials are barriers to heating. Design, construction, conductivity, packaging and loading of any device that will need to be sterilized are critically important considerations.

When designing devices, it is important to make sure that heat can access all areas that need to be sterile, or that inaccessible areas can be pre-heated

Table 2.11 Some typical uses (applications) of dry heat

Depyrogenation of glassware
Sterilization of glassware, glass syringes, some oils, greases, powders and ceramics
Other uses:
Spacecraft decontamination
Medical devices, silicone prostheses (e.g. silicone mammary prostheses), themoresistant powders and drug/device combinations
Moisture-sensitive chemicals and materials used in medical devices
Dental instruments and items
Some active electrical components
Drying 'wet' materials
Laboratory equipment and materials

prior to sterilization (e.g. stoppers in bottles). Evacuation or moisture removal may need to be controlled. If a vacuum process or dehydration support system is used, the potential influence of a vacuum or dehydration on the device, component or material should be taken into account. Dry heat sterilization over an extended period may gradually soften or distort certain materials. The presence of packaging can influence the process. Wrapped items require longer times for adequate heating. Non-liquid products typically need permeable packaging such as Tyvek®, muslin or paper. Air pockets may reduce time to heat, and some packages may require an external air pressure to balance internal pressure within the packages to prevent bursting on heating.

2.5.1 Materials that can be sterilized using dry heat

Dry heat sterilization in the temperature range 105–135°C can potentially sterilize materials that are adversely affected by moisture, hydration, corrosion or erosion in moist heat. Typical materials sterilized using dry heat[30] are listed in Tables 2.12 and 2.13. Dry heat cannot sterilize aqueous liquids, whereas moist heat can, but it can be used to sterilize electrical components whereas moist heat cannot.

Dry heat sterilization offers an important alternative to other sterilization options. For example, acetal, oils, powders, polysulfones, silicones and

Table 2.12 Some typical healthcare materials that have been sterilized with dry heat

Acetals
Ceramics
Cutting-edge instruments (withstand higher dry temperatures than steam temperatures)
Dental instruments
Glass syringes
Glass suction containers
Glassware – ampoules, vials, test tubes, flasks
Glycerine
Heat-resistant electronics
Metal instruments and trays
Metal needles
Oils
Papers (certain ones)
Petroleum
Polymers – acetal, polymethylpentene, some nylons, polysulfone
Powders
Silcone prosthesis (e.g. mammary glands)
Teflons
Earlier spacecraft circuit boards, components, metals, polymers and materials

Table 2.13 Potential dry heat sterilizable items, polymers and materials

Polymer or material type	Tentative maximum heat temperatures (°C)
Acetal (ACL), delrin or polyoxymethylene	Up to 121°C
Aluminium	Up to 190°C
Cellulose acetate (non-load)	Up to 120°C
Cellulose acetate butyrate (non-load)	Up to 130°C
Cellophane (e.g. cuprophane)	Varies
Ceramics (e.g. aluminium oxide and silica)	Ultra high
Electronics	Varies
Glass	> 190°C
Grease	Depends upon the type of grease
Ethylene-chlorotrifluoroethylene copolymer (ECTFE)	Up to 150°C
Ethylene-tetrafluoroethylene ETFE	Up to 150°C
Fibers (e.g. glass, quartz)	
Fluoro polymers (most Teflons)	Varies (see polymers)
High-density polyethylene (HDPE)	Up to 120°C
Instruments	Up to 190°C*
Metals (note metal temper may occur above 190°C)*	Up to 190°C
Muslin	Up to 160°C
Nylon (polyamide – heat-stabilized grades)	Up to 130°C
Needles	Up to 190°C
Non-aqueous solvents (e.g. low temperature dehydrating dimethyl sulfoxide (DMSO))	
Oils	Depends upon the oil
Paper (varies depending upon paper)	Up to 134°C
Petrolatum gauze	Up to 160°C
Polycarbonate (PC)	Up to 170°C
Polyethylene (HDPE and XLP)	Up to 120°C
Polyetherimide	Up to 134°C
Polyetherketone (PEI)	Up to 250°C
Polyetheretherketone	Excellent
Polyethylene terephthalate copolymer (PETG)	Up to 134°C
Polymethylpentene (PMP or TPX)	Up to 170°C
Polypropylene (PP)	Up to 135°C – no stacking
Polypropylenecopolymer (PPCO)	Up to 120–135°C
Poly phenyl oxides (PPO), varies	Varies between 100°C and 148°C
Powders	Depends upon powder
Polysulfone (PSF)	Up to 160°C
Polyurethane (PU-aromatic)	Varies depending upon grade and loads
Polyvinyl chloride (flexible-non-load, varies)	Up to 120°C
Polyvinylidene fluoride (PVF)	Up to 125°C

*The brittleness of a metal may be reduced by tempering through heating it; but this may be associated with a reduction of hardness of the metal but an increase in ductility, elasticity or plasticity. Low tempering temperatures such as 190°C may only relieve some of the internal stresses, decreasing brittleness while maintaining a majority of the hardness; however, tempering temperature varies with the variety of steel. One cannot judge temper temperatures of alloy steels by temper colors. Hardness is often used to describe strength or rigidity but, in metallurgy, the term is typically used to describe resistance to scratching or abrasion. Brittleness describes a metal's tendency to break before bending or deforming either elastically or plastically.

Teflon® are excellent candidates for dry heat sterilization. Some materials (e.g. Teflon®, acetal) can be easily damaged, or cross-linked (silicone) by radiation sterilization. Oil, collagen or powder filled devices may be sterilized by dry heat but not by chemical methods (e.g. ethylene oxide, hydrogen peroxide, plasma, ozone) and moist heat.

Dry heat is also used for sterilizing petroleum jellies, surgical catguts, surgical instruments and glassware, including vials for pharmaceutical drugs.[30,32] It is used in sterilizing dental instruments to minimize the corrosion of sharp items. It is commonly used in laboratories for depyrogenation of glassware intended to be used in pyrogen testing. The high temperature used during dry heat sterilization can, however, lead to creep, crazing, softening, distortion, oxidation or degradation of some materials. Materials that can be easily damaged by dry heat include ABS, acrylics, plasticized PVC, styrene (PS) (Paryls), low density PE and polyethylene terephthalate (PET). The use of heat stabilizers in polymers can overcome the limitations of traditional dry heat sterilization.

2.5.2 How dry heat sterilization affects microorganisms

Dry heat can inactivate all microorganisms including pyrogens, but it is less effective against prions or *Pyronema* as steam or moist heat. However, at extremely high temperatures (330°C), where heat destroys everything down to carbons, dry heat becomes a virtually 'absolute' sterilization method. A variety of factors influence the ability of dry heat to sterilize or kill microbes (Table 2.14), and there are several ways that the microbiocidal effectiveness of dry heat can be impaired (Table 2.15). Bioburden, for example, affects lethality. As with some ethylene oxide sterilization and irradiation methods, non-logarithmic inactivation with dry heat, such as tailing, can occur. Consequently, dry heat is best performed with low presterilization bioburden levels, below 1000 cfu/device (Fig. 2.7).

Table 2.14 Factors influencing the ability of steam to kill microbes with dry heat

Related to lethal steam	Related to organisms to be killed
Intensity (temperature)	Types of microbes (e.g. spores, prions)
Exposure time (longer than with steam)	Number of microbes to be killed
Dehydration	Level of sterility assurance (probability)
Presence of moisture	Previous history of microbes to be killed
Distribution of heat	Places that harbor or protect organism
Less than stated temperature	Barriers to the transfer of heat
Transfer of heat (e.g. via convection, conduction, infrared)	Initial and subsequent water activity

Table 2.15 Examples that may impair microbial effectiveness to dry heat

- Occluded spores
- Insulation (barriers) to transfer of heat
- Load mass, configuration and heat sink
- Non-conductivity of heat by material or environment
- Impediments to transfer of heat
- Insufficient time, for heat-up as well as exposure and cool-down
- High bioburden in excess of 1000 cfu
- High water activity
- Immediate micro environment
- Heterogeneous population of microbes
- Microbes/spores entrapped in materials, polymers
- Variation in heated environment (e.g. air, other gases, oil, distance from a conducting surface)

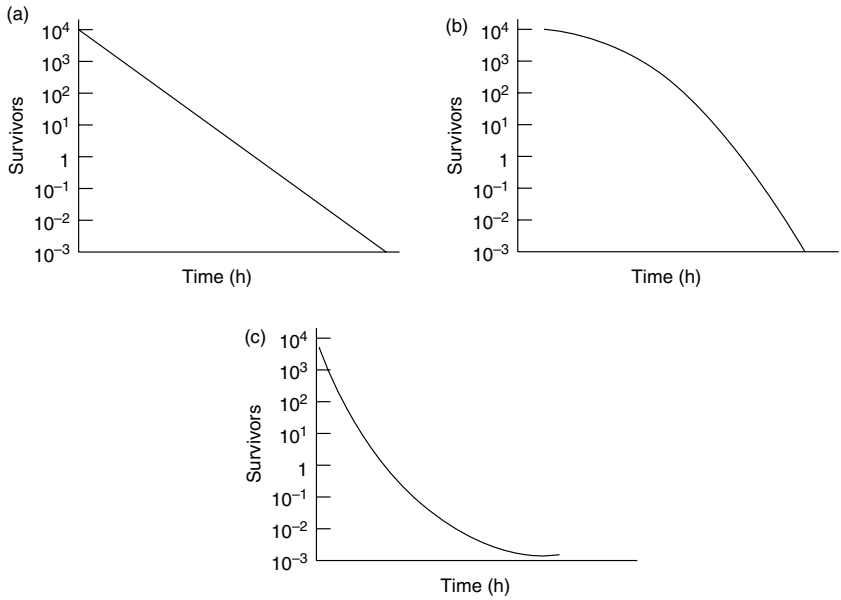
Dehydration enhances the dry heat process.[33] Under extremely dry conditions, less than 0.1 Aw, dry heat inactivates microorganisms primarily by oxidation but also through dehydration.[33,34] Dry heat sterilization temperatures as low as 105–135°C[35] can kill microbes in a day or less. The process can be as fast as 1 or 2 s at 330°C.[31] Chemicals like DMSO (e.g. including alcohols, ethers, ketones) and vacuum conditions that dehydrate product will reduce the time and temperature taken for sterilization (e.g. <140°C) without the corrosion and hydration effects of steam.[36]

2.5.3 Mechanism of dry heat sterilization

Dry heat sterilization uses heated air or another dry heat source. Dry heat can be delivered through convection, conduction or thermal irradiation. Sterilization by dry heat is achieved by exposure to elevated temperatures, typically 2 h at 160°C, 1 h at 170°C, 30 min at 180°C (see Table 2.16). Some microorganisms may be inactivated at lower or higher temperatures. For example, dry heat can sterilize down to 105°C, but with much longer exposure times.[35] To achieve dry heat sterilization requires removing all moisture and ensuring areas to be sterilized are exposed to elevated temperatures for a given period of time. It is important to prevent variation of temperature in different parts of the product especially in difficult-to-penetrate areas.

2.5.4 Key steps and technologies in dry heat sterilization

Dry heat sterilization is simpler, has fewer parameters and requires less sophisticated equipment and facilities than steam, ethylene oxide sterilization, hydrogen peroxide, ozone, plasma or ionizing irradiation. It is important



A theoretical example of order of death of a bacterial population (applicable for either physical or chemical treatment)

Time increment	Bacteria living at beginning of time increment	Bacteria killed during one time increment	Bacteria surviving at end of time increment	Logarithm of survivors
First	1 000 000	900 000	100 000	5
Second	100 000	90 000	10 000	4
Third	10 000	9000	1000	3
Fourth	1000	900	100	2
Fifth	100	90	10	1
Sixth	10	9	1	0
Seventh	1	0.9	0.1	–1
Eighth	0.1	0.09	0.01	–2
Ninth	0.01	0.009	0.001	–3
Tenth	0.001	0.0009	0.0001	–4
Eleventh	0.0001	0.00009	0.00001	–5
Twelfth	0.00001	0.000009	0.000001	–6

2.7 Theory and variation of microbial death destruction. While microbes are assumed to die logarithmically, there may be variation to this conclusion. Be aware of what you are observing. Is the survival curve a straight line and logarithmic, or not? (a) Logarithmic death: homogeneous population, uniform cell distribution; (b) retarded initial rate: dormant spores or heterogeneous population and (c) reduced or delayed final rate: clumped or heterogenous resistant cells.

Table 2.16 Examples of dry heat sterilization temperature and time

Temperature (°C)	Time (overkill)*
330	1.15 s
190	6 min**
190	12 min (with package)
180	30 min
170	60 min
160	120 min
150	180 min (3 h)
105–135	8 h to overnite

Notes: All of the above temperatures and times depend upon load, bioburden level, time to penetrate and validation approaches (e.g. overkill, bioburden, etc.). Lesser or reduced temperatures and times may result based on bioburden control, resistance, sterility assurance level, lethality measurements and improved heating methods.

* Overkill implies 10^6 resistant spores are inactivated with a 10^{-6} probability of survival or inactivation of 10^{12} total spores.

** Cox sterilizer uses forced heated air at approximately 2500 ft/min to heat. Infrared irradiation, another type of dry heat sterilizer, can heat materials quickly at infrared wavelengths.
At extremely high temperatures, heat can destroy all organic matter, resulting in an absolute sterilization condition. Prions, composed of folded proteins, are the most heat resistant infectious agents; normal incineration temperatures are not hot enough to inactivate them.

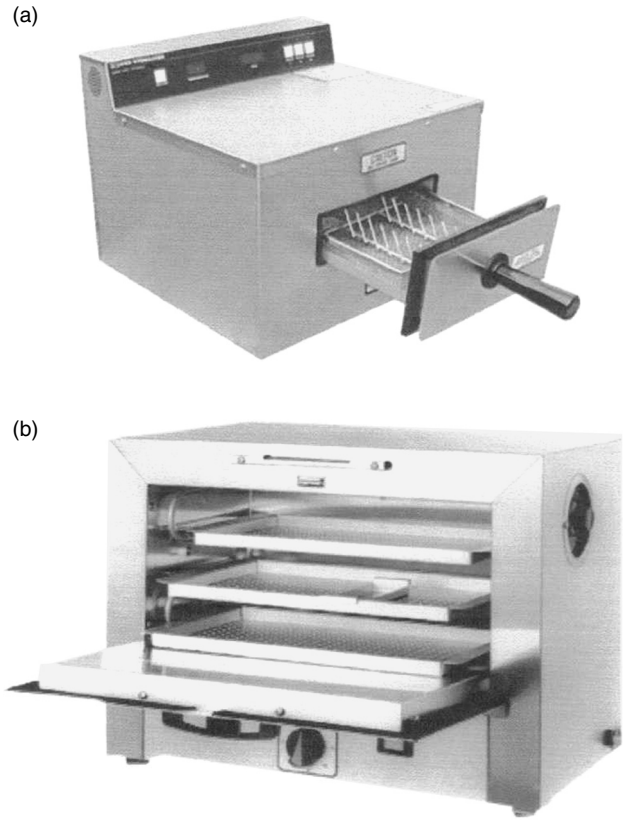
to position a product during loading so that dry heat can be easily dispersed and disseminated, and reach all surfaces to be sterilized.

The heat-up process for dry heat sterilization can be very lengthy. Heating by infrared (thermal irradiation tunnels) is a more rapid approach, but heating by oven convection can be slow. The removal or displacement of cool air by convection is critical under normal dry heat sterilization. This is sometimes created through circulation or evacuation. Heating by conduction is another means. The process requires a controlled cool-down period following exposure where hot heat is allowed to dissipate.

Types of dry heat sterilizers

The simplest dry heat sterilizers are ovens. Gravity convection ovens are the simplest method. As air is heated it expands and hot air rises, displacing cooler air. This is not the best dry heat sterilizer. The mechanical convection oven or forced convection oven has a blower that actively forces heated air in all areas of the oven. It is more efficient than the gravity convection oven. The Cox Rapid Heat Transfer Sterilizer (see Fig. 2.8) is one of the fastest sterilizers. It moves heated air at a rate of 2500–3000 feet/min at 190°C (375°F) for 3–6 min.[37]

More sophisticated and complicated methods include infrared radiation (IR), and rapid heat transfer and continuous belt systems. IR is one of the simplest



2.8 Two commercial dry heat sterilizers: (a) Cox Rapid Heat Transfer Sterilizer, 6 min unwrapped at 375°F (190°C), 12 min wrapped and (b) Wayne S1000 dry heat sterilizer, standard 160–180°C oven for instruments.

means of heating and sterilizing, but items must have direct contact with the IR rays or heat transfer may not be uniform. Continuous belt sterilizers or radiant heat tunnels are typically forced air convection or IR systems that use continuous moving belts through a tunnel to heat and sterilize items as they pass.

2.6 Testing and validating dry heat sterilization

Products sterilized with dry heat should be easy to release based upon acceptable time–temperature parametric data. The relationship between the rate of destruction of bacterial spores by dry heat temperature is given by the Arrhenius equation:[31]

$$\log 10k = -\frac{E}{2.3RT} + A$$

Using spores of *Bacillus atrophaeus (subtilis var. niger (globigii)),* it has been found that the energy of activation is low (11 000 cal/mole, approx) and the value *A* is 5.26, thus supporting the view that dry heat sterilization can be described as a first-order chemical reaction.[31] Consequently, the mathematical kinetics applied in steam sterilization can be applied or adapted to dry heat. Recognizing that dry heat sterilization follows a first-order chemical reaction, the classical Arrhenius equation allows for the possibility of correlating time to dry heat sterilization.

Demonstration of microbial effectiveness is based upon factors such as knowledge of product bioburden (ISO-11737-1)[20] and microbial resistance (ISO-11737-2).[21] Microorganisms with a high resistance can be used as BI to validate a dry heat process. A BI (also known as a PCD) for dry heat is typically a carrier or dried suspension consisting of a known concentration of spores (e.g. *Bacillus atrophaeus* (*subtilis var. niger*)) that has been demonstrated to be resistant to dry heat sterilization. It can be used to predict lethality because the spores are more resistant than the bioburden on the product. BI results typically take three to seven days at 30–35°C, but more rapid indicators may be available (e.g. 4 h or less). The PCD or BI is used to assess the performance of the process with fractional (i.e. abbreviated) cycle exposure to show lethality, or half-cycles to demonstrate total inactivation, with a 10^{-6} probability of assurance under full or routine cycles. Validation of dry heat can be performed per ISO 20857.[38] There are typically fewer types of spores available to use as BIs to monitor dry heat sterilization (Table 2.17) than for steam sterilization.

2.6.1 Advantages and disadvantages of dry heat sterilization

To analyze and determine the rationale for applying or using dry heat sterilization requires consideration of the advantages and disadvantages of the technique. The advantages of dry heat sterilization[37,39,40] include the following:

Table 2.17 Conventional dry heat biological indicator(s)

Standard dry heat BI: *Bacillus atrophaeus* (*subtilis var. niger*) ATCC 9372/NCTC 10073
See ISO 11138 for description and details
An industrial moist and dry heat-resistant spore or BI: *Bacillus subtilis 5230* or ATCC 35021

Note: Vegetative microbes have much higher resistance to dry heat than to steam heat. Also there can be other spore species with higher resistance than *Bacillus atrophaeus* or *subtilis 5230*.

- Dry heat is a relatively simple method; it does not require consumables or leave toxic residues.
- Dry heat sterilization can be used for materials and products that steam cannot penetrate (e.g. powders, oils).
- Dry heat can be used where hydration is undesirable.
- Dry heat causes less corrosion or dulling of sharp instruments than steam.
- Dry heat at elevated temperatures and exposures can depyrogenate (deactivate end toxins).
- It can be used to sterilize some electronics, which are inactivated by steam, high humidity EO/formaldehyde, or irradiation.
- It can be used to sterilize silicone implants that are cross-linked by radiation, impermeable to steam or peroxides and absorb EO.
- It can sterilize glassware without eroding surfaces. It can sterilize metals without corrosion.
- Dry heat will eventually penetrate metal instruments and items that cannot be disassembled through conduction, etc. Difficult-to-heat components can be pre-sterilized with thermal irradiation or convection heat.
- Oxidation can be eliminated or reduced by use of non-oxygen gases (e.g. nitrogen).

The disadvantages of dry heat sterilization[40] include the following:

- Dry heat sterilization is less effective than steam heat at the same temperature (esp. against prions).
- Long processing times (long heat-up time, long exposure).
- High temperatures mean there are fewer polymers that can be sterilized by this method, due to problems of melting, distortion and degradation.
- The packaging materials that can be used are limited because of the high heat.
- Packaging must be kept to a minimum to allow for heat transfer. It must be heat penetrable and resistant. Dry heat cannot deactivate prions.
- Oxidation can occur in some materials.

2.7 Conclusions

Traditionally, sterilization methods using saturated steam under pressure or dry heat have been considered among the most reliable and should be used whenever possible. Other sterilization methods include filtration, ionizing radiation (gamma and electron-beam radiation), gas (ethylene oxide, hydrogen peroxide, and ozone sterilization) and glutaraldehyde. However, the numbers of agents capable of sterilizing healthcare products without adverse effects or damage are relatively few. Sterilization without some limitations is

virtually impossible. High-temperature heating can distort, corrode and melt; radiation is extremely hazardous and can degrade and prevent the reuse of many materials; chemical sterilization may use hazardous substances, can leave toxic residues and have limited penetration; hydrogen peroxide can destroy materials such as paper, rubber, etc.; and ozone can oxidize certain materials. It is likely that steam and dry heat will be used to sterilize more materials[41,42] in the future at lower temperatures in combination with other agents (e.g. acids to modify pH).[43] Other developments will include more heat-tolerant materials (with additional cross-linking),[30,44] and heat stable organic materials for electronics (e.g. transitors) robust enough under high temperature, and implantable.[45] Heat sterilization is an important challenge and polymers plus materials known to be heat sterilizable and compatible have intrinsic long-term advantages. Heat sterilization enables biomaterials and devices to be completely sterilized, through long lumens, crevices, etc. Compared to other methods, it is inexpensive, enables resterilization, and is more readily available and accessible in healthcare facilities.

Despite the common practice of encompassing or describing steam and dry heat sterilization as 'heat sterilization', there remain unique differences as well as similarities between steam and dry heat sterilization (Table 2.18)

Table 2.18 Comparison of various factors of steam and dry heat sterilization

Factor	Moist heat	Dry heat
Compatibility	Moisture/heat resistance	Require heat stable
Materials	Stable moisture/heat	No aqueous liquid
Packaging	Breathable	Permeable
Release reliability	Excellent	Very good
Cost	Moderate	Low
Consumable	Low (water)	None, except that required for heat
Safety	Moderate	Moderate
Toxicity	Low (with no additives)	Low–none
Penetration	Good	Excellent given time
Lethality	Excellent	Depends upon temperature
Process time	Varies with temperature	Varies with temperature – seconds to hours depending on other factors
Release time	Varies with BIs Can be process control released	Can be parametric release
Complexity or simplicity	Steam is more complex than dry heat	Dry heat is simple
Inactivation or destroy	Can inactivate prions, but not pyrogens	Can destroy pyrogen, but not prions
Availability	Wide	Limited in medical devices

that need to be considered to determine which method should be applied under different circumstances. Each type of heat sterilization has its advantages and disadvantages (e.g. speed, availability, cost, wetting, corroding, penetration, etc.). The selection of the particular process type is dependent upon a variety of factors such as cost, simplicity, type of product, material, type of load and end use characteristics of the product.

Heat sterilization at lower temperatures will allow more heat-sensitive polymers to be tolerant and sterilizable because new sterilization techniques are for niche applications, provide less penetration and are on a small scale. Heat sterilization uses no toxic chemicals, does not generate toxic waste and is thus environmentally safe. Polymers and packaging materials continue to become more heat stable, heat sterilizable and less costly because of demand not only for medical devices but also for other applications. In particular, for example, heat-resistant fluoropolymers, organic materials and drug-device combination products should provide cost-effective solutions to the ever-growing demands of biocompatibility and modern medical technology.

2.8 Sources of further information

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3

Sterilisation of healthcare products by ionising radiation: principles and standards

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Abstract: The use of ionising radiation to provide a terminal sterilisation process to a sealed package is an attractive option for many healthcare products such as syringes, sutures and tissue allografts. It is also seen as an efficient and validatable approach for the sterilisation of expensive, low production volume, healthcare products such as drug-device combination products. This chapter outlines the effects of radiation on matter and addresses some of the technical issues in selecting appropriate radiation sources. It also provides useful information on the various international standards and irradiation protocols that have been developed to ensure that the desired sterility assurance levels can be achieved and validated.

Key words: ionising radiation, sterilisation, international standards, biomaterials.

3.1 Introduction

Healthcare products include a wide range of products including syringes, catheters, dressings, sutures, tissue allografts, proteins, enzymes, drugs, polysaccharides, liposomes and bones. In addition, combinations of these and other components such as metals and polymers are used to produce drug-device combination products, an area of both rapid development and growth in the pharmaceutical industry. Pharmaceuticals, including drugs and devices, are sterilised by a range of techniques, including dry heat, ethylene oxide, hydrogen peroxide, air-steam mixtures, steam, steam-in-place, gas plasma, filtration and formaldehyde and ionising radiation (Agalloco and Akers, 1993; Nordhauser *et al.*, 1998).

The choice of sterilisation technique will depend upon many factors, particularly paying regard to effectiveness in achieving a desired level of sterility, applicability to both large- and small-scale production facilities, validation of the process and potential of the process to damage the healthcare product. The use of large-scale sterilisation facilities to sterilise small production runs of expensive items such as drug-device combination products, for example, is unlikely to be cost effective and is also difficult to validate with regard to the sterility assurance level (SAL).

The attraction of using ionising radiation for the sterilisation of a sealed package containing a healthcare product is clear and this approach is now widely used to sterilise mass-produced items, such as medical syringes, sutures, needles and dressings, where damage to the product by ionising radiation is either unlikely or has little effect on the effectiveness and safety of the product. International standards are now available to ensure the effectiveness of terminal sterilisation of healthcare products by ionising radiation, typically at sterility assurance levels of 1: 10^6 (ISO, 1995a, 1995b, 1996, 2006a, 2006b, 2006c; AAMI, 2001). These are particularly applicable to mass-produced manufactured items. These standards have also formed the basis of a Code of Practice (Parsons *et al.*, 2005; IAEA, 2008) for the terminal sterilisation of tissue allografts, where items such as bone and amnion are diverse both in origin and nature, and as such require other considerations to be taken into account when attempting to use the above international standards to achieve a specified SAL.

The use of ionising radiation to sterilise healthcare products is particularly attractive for many applications. Terminal sterilisation of relatively clean products in a sealed package combined with a statistical approach to dose setting to achieve a desired sterility assurance level are the major advantages of this technique. It can be applied to both large- and small-scale production runs with relatively easy and demonstrable validation procedures. As with other sterilisation techniques, damage to the healthcare product, particularly to sensitive healthcare products such as proteins, enzymes and drugs, must be minimised and be constrained within acceptable limits. The aim of all sterilisation processes is to reduce bacterial and viral contamination to acceptable levels while retaining the integrity and functionality of the product. In order to devise suitable sterilisation processes using radiation, it is therefore essential to understand the principles of radiation chemistry and how sterilisation processes using ionising radiation can be validated to ensure they meet these objectives.

3.2 Interaction of ionising radiation with matter

The main sources of radiation used to sterilise biomaterials are: (a) high-energy photon sources such as X-ray machines, and cobalt-60 and (b) high-energy electrons from electron accelerators. Both types ionise molecules but via different processes, which affect their practical application to sterilisation.

3.2.1 The ionisation of molecules

If the energy of a particle or photon exceeds the ionisation potential of a molecule, then, in principle, ionisation may occur. In practice, sources of

ionising radiation have energies which greatly exceed the ionisation potentials of all molecules and are usually classified by the way in which they are produced. For the purposes of sterilisation of healthcare products, both high-energy photon and high-energy electron sources are used commercially. The ways in which high-energy photons and high-energy electrons interact with matter are substantially different and are outlined below.

3.2.2 High-energy photons

High-energy photons, produced by either X-ray machines or by gamma irradiators, interact with matter in three distinct processes: via the photoelectric effect, via Compton and other scattering processes and via a pair formation process. The contribution of each process to the absorption of photons depends on both the energy of the photons and on the atomic number of the stopping matter.

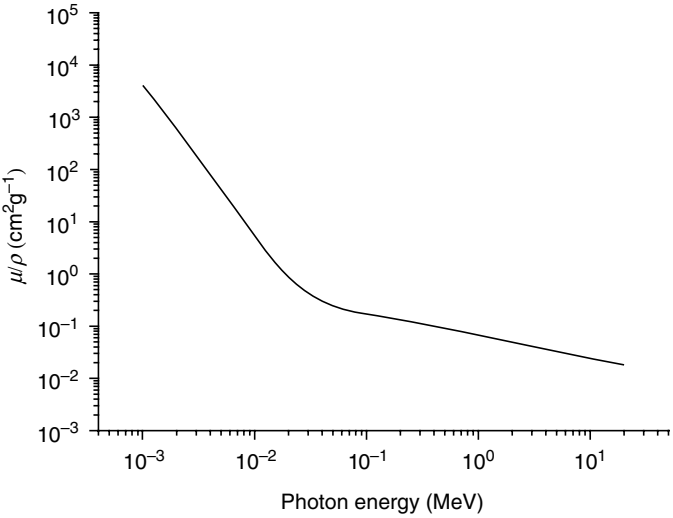
For low-energy photons interacting with water, the photoelectric effect is dominant at 0.01 MeV and tails off at 0.1 MeV. In this process, the photon is completely absorbed by the water molecules and a photoelectron is ejected. At higher energies, Compton scattering is the dominant process in water over a wide range of photon energies (approximately 0.1–10 MeV). In Compton scattering, only a fraction of the photon energy is absorbed to produce an ejected electron and so the degraded, scattered photon continues to ionise more water molecules. At high energies in excess of twice the rest mass of the electron – that is, in excess of 1.02 MeV – the incident photons can also be absorbed and in doing so produce a positron and electron pair.

As a beam of high-energy photons with incident intensity $I_{0,}$ passes through matter, the loss of intensity may be calculated using the Beer–Lambert equation:

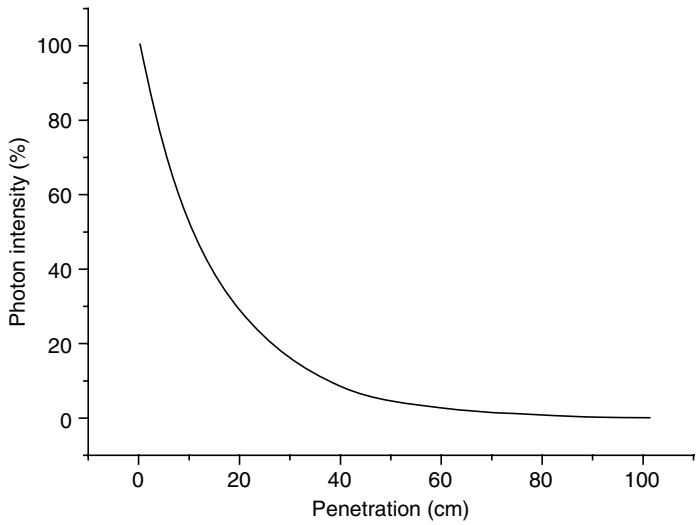
$$I = I_0 e^{-(\mu/\rho)x\rho}$$

where x denotes the path length (cm), μ/ρ is the total mass attenuation coefficient ($cm^2\ g^{-1}$) and ρ ($g\ cm^{-3}$) is the density of the matter. In this equation, the total mass attenuation coefficient takes into account the contributions from all three photon absorption processes – that is, from the photoelectric effect, Compton scattering and pair formation. Calculations of the total mass absorption coefficients for a wide range of photon energies absorbed by specific atoms, compounds and mixtures are available, for example, from the National Institute of Standards and Technology (Hubbell, 1977, 1985). Figure 3.1 shows a plot derived from such calculations for water.

Applying values of μ/ρ for water taken from Fig. 3.1 of $6.323 \times 10^{-2}\ cm^2\ g^{-1}$ at 1.25 MeV (as an approximate value for the 1.173 and 1.332 MeV gamma rays emitted by ^{60}Co), Fig. 3.2 shows the effect of depth of water ($\rho = 1\ g\ cm^{-3}$)



3.1 Mass attenuation coefficients (μ/ρ; cm^2/g) for water for high-energy photons (Hubbell, 1977, 1985).

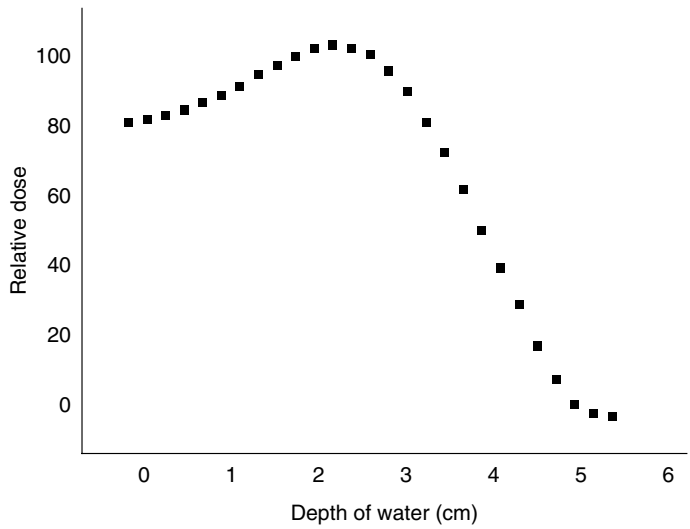


3.2 Penetration of 1.25 MeV photons through water.

on the intensity (I) of these gamma rays. It is clear, therefore, from Fig. 3.2 that high-energy photons are highly penetrating and can provide well-distributed and uniform sterilisation doses of radiation to large packages of healthcare products. It is important to note that high-energy photons lose their intensity exponentially and, therefore, unlike high-energy electrons, they do not have a finite range as they pass through matter.

3.2.3 High-energy electrons

High-energy electrons also cause ionisations in atoms and molecules as they pass through matter. The mechanism by which they lose energy is, however, different to those involved in the loss of energy by photons (see Section 3.2.2). The loss of energy as a function of distance travelled through matter, denoted by the term 'stopping power', is described by the Bethe equation in which the rate of change of energy loss with distance depends both on the energy of the electron and the electron density of the stopping matter. This equation shows that the stopping power increases as the electron energy decreases. A consequence of this is that electrons deposit more energy per unit distance as they slow down and thus have a finite range. High-energy electrons are, thus, much less penetrating than high-energy photons and produce much denser ionisation within matter, with the secondary electrons produced by the primary ionisation process being produced in a cascade and having sufficient energy to bring about many more ionisations. The radiation dose thus varies with penetration depth in a characteristic way with the maximum dose being dependent upon electron energy and always occurring between the incident surface and the range of the electron. In water, for example, a 10 MeV electron will penetrate only about 5.2 cm, although the absorbed radiation (usually expressed in Grays (Gy) where 1 Gy = 1 J kg^{-1}) will be relatively small about 4 cm. This is illustrated in Fig. 3.3.



3.3 Penetration of 10 MeV electrons through water. (*Source*: Data supplied by Andrew Stirling, I-Ax Technologies, Inc., Ottawa, Canada.)

High-energy electrons can also produce high-energy photons in the form of characteristic X-rays and bremsstrahlung radiation. The probability of producing these photons is dependent upon the atomic number of the matter absorbing the electrons and is significant for heavy metals such as tungsten and molybdenum. Characteristic X-rays are produced when electrons eject electrons from the inner atomic shells of the heavy metal target – the X-rays are emitted when other higher-energy electrons within the metal occupy the vacancies produced by the initial electron ejection processes. These X-rays have narrow bands of energy and are characteristic of the heavy metal target. Bremsstrahlung radiation is produced when the incident electrons interact with the electric field of the heavy metal nucleus and has energy from zero up to that of the incident electrons. The maximum intensity of bremsstrahlung radiation occurs at approximately one third of the maximum energy, the energy of the incident electrons.

3.3 Sources of ionising radiation

Both high-energy photon sources and high-energy electron accelerators are in common use for the sterilisation of biomaterials. The choice of sterilisation source will depend upon a number of factors which are discussed in the following section.

3.3.1 Gamma radiation and X-ray sources

Cobalt-60 (60Co) and caesium-137 (^{137}Cs) are the most widely used sources of gamma radiation. ^{60}Co produces gamma rays with energies of 1.173 and 1.332 MeV and has a half-life of 5.27 years, whereas ^{137}Cs produces gamma rays with an energy of 0.662 MeV and has a longer half-life of 30.1 years. For both isotopes, the gamma rays energies are not high enough to induce radioactivity in the irradiated products, which would otherwise be a serious disadvantage to a sterilisation process. In industrial practice, the use of ^{137}Cs has been limited to small self-contained, dry storage irradiators used primarily for the irradiation of blood and for insect sterilisation.

In principle, X-rays may also be used for sterilisation. For example, high-energy electrons produced by an accelerator could be used to produce high-energy photons (e.g., X-rays produced by bombarding a tungsten target). In practice, however, the costs of establishing and running such a facility are relatively high with only low conversion of electron beam power to X-ray beam power (I-Ax Technologies Inc., 2008). The use of high-energy electron sources to produce high-energy X-rays is also limited by the potential for producing radioactivity in the irradiated product. This can occur via a number of processes, including photo-disintegration, neutron activation and

photo-activation. However, extensive research has shown that below certain energy thresholds, any induced radioactivity is insignificant compared with that which is naturally present. These limits have been agreed on by the Joint Expert Committee on Irradiated Foods of the UN Food and Agriculture Organization, the World Health Organization and the International Atomic Energy Agency. They have also been accepted by the USFDA and other national bodies. These limits are currently: 10 MeV for electrons and 7.5 MeV for high-energy photons.

3.3.2 High-energy electron sources

High-energy electrons are produced industrially using electron accelerators. Two types are in common use: DC accelerators and accelerators based on radio frequency (rf) power technology. For the former type, the most common commercially available types are the Dynamitron^{R} and the Insulated Core Transformer supplying up to 5MeV electrons. Higher-energy electrons are produced by the rf accelerators, easily reaching energies of 10 MeV. Radio frequency accelerators, which use a series of rf cavities, are called linacs and can either be S-band (operating at an rf of 3 GHz) or L-band (1 GHz). S-band accelerators produce beam powers up to 20 kW, whereas L-band machines can produce beam powers in excess of 20 kW. A more compact rf accelerator, the Rhodotron®, uses radial accelerating fields.

3.3.3 Commercial radiation sources

Since the mid-1950s, there has been a rapid growth in the use of ionising radiation to sterilise or reduce the microbial bioburden of a range of industrial and agricultural products. The growth was largely stimulated by the need for single-use medical devices. Both gamma ray sources (^{60}Co) and electron beam sources are used currently for industrial sterilisation, and have been developed either as 'in-house' facilities or as outsourced contract services. Up until 2006, about 65% of sterilisation activity was provided by outsourced contract service providers (Masefield *et al*., 2006).

In a worldwide survey of ^{60}Co radiation sources made by the International Atomic Energy Agency (IAEA), 123 radiation processing facilities were listed in a directory (IAEA, 2004). Of the 123 facilities, 104 were used for the sterilisation of healthcare products such as medical devices, biological tissues and sanitary materials at a rate of 336 000 m^3 per annum. In a separate survey of industrial electron beam facilities made by I-Ax Technologies Inc., a total of 42 facilities were being used for sterilisation purposes (14 in North America; 20 in Europe and 8 in Asia) (I-Ax Technologies Inc., 2008). The above data reflect the current preference of a gamma radiation source

for sterilising a wide range of products. This choice of source is seen to provide a flexible, versatile and cost-effective method of sterilisation. However, for low-density products with a uniform composition and compact packaging, electron beam accelerators can provide much faster processing. The use of X-ray sources as an industrial method of sterilisation is emerging on 1 April 2008. IBA and LEONI Studer jointly announced the construction of a new X-ray sterilisation facility using the latest Rhodotron® TT-1000 system at the LEONI Studer Hard premises in Däniken, Switzerland. This will be the first facility worldwide capable of sterilising large amounts of medical devices directly on pallets, using an X-ray system.

3.4 Validation and international standards of sterilisation by ionising radiation

As with all methods of sterilisation, it is imperative that internationally accepted validation processes are adopted. These protocols may vary in different parts of the world with major protocols usually being developed in Europe and the USA. The following section outlines the principles involved in such protocols for sterilisation by irradiation.

3.4.1 Principles

The main aim of sterilisation of healthcare and related products is to reduce the level of pathogens to an acceptable, safe level. In doing so, it is clearly important to minimise damage to the product itself. The radiation chemical principles and the methods derived therefrom have been summarised above.

The action of radiation on bacteria, viruses and spores has received much attention in the research literature, largely, in the case of cells, as part of the process of understanding the mechanisms of radiotherapy. Cells, including bacterial cells, are killed by ionising radiation through damage to DNA. The damage may be attributable to both the indirect effect, arising from water-derived free radicals produced within the cell, and also from the direct effect of radiation on DNA within the cell nucleus. It is unlikely that water-derived free radicals formed outside the cell are lethal. The proportions of indirect to direct effect within cells and viruses have also been the subject of much study and estimates have been made which are close to 50:50 (e.g., see von Sonntag, 1987; Krisch *et al.*, 1991). That the lethal effects of radiation occur within the cell or virus is a distinct advantage for sterilisation by radiation of healthcare products in solution. Thus, water-derived free radicals produced outside the cells or viruses can be scavenged. At the same time, the direct effect of radiation on DNA still occurs and so kills cells. There may be some

effect of free radical scavengers incorporated within the cell which might reduce the rate of killing and this can be tested and accounted for. The effect of absorbed dose on the inactivation of a population of a specific cell or virus is normally accounted for quantitatively by an exponential relationship:

$$N = N_0\, 10^{-(D/D_{10})}$$

where N represents the number of survivors at a dose D, N_0 is the original number of cells or viruses and D_{10} is the dose required to reduce the number of cells or viruses to 10%. Differences in sizes of the genomes for bacteria, spores and viruses lead to differences in sensitivity to radiation. In general, D_{10} values for bacteria and spores are lower than those for viruses. Typical D_{10} values for bacteria, for example, range from 1 to 4 kGy whereas the typical range for viruses is about 3–8 kGy. It should be assumed that D_{10} values are temperature-dependent. For the HIV-1 virus, for example, the D_{10} value was found to be 7.2 kGy at room temperature and 8.3 kGy at –80°C (Hernigou *et al*., 2000). Other factors may also affect the D_{10} values and it is therefore advisable to determine these values for the particular set of sterilisation conditions.

3.4.2 International standards

The quantitative relationship between cell or virus survival and also the ability of commercial gamma radiation sources to deliver accurate doses of radiation enables methods to be developed which can achieve specific SAL for healthcare products. These methods were first developed for manufactured healthcare products, such as syringes, sutures, needles and items produced in large numbers. In the seminal ISO documents on these methods (ISO (International Organization for Standardization), 1995a, 1995b), two methods were used to establish radiation doses to achieve SAL values of 10^{-6} (i.e. a probability of 1 in 10^6 of there being one survival colony forming unit (cfu) – in the case of bacteria). Method 1 relied on knowing the bioburden on the product before irradiation – that is, the cfu values for each type of bacteria should be known. In this method, for bacteria, a standard distribution of resistance (SDR) was assumed, as given in Table 3.1 below. These data were then used to establish a verification dose to achieve a SAL of 10^{-2}.

Table 3.1 Microbial standard distribution of resistance

D_{10} (kGy)	1.0	1.5	2.0	2.5	2.8	3.1	3.4	3.7	4.0	4.2
%	65.487	22.493	6.302	3.179	1.213	0.786	0.350	0.111	0.072	0.007

Source: Whitby and Gelda (1979).

Delivery of the verification dose and subsequent confirmation that no colony-forming units survive, then allowed a sterilisation dose to achieve a SAL of 10^{-6} to be calculated. The method involved a statistical approach to setting the sterilisation dose, requiring the use of relatively large numbers of samples from three batches (130) for the establishment of the initial bioburden and verification dose. In Method 2, no assumptions were required concerning the numbers and types of bioburden. Instead, incremental doses were given to samples of the product and the remaining survivors measured. Again, a relatively large number of samples (280) was required to establish a verification dose for an SAL of 10^{-2}, from which the sterilisation dose required to achieve a SAL of 10^{-6} could be calculated.

These seminal international standards ISO 11137:1995 (ISO, 1995b) have now been cancelled and replaced by ISO 11137, Parts 1–3, 2006 (ISO, 2006a, 2006b, 2006c), thereby allowing revisions of the Methods 1 and 2 and also inclusion of a new method, the VD_{max} method.

In Part 1 of the revised standards, the requirements for development, validation and routine control of a sterilisation process for medical devices are set out, describing the requirements to ensure that the activities associated with the process of radiation sterilisation are performed at the required standard, including calibration, maintenance, product definition, process definition, installation qualification, operational qualification and performance qualification. It also emphasises that attention should be given to other aspects of the whole sterilisation process, from raw material to the final sealed, sterilised product package. Such considerations include:

- the microbiological status of raw materials;
- the validation and routine control of any cleaning and disinfection procedures used on the product;
- the control of the environment in which the product is manufactured, assembled and packaged;
- the control of equipment and processes;
- the control of personnel and their hygiene;
- the manner and materials in which the product is packaged;
- the conditions under which the product is stored.

Part 3 of ISO 11137:2006 gives guidance on how the dosimetric requirements of the ISO should be met. The measurement of dose is central to the sterilisation process. An accurate and precise dose delivered to a product whose initial bioburden is known enables a statistical approach to be taken for the achievement of a specified sterility assurance level, the central feature of the sterilisation of medical products by ionising radiation. Radiation dose is measured during all stages of development, validation and routine monitoring of the sterilisation process. It is important to demonstrate that

dose measurement can be related to an international standard, that the uncertainty of measurement is known and that the influence of temperature, humidity and other environmental considerations on dosimeter response is known and taken into account.

Dose mapping is a particularly important parameter in the determination of the uncertainty of the dose delivered to products. The mapping process is essentially a measurement of the variation of delivered dose within the radiation containers in which products are irradiated. The variation of dose can be influenced by the density of products where a low density-product would not significantly shield dose from other products within the container. Other considerations include the size of products and their spatial arrangement within the radiation container. Dose mapping considerations also vary according to the type of irradiation facility, gamma, electron beam and X-ray .The main outcome of a thorough and proper consideration of dosimetry in a sterilisation process is the establishment of minimum and maximum doses delivered to containers. These limits are clearly important for establishing doses which will guarantee sterilisation of any sealed product package to the specified SAL.

In Part 2 of ISO 11137 (ISO 11137, 2006b), the methods for establishing the sterilisation dose have been both revised and amended to include an approach based on the VD_{max} method (AAMI, 2001). This document also introduces the concept of product families, the grouping of which is largely dependent upon the number and types of microorganism present on or in the product. The criteria for including a product within a product family also includes other parameters which may affect bioburden, such as the nature and sources of raw materials, the components, the product design and size, the manufacturing process, the manufacturing equipment, the manufacturing environment and location. The ISO also categorises the types of manufactured items that can be sterilised. These are: individual healthcare products in their packaging systems; a set of products within a packaging system to form a healthcare product; a number of identical healthcare products in their packaging system; and a kit comprising a variety of procedure-related healthcare products. Guidance on the selection of items within these categories is then given for the purposes of dose setting and dose substantiation.

An important aspect of establishing the sterilisation dose is the decision whether to test whole individual products within the above product categories, or to test a portion instead, a sample item portion (SIP). The latter may be taken when it is otherwise impracticable to test the whole product, providing the average bioburden of the individual product is greater than 1 colony-forming unit (cfu). The value of the SIP is a fraction whose value can be calculated using the guidelines of the ISO. Thus, for a powder, its mass can be used. The adequacy of the SIP must be demonstrated – out of 20

non-irradiated SIPs, at least 17 should yield positive tests of sterility – that is, 17 should show detectable microbial growth. Tests of sterility should be conducted in accordance with ISO 11737-1 and ISO 11737-2.

In Part 2 of ISO 11137:2006, a number of approaches to setting the sterilisation dose is given and thus allows the manufacturer of healthcare products a considerable degree of flexibility to achieve the desired level of SAL. Essentially, there are three types of method: Method 1, Method 2 and VD_{max} methods.

In Method 1, the determination of the sterilisation dose depends on experimental verification that the radiation resistance of the product bioburden is less than or equal to the resistance of a standard distribution of resistances, as detailed in the Table 3.1 (Whitby and Gelda, 1979). The method is based upon determining the initial bioburden of the unirradiated product using at least ten product items from each of three independent production batches. Using an appropriate average bioburden, the dose required to yield an SAL of 10^{-2} can be calculated using an extended form of the single exponential D_{10} equation given above applied to the SDR in Table 3.1. Tables of radiation doses for combinations of bioburden and SAL for the SDR are given in ISO 11137-2, for a wide range of bioburden values for each of the SAL values in the range, 10^{-2}–10^{-6}. The verification dose test to achieve an SAL of 10^{-2} requires, thus, 100 products items from a single batch to be irradiated and sterility tests carried out on the product items. The verification dose test may be accepted if there are no more than two positive tests of sterility within the 100 items, and provided that the actual dose or range of doses delivered to the items are within limits set out in the ISO. The sterilisation dose required, for example, to achieve an SAL of 10^{-6} can then be calculated using the latter tables. If SIPs are used, the average bioburden for the whole product must first be calculated before using the tables to calculate the sterilisation dose. This version of Method 1 applies to products with an average bioburden ≥ 1.0 for multiple production batches. ISO 11137-2:2006 also gives amendment of this procedure for both single production batches (bioburden ≥ 1.0) and for products with average bioburden in the range 0.1–0.9 for either single or multiple batches.

In Method 2, no assumptions are made about the radiation resistance of contaminating microorganisms and there are no requirements to measure the initial bioburden. Instead, incremental doses are given to a number of products in order to estimate the dose at which only one in 100 products would be expected to be non-sterile. At this dose, the D_{10} value (the dose required to reduce the number of microorganisms to 10% of this value) for the remaining microorganisms should be more homogeneous, and it is this value which is then used to calculate the dose to achieve higher level of sterility assurance, typically 1 in 10^6. At each incremental dose, the number of positive tests of sterility is recorded – this number decreasing as the dose

is increased. There are two variations of Method 2, viz. Methods 2A and 2B. The former is used more generally while the latter is used for products with a low and consistent bioburden.

In Method 2A, 280 product items are selected from each of the three independent production batches. From each production batch so selected, 20 product items are irradiated at each of at least nine doses starting at 2 kGy and increasing the dose by 2 kGy increments. For each product item, the number of positive tests of sterility is recorded and then this information is used to determine the dose to provide a sterility assurance level of 10^{-2} for the test. It is this dose which is subsequently used in a verification dose experiment on a further 100 product items. In this latter test, the number of positive tests of sterility is recorded and, depending upon the number found in the range 0–15, a sterilisation dose to achieve an SAL of 10^{-6} may be calculated. For a number of positive sterility tests in excess of 15, the cause should be determined and corrective action implemented before a new determination of a sterilisation dose could take place.

Method 2B is similar to that of 2A in that incremental doses are again used to test the actual radiation resistances on the products. In this case, however, the entire product should be used (SIP = 1), the number of positive tests of sterility should not exceed 14 in the incremental dose tests and the estimate of the dose required to produce an SAL of 10^{-2} should not exceed 5.5 kGy.

In the third method of ISO 11137, Part 2 (ISO 11137, 2006b), the VD_{max} method, there are similarities with Method 1 in that the initial bioburden values on product samples are required and that the SDR is the basis of the assumed radiation resistance. By taking into account the distribution of radiation resistances in the SDR, a verification dose experiment carried out on only ten product items is calculated – that is, for a SAL of 10^{-1} which is characteristic of both bioburden level and the associated maximal resistance. Thus, components of the SDR of high D_{10} value are used to determine the sterilisation dose and so ensure a greater degree of conservativeness of the SDR is preserved. The method is designed not only to provide this degree of assurance but also to facilitate the use of fewer product items for testing – in this case, only ten items. In practice, the VD_{max} dose is calculated using the average bioburden level and then ten product items from each of the three independent production batches are exposed to this dose and each item subjected to a test of sterility. If there is no more than one positive test of sterility in the ten tests, the pre-selected sterilisation dose is substantiated. The pre-selected sterilisation doses are 15 and 25 kGy, the former applicable only for product bioburdens less than or equal to 1.5, while the latter is applicable to average bioburden levels of less than or equal to 1000. Modifications of the VD_{max} methods may also be used for items from a single production batch.

3.5 Conclusions and future trends

There is an increasing realisation that the established standard sterilisation dose of 25 kGy confers no guarantee of sterility. This is particularly so for viral contamination where the D_{10} values are generally higher than for bacteria. Good manufacturing practice, where the statistically averaged bioburden is low and the distribution of pathogens is known both in types and intensities, is likely to allow the use of doses lower than 25 kGy to achieve sterility assurance levels of $1{:}10^6$. Recent revisions of the ISO Standards (ISO, 2006a, 2006b, 2006c) facilitate the use of both lower doses of sterilisation and the use of fewer samples for dose validation purposes, while retaining the integrity of the approach to attain sterility assurance levels as high as $1{:}10^6$. These revisions are likely to be of particular interest and significance to the drug-device industry where low volumes of expensive products present a challenge to the cost effectiveness of sterilisation processes designed for much larger product volumes.

3.6 Sources of further information and advice

To gain a good insight into the mechanistic aspects of the radiation chemistry and biochemistry of cells, viruses and their components, *The Chemical Basis of Radiation Biology* by von Sonntag is recommended (von Sonntag, 1987). For guidance on standards to be adopted for the sterilisation of healthcare and tissue allografts, the ISO and IAEA references contained in this chapter are essential reading. Finally, the websites of the International Irradiation Association and of the International Atomic Energy Agency are very useful to keep abreast of current practices in sterilisation and for international meetings relevant to sterilisation by ionising radiation.

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4

Ethylene oxide (EO) sterilization of healthcare products

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Abstract: Ethylene oxide is a dominant agent in the sterilization of medical devices due to its effectiveness and compatibility with most materials. The advantages and disadvantages, as well as its recommended uses, are explored in this chapter. The variables and their relevance on process optimization are described, the types of processing cycles are detailed and emphasis is given to the design and validation of the sterilization process.

Key words: medical devices, ethylene oxide sterilization, process design, process validation.

4.1 Introduction

Used as a fumigant for insects in the early twentieth century, ethylene oxide (EO) was recognized as an anti-bacterial agent around 1929. Initially it was used for sterilization of spices, and in the 1940s it started being used as a low-temperature sterilizing agent for healthcare products (Rogers, 2005).

Nowadays, ethylene oxide is still a dominant sterilization agent used in the medical device (MD) industry, with a continuous growth tendency, especially due to its effectiveness and compatibility with most materials. It is widely used, because it avoids heat and radiolytic stress often associated to sterilization with steam or radiation. This last point is especially important due to the diversity of developed products, designs, type of materials and packaging configurations demanded by the current market. This technique also has disadvantages, related to EO toxicity, that require special care for the protection of workers and patients, which has led several countries to limit its use, especially in healthcare centers. This topic will be further explored.

This chapter provides a framework for understanding the basic principles of EO sterilization. The advantages and the disadvantages of this sterilization methodology and its recommended uses are described. The EO sterilization mechanism is explained and the variables that influence process lethality are discussed, as well as their relevance to process optimization. The EO processing cycles are detailed and emphasis is given to the design

and validation of the sterilization process, including the microbiological assessment, which is the most challenging in the validation context.

4.2 Advantages of ethylene oxide (EO) sterilization

Ethylene oxide is an 'ideal' gaseous sterilant because of its characteristically high diffusivity through solid matrixes (Ernest, 1973; Rogers, 2005). The main advantages of this sterilization methodology are its effectiveness and compatibility with most materials, as well as its flexibility, which results from the dependency on several factors, such as concentration, temperature, humidity and time (and their combinations).

In comparison with other methods, the differential advantage of EO is that it can sterilize heat-, moisture- and/or radiation-sensitive medical items without deleterious effects on the materials. For many MDs, and in particular thermolabile plastic, elastomer polymeric materials and most electronic devices and biomaterials, EO is the sterilant of choice, and quite often the only acceptable sterilization method (Ernest, 1973; Handlos, 1980; Rogers, 2005). Considering the exponential market growth of custom procedure packs (that combine a diversity of products and range of polymers) for use in specific medical and surgical procedures, the probability of incompatibility between material/sterilization process increases, which results in an increased use of EO.

Ethylene oxide sterilization is mainly applied to the MDs industry, with other significant applications in pharmaceuticals and cosmetics, particularly for some chemical compounds and/or packaging materials before aseptic processing. The use of EO as a terminal sterilization process for pharmaceuticals can be limited: (i) the EO process might alkylate or hydrolyze the molecules, (ii) the relatively long process times at 40°C/104°F to 60°C/140°F might cause some thermal degradation and (iii) components of the formulation that have low boiling points might evaporate, due to vacuum pulses (AAMI TIR 17, 2008). The penetration of EO in liquids or powders depends upon the amount in containers: if the material is spread thin, the gas will penetrate, but this will not occur if a bulk volume is considered. This explains why EO sterilization is not commonly recommended for liquid or powder products.

The effectiveness and reliability of EO sterilization is undeniable. The powerful microbicidal, virucidal and fungicidal activity of this agent has been demonstrated in several studies and summarized in published reports (Parisi and Young, 1991; Ries *et al.*, 1996; Alfa *et al.*, 1996, 1997, 1998a, 1998b; Rutala *et al.*, 1998). The microbicidal activity of EO is the result of alkylation of side chains of enzymes, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) (e.g. OH, COOH, SH and NH). The alkylation (replacement of a hydrogen atom with an alkyl group) interferes with the normal cellular metabolism and reproductive processes, which renders a non-viability of affected microbes (Poothulil *et al.*, 1975; Swenberg *et al.*, 2000).

EO properties are well understood, and knowledgeable users can quickly develop and validate effective sterilization processes.

4.3 Disadvantages of ethylene oxide (EO) sterilization

The disadvantages associated with EO sterilization are the lengthy cycle, the cost, and its potential hazards to patients, staff and environment, as well as the risks of handling a flammable and explosive gas. Due to its complexity and hazardous potential, it requires a properly designed area (promoting an efficient work flow), sophisticated technology and equipment, feasible and ongoing engineering controls, safe work practices and trained staff. Detectors are required to protect staff workers, especially since this gas is colorless and odorless until a level of 430 ppm, much above its toxic level. Moreover, careful aeration of MDs is required since absorbed EO can leave toxic residues on them. However, EO disadvantages have been mainly overcome by equipment and facilities investments, which have dramatically improved the process efficiency while guarantying workers' security and environmental protection. The processing equipment of modern plants consists of tightly closed, highly automated and controlled systems. Currently, the EO sterilizers combine sterilization and aeration in the same chamber or in a continuous chamber (in automatic communication with the sterilizer), achieving a nonstop process that minimizes the potential occupational exposure to EO. The AAMI TIR 15 (2009) and EN 1422 (2009) standards provide guidelines for design and selection of an appropriate sterilization equipment and facility for attaining a safe, reliable and effective process. Although the process is complex (due to the EO inherent toxicity and flammable properties, as well as the large number of variables involved), and requires knowledge and careful monitoring, an efficient sterilization process can be achieved by skilled users.

The capacity of EO sterilizers varies from table-top size to very large floor-loading chambers, but, due to the inherent risks associated with EO, this technology is becoming more and more industrial and its use in healthcare units is decreasing.

Its complexity, numerous speculative risks and misconceptions led to unfair criticism and disapproval of the EO process. Despite many predictions about its demise as a sterilization alternative, it is still a dominant mode of sterilization and it continues to be used for increasing volumes of MDs. Nowadays, EO can be used safely with minimal hazard and its benefits continue to outweigh its inherent risks (ANSI/AAMI ST 41, 2008).

4.3.1 Time and cost

The extended process time of EO sterilization is mainly due to quarantine period of the biological indicators (BIs) required for clearance and approval

(conventional release), and aeration time for EO residues removal. Recent technological advances have greatly reduced the cycle time of EO sterilization, due to the development of parametric release methods and aeration processes optimization. Proper EO handling requires sophisticated equipment, automatic controls (that preclude human error) and careful monitoring, which results in high operational handling cost. This topic will be further discussed.

4.3.2 Risk to patients

The large variability of the rate and extent of EO adsorption and desorption by the different polymers used in the MD industry requires careful verification that EO residues and by-products in MDs are below hazardous levels before their use on the patients.

The ISO 10993-7 (2008) specifies the allowable limits for residual EO and for its by-product, ethylene chlorohydrin (ECH), which is formed due to the EO reaction with chloride radicals. These chemicals are particularly relevant, since the exposure to devices that have been improperly aerated can cause irritation and, eventually, burns (ANSI/AAMI ST 41, 2008). Another by-product, formed by the reaction of EO with water, is ethylene glycol (EG). However, no exposure limits for EG are defined because studies have shown that when EO residues are controlled (as required by ISO 10993-7), it is unlikely that biologically significant residues of EG would be present (ISO 10993-7, ANSI/AAMI ST 41, 2008).

The limits for EO residues were established using health-based risk assessment studies (aiming at a minimal risk to patients during standard use of the product), taking into account the contact time with the device (limited exposure – daily; prolonged exposure – monthly; permanent exposure; although certain exceptions occur for particular devices). The ISO 10993-7 (2008) also outlines suitable methods for the extraction of residues from products (using exhaustive extraction and simulated-use procedures), details the subsequent analysis via gas liquid chromatography and provides the procedures for determining compliance and subsequent MD release to the market. The objective of simulated-use procedures is to quantify the 'bioavailable' EO residues, which is the amount of EO that may be assimilated by the body; therefore, water extraction is carried out under conditions that represent the intended use of product (at room temperature, 22°C/72°F, or body temperature, 37°C/99°F). With exhaustive extraction (thermal extraction followed by headspace analysis and solvent extraction procedures, with either headspace gas analysis or chromatography of the solvent extract), the intention is to recover the entire residual content of a device. Simulated-use methods are commonly used for devices with limited

potential patient exposure, while exhaustive methods are appropriate for prolonged or permanent exposure devices.

It is important to carefully study the method for residuals quantification. There are no general rules and each specific material has its own characteristics (AAMI TIR 19, 1998, 1999; ISO 10993-7, 2008). Tests can be conducted at the final desired aeration time-point, or an EO dissipation curve can be established by periodic sampling and analysis of the product. Release is based on the time after sterilization when the regression line intercepts the maximum allowable residue. These data can be used to establish quarantine times prior to product release, or to provide additional information about the influence of manufacturing, packaging or sterilization processes on product EO levels. The adsorption and degassing of EO from sterilized products is influenced by several factors, and the conditions under which degassing occurs has a high influence on EO residues diffusivity. This issue will be further discussed in a later section (Booth, 2000; ISO 10993-7, 2008).

Despite well-known EO toxicity, there are large uncertainties associated with the current quantitative risk assessment studies that establish the undesirable effects of EO residues on patients' health. However, the most important is that EO potential risks were always estimated conservatively, which means that its effects are overestimated. The residues of EO should be kept as low as feasible, and cannot exceed the limits defined by ISO 10993-7 (2008); EO potential risks become trivially small or even zero if doses are significantly low (Mendes *et al.*, 2007, 2008).

4.3.3 Workplace considerations

When EO sterilization equipment is elected, provisions should be made for compliance with Occupational Safety Health Administration (OSHA) safety standards and state regulations. Workplace exposure to EO is regulated by OSHA through standard 29 CFR 1910.1047 (ANSI/AAMI ST 41, 2008; AAMI TIR 15, 2009).

Health risks

Acute overexposure to EO may result in irritation (e.g. to skin or mucous, eyes, gastrointestinal or respiratory tracts) and central nervous system depression. Chronic (long-term) exposure to EO has been linked to an increased risk of cancer and reproductive effects, neurotoxicity, fetotoxicity and spontaneous abortion. In various *in vitro* and animal studies, EO has been demonstrated to be carcinogenic; findings in humans and experimental animals exposed to EO airborne concentrations also indicate damage of the genetic material (DNA), due to its alkylating properties. Currently, limited studies on chronic effects in humans, resulting from exposure to EO, suggest

a causal association with leukemia, although the environmental EO concentration is questionable. Despite being classified by the Environmental Protection Agency (EPA) as Group B1 (probable human carcinogen), recent epidemiological studies of controlled/occupational exposure to EO did not demonstrate potential cancer risk in workers (29 CFR Part 1910.1047, n.d.; ANSI/AAMI ST 41, 2008; Valdez-Flores *et al.*, 2010).

Occupational exposure limits

Workers' exposure to EO should be kept as low as feasible. OSHA has established a permissible exposure limit (PEL) of 1 part per million (ppm) airborne EO in the workplace, and an 'action level' of 0.5 ppm, expressed as a time-weighted average (TWA), for an 8 h work shift in a 40 h work week. Exceptionally, exposures above 1 ppm are allowed if they are compensated by equal or longer exposures below the limit, during the same 8 h work day. The short-term exposure limit (STEL) is 5 ppm, expressed as a 15 min TWA, and OSHA has also established a PEL of 5 ppm for ethylene chlorohydrin in the workplace (ANSI/AAMI ST 41, 2008). Workers who are or will be exposed at or above the action level (0.5 ppm) for 30 or more days per year should be submitted to medical examination and clinical analysis control, at least annually (ANSI/AAMI ST 41, 2008).

Environmental and employee monitoring

In order to ensure a safe and healthy work environment and to establish compliance with regulated limits and voluntary guidelines on occupational exposure to EO, airborne EO concentrations must be monitored in the workplace. Two general types of monitoring are performed in EO sterilization facilities: personnel monitoring (devices used by operators) and area monitoring.

Personnel monitoring aims at determining airborne contaminants in the employee breathing zone (EBZ), which is assumed to be the amount actually inhaled. The two most popular methods that have been used for EO exposure determination are charcoal tubes and passive dosimeters. Tedlar gas-sampling bags, impingers and detector tubes are examples of other personnel monitoring systems. In addition, there are several commercially available real-time continuous monitoring analyzers, portable and directly readable.

Area monitoring is performed for determination of environmental EO concentration in a particular workplace area. The following types of area monitoring devices are currently available: metal oxide semiconductors, electrochemical sensors, gas chromatographs, infrared spectrophotometers, photoionization detectors and gas detector tubes. The continuous

monitoring of the work place environment can also be interfaced with controls to increase ventilation when the OSHA action level, PEL or STEL is exceeded.

Each method has its own specific limitations and this topic is explored in ANSI/AAMI ST 41 (2008).

Personal protective clothing and equipment

If eye or skin contact with EO or EO mixtures might occur, such as during sterilizer maintenance, EO cylinder changing, or by EO leak or spill, appropriate personal protective equipment (PPE) must be used (29 CFR Part 1910.132, 29 CFR Part 1910.133, n.d.).

When excessive EO exposure could occur, personnel should use an adequate respirator, certified by the National Institute for Occupational Safety and Health (NIOSH). The handling of liquid EO requires impermeable clothing (coveralls or similar full-body work clothing, gloves, head coverings, face shields or splash-proof safety goggles) and impermeable shoes. Rubber and leather must be avoided, since liquid EO readily penetrates these materials (ANSI/AAMI ST 41, 2008).

4.3.4 Environmental impact

The state regulations and the papers published by the EPA must be followed in order to control the potential environmental risks. In addition, the risks associated with handling a flammable and explosive gas also need to be considered.

Emission control systems

Several countries have recently introduced regulations to limit the amount of EO released in the atmosphere (Guidelines for the Reduction of Ethylene Oxide Releases from Sterilization Applications, Environment Canada, www.ec.gc.ca). The most important systems for reducing EO emissions are catalytic converters and acid water scrubbers. The first system is the most efficient and operates at relatively low temperatures (121–288°C/250–550 °F) to flamelessly convert EO to carbon dioxide (CO_2) and water vapor. The second one basically consists of a bath where effluent EO gas reacts with acid water, converting it into EG.

In addition, absorption systems (e.g. filtering media) can be used to absorb EO in low concentrations and some systems also operate with recovery (or reclamation), which means that the gas is reprocessed for reuse rather than discharged into the atmosphere. Besides the reduced costs of this option, it is not very common due to its inherent complexity and associated risks (ANSI/AAMI ST 41, 2008).

Recommendations for working with a flammable and explosive gas

Ethylene oxide is flammable and can be highly explosive when pure. Its range of flammability, as a mixture in air, extends from 3.6% to 100% by gaseous volume. When 100% EO or flammable blends of EO are used, electrical accessories should comply with Class I, Division 2, Group B electrical requirements, as stated by the National Fire Protection Association (NFPA) in NFPA 70 (2008) or equivalent; the sterilizer interior should comply with Class I, Division 1, Group B electrical requirements stated within NFPA 70 (2008) or equivalent; the equipment and piping should be grounded in accordance with NFPA 70 (2008) or equivalent. In facilities constructed after 1995 and where NFPA standards are under a jurisdiction, the storage, handling and use of EO shall comply with NFPA 560 (2007).

It is recommended that the chamber environment should remain within the non-flammable zone; therefore, the flammability calculations shall be considered when designing sterilization cycles (AAMI TIR 15, 2009). The mixture of EO and chlorofluorocarbon-12 (CFC-12), referred to as 12/88 EO (mixture of 12% EO and 88% CFC-12) and most commonly used in the late twentieth century, was banned in December 1995 under provisions of the Clean Air Act. The scientific evidence that linked the gas mixture to the destruction of the earth's ozone layer was the basis of the decision (ANSI/AAMI ST 41, 2008).

Nowadays, EO cycles with nitrogen are common and sterilant mixtures of EO with hydrochlorofluorocarbons (HCFCs) or of EO with CO_2 can also be used to reduce the potential flammability of EO. HCFCs also cause some depletion of the earth's ozone layer, although to a much lesser extent than CFC-12, and the international agreements call for it to be phased out completely in 2015 (ANSI/AAMI ST 41, 2008; AAMI TIR 15, 2009).

Ethylene oxide processing cycles

Ethylene oxide may be used pure or diluted with HCFCs or CO_2, and these latter solutions are neither as effective nor as cost efficient as 100% EO. Typically, large-scale industrial units use pure EO, while the blends are used in smaller laboratories and in healthcare facilities.

The typical EO processing cycles are (29 CFR Part 1910.1047, n.d.; Ernest, 1973; Rogers, 2005; AAMI TIR 17, 2008; AAMI TIR 15, 2009):

- 100% EO cycles with/without nitrogen. This is the typical industrial cycle. Its advantages are related to its lower cost (than the non-flammable blends), its adequacy for sensitive materials (due to lower damage) and to the reduction of potential hazards due to environmental EO exposure (potential gas leakage is minimized). In addition, despite requiring intrinsically explosion-safe equipment and instrumentation,

this solution does not require a pressure vessel, since chamber pressures are below atmospheric.

- Standard EO/HCFC cycles. These non-flammable gas mixtures provide safe working conditions and this solution is useful in non-explosive facilities. Their use is being restricted due to the ozone depletive properties of HCFCs. The common blends are: (i) 8.6%w EO/91.4%w HCFC-124, (ii) 10%w EO/90%w undisclosed HCFCs and (iii) 10%w EO/27%w HCFC-22/63%w HCFC-124.
- EO/CO_2 (high-pressure) cycles. These non-flammable gas blends are less expensive than EO/HCFC blends. The disadvantage of this solution is the high-pressure process that is required to achieve an effective sterilization concentration, and the inherent reduction of the EO sterilization efficacy. The common blends are: (i) 8.5% EO/91.5% CO_2 (w/w), (ii) 20% EO/80% CO_2 (w/w) and (iii) 30% EO/70% CO_2 (w/w).

4.4 Sterilization process characterization

The basic EO sterilization cycle consists of five stages – that is, preconditioning and humidification, gas introduction, exposure, evacuation and air washes (ISO 11135-1, 2007).

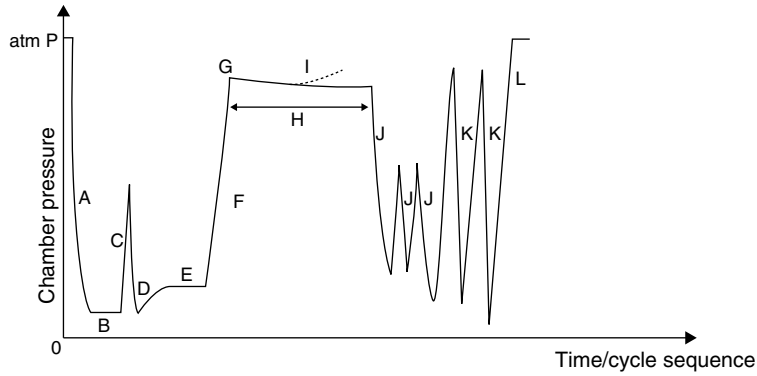
4.4.1 Preconditioning area (outside sterilizer chamber)

The preconditioning facilities (typical in industrial processes) provide heat and humidification to the product (and to microorganisms) through assisted air circulation, shortening the cycle time and equalizing the temperature and humidity of the loads during winter and summer. The time required for adequate temperature and humidity balance of the load should be evaluated in the coldest seasons or by using a refrigerated load to simulate the lowest temperature to which the product may be exposed before preconditioning (ISO 11135-1, 2007; AAMI TIR 15, 2009).

4.4.2 Typical industrial sterilization cycle

Vacuum cycles are the preferred choice (due to the reasons previously explained) and the use of pure EO as sterilizing agent, together with nitrogen as inert gas, is increasing. The basic steps of a hypothetical and typical 100% EO sterilization cycle, also called deep vacuum cycle, are explained in Fig. 4.1 and a short description in terms of cycle optimization is also given.

(A) *Air removal – vacuum*. Air is withdrawn from chamber. This step is necessary so that subsequent EO injection will not pass through significant



4.1 A hypothetical and typical 100% EO process cycle.

flammable limits or explosive conditions, and the deeper the vacuum the higher the moisture diffusion.

(B) *Leak test*. The chamber tightness is checked before injecting EO.

(C, D) *Nitrogen flushes*. Nitrogen injections and evacuations can be used to reduce the oxygen concentration in the chamber. Inert atmospheres are attained, which are safe for EO injection.

(E) *Conditioning – steam injection for temperature and humidity stabilization*. The purpose of humidification is to drive the moisture deep into and through the materials and heat up the sterilization load. Humidification is performed under vacuum and prior to admission of EO because the water vapor molecules diffuse slower than the EO molecules. Also, water readily reacts with EO through hydrogen bonding, which would create aggregates that impede the diffusivity of water vapor. Humidification can be achieved by static or dynamic environmental conditioning.

- Static humidification: steam is injected into the sterilizer until a certain pressure, or a target relative humidity level, is achieved in the sterilization chamber. During the humidity stabilization, as the load mass adsorbs the injected moisture, chamber pressure is maintained by steam injection.
- Dynamic environmental conditioning (DEC): DEC is a more effective process designed to heat up the load using flowing steam as the heating medium. The amount of heat available is dependent on the operational pressure during the DEC phase. This process follows a steam-bleed principle, because steam is injected in a steady flow as the chamber is being evacuated, and in this way the air is removed or displaced by the action of steam injection.

  Two common methods for delivering DEC are pulsed-steam injection (also known as stepped conditioning because vacuum pull is alternated

with steam injection) and continuous steam injection (because a steam injection is kept constant while the vacuum pump is activated).

(F) *Sterilant injection*. The EO and nitrogen injections are in equilibrium in order to provide the required EO concentration and a non-flammable mixture.

(H, I) *Exposure time*. A forced recirculation is important for keeping homogeneous sterilization conditions during this step. If the original chamber pressure is to be maintained throughout the exposure, it can be done by using either EO or inert gas/nitrogen make-ups or additions. When nitrogen is used to maintain the chamber pressure, the recirculation system will be sufficient to minimize the risk of the inert and EO gases stratification, and to avoid the potential effect of reduced EO concentration (which could affect the lethality rate).

(J) *Flushing – nitrogen rinsing step*. The EO is purged to remove the residual sterilant and the chamber is flooded with nitrogen to keep inert atmospheres inside the sterilizer; successive operations may be performed.

(K) *Flushing – air rinsing step*. The EO is removed from the chamber by consecutive vacuums and injections of filtered sterile air.

(L) *Air admission*: Last air rinsing brings the chamber back to atmospheric pressure.

Aeration

Aeration can be performed in the sterilizer or in a separate aeration chamber or room, under controlled conditions. All EO sterilized materials should be properly aerated before handling and use. The aeration time depends on many variables, including:

- composition, density, porosity, dimensions, surface area and design configuration of the material. Metal and glass are two materials that retain very low EO quantities. Polymers adsorb and desorb EO at higher but variable rates. It is described that polyvinyl chloride, polystyrene and rubber retain more EO than polyethylene, polyurethane, silicone, acrylic butyl styrene and polycarbonate, and the latter retain more EO than nylon, paper or cotton;
- packaging material (wrapping material and/or sterilization container system);
- sterilizing conditions (i.e. temperature, sterilant concentration, exposure time);
- aeration conditions;
- size, configuration of the load, and number of highly EO-absorptive materials being aerated; and

- acceptable limits of residues for the intended use of the device (Ernest, 1973; Handlos, 1980; Scott, 1982; Aeschlimann, 1984; Muzeni, 1985; Vink and Pleijsier, 1986; Buben *et al.*, 1999; Booth, 2000; Lucas *et al.*, 2003; Rogers, 2005; Mendes *et al.*, 2007, 2008; ISO 11135-1, 2007; ANSI/AAMI ST 41, 2008; ISO 10993-7, 2008; AAMI TIR 15, 2009; AAMI TIR 16, 2009).

4.4.3 Process variables

Ethylene oxide sterilization is a complex multi-parameter process. The effectiveness of an EO sterilization process is influenced by many variables and each one may be varied, this affecting the other dependent parameters. An effective process design requires an understanding of the process parameters and the interrelationships between them and the products. The most significant variables are outlined below (ANSI/AAMI ST 41, 2008; AAMI TIR 17, 2008; AAMI TIR 16, 2009).

Pressure

Initial vacuum level interferes with the sterilization efficacy because the residual air in the load hinders moisture diffusion, and consequently affects heat and gas transfer into the product. Besides the pressure depth, the process specification also involves the establishment of the gas injection and evacuation rates due to their effect on the cycle lethality, as well as due to the potential for package and product damage.

Shallow vacuum processes (nitrogen soft cycles), in which the vacuum levels are at or around 1/2 of atmospheric pressure, are designed for sterilizing pressure-sensitive materials. Deep vacuum processes are adequate for sterilizing loads that do not contain pressure-sensitive materials.

Ethylene oxide concentration

The EO concentration can be directly measured in the headspace chamber by infrared (IR) spectroscopy, gas chromatography (GC) and microwave spectroscopy, or can be calculated (Mendes *et al.*, 2007; AAMI TIR 15, 2009). The higher the concentration, the faster the sterilization process; however, higher concentrations will lead to higher EO residuals and consequently to increased aeration times. Since this variable interferes with the microbial inactivation kinetics and with outgassing, the process optimization also requires considerations about the material (i.e. EO absorption and retention characteristics).

As the EO concentration increases from 50 to 500 mg/L, there is a significant increase of the microbial death rate. At concentrations above 800 mg/L,

the rates do not increase significantly. The use of concentrations between 400 and 650 mg/L is recommended for achieving microbiological lethality in most products within a reasonable and practical exposure time, and without disregarding the EO residuals.

Temperature

Temperature has a significant influence on microbial lethality and affects the EO diffusion through cell walls and packaging materials. High-density loads and items composed of materials with low thermal diffusivity require longer heat-up time. Microbial death rate depends on temperature and, consequently, if high temperatures are used, the cycle time can be reduced. However, it is important to consider the maximum temperature the product and the package can withstand. Typical operational temperature values are above 35°C/95°F and below 60°C/140°F. It is consensual that a Q_{10} value of 2, which means that a 10°C/50°F change would affect lethality by a factor of 2.

Humidity

Relative humidity may be directly measured or calculated (AAMI TIR 15, 2009). This parameter plays a critical role in EO sterilization processes and is the most complex of the controllable variables because it influences the gas diffusion. An inadequate humidification is the major contributory cause for most microbiological failures of EO processes.

A level of relative humidity (RH) above 30–35% and below 85–90% (in the chamber) is commonly used to achieve an effective EO sterilization, and particular consideration should also be taken due to product limitations. Excessive moisture should be avoided throughout the cycle because it inhibits sterilization (drops of water protect microorganisms from EO action) and it increases the possibility of EG generation.

Exposure time

The time necessary to provide the required sterility assurance level (SAL) is primarily related to gas concentration and temperature. It should be taken into consideration that the lethality occurs not only during the exposure time, but also during the sterilant injection time (this including the nitrogen blanket injection, if used) and the sterilant removal time.

Aeration

Aeration after processing is important for the removal of EO residuals. Temperature (usually between 37°C and 50°C, or 99°F and 122°F), dwell

time, rate and number of air changes, air flow rates and patterns (conditioned by the loading characteristics) will affect the EO diffusion from the product load. Different aeration technologies have been reported, such as pulsed vacuums post-process and heat addition, steam addition and removal, combinations of different gases and pressure set points, and newer developments, such as microwave desorption.

Packaging

The product packaging should be permeable to gas and humidity, should allow aeration after cycle completion and should be capable of tolerating vacuum/pressure differentials and evacuation/pressurization rates. The material itself, the layers of packaging (number of barriers) and the material density influence permeation.

Device

The type of materials, complexity and design of the devices influence the EO and humidity penetration.

Load

The load density and configuration influences the EO and thermal diffusion.

Microbiological contamination

It is important to keep the cleanliness of the device itself and of the packaging under control. An environmental monitoring program should be established to monitor the cleanliness levels (Ernest, 1973; Booth, 2000; Rogers, 2005; Mendes *et al.*, 2007; AAMI TIR 17, 2008; ANSI/AAMI ST 41, 2008; AAMI TIR 15, 2009; AAMI TIR 16, 2009).

4.5 Process definition

The main goal of cycle development studies is to attain a desired microbial lethality in the product, while maintaining its functionality and safety, as well as package integrity. These studies may be conducted in a small development vessel or in a large production chamber. The use of a research sterilization vessel provides a more effective process control and easier and faster sample removal (Booth, 2000; ISO 11135-1, 2007; AAMI TIR 17, 2008; AAMI TIR 16, 2009). BIs provide a unique direct measure of the process lethality. The bacterial spore, especially *Bacillus subtilis niger* (reclassified as *Bacillus atrophaeus*), is the most resistant microorganism and consequently it is the recommended BI (Mendes *et al.*, 2007; ANSI/AAMI ST 41, 2008).

4.5.1 Lethality modeling

The mathematical modeling of the EO sterilization cycle allows the definition of optimal inactivation conditions, which is particularly important for industry. The accurate prediction of D-values and process times, required for a target SAL, allows cycle times and/or EO concentration reduction, as well as the comparison of effectiveness and equivalency of different sterilization processes. Furthermore, lethality modeling contributes to process efficiency and flexibility, and the parametric release is much more scientifically supported (Mendes *et al.*, 2007).

To integrate mathematically the dynamic temperature and concentration conditions effects on inactivation, Rodriguez *et al.* (2001) developed the following model for BI spores of *Bacillus subtilis niger*:

$$N(t) = \frac{N(t=0)}{e^{\left[k_{T_R}\int_0^t C(t)^n \times 10^{\frac{T(t)-T_R}{z}} \mathrm{d}t\right]}} \qquad [4.1]$$

where $N(t)$, $C(t)$ and $T(t)$ are the number of survivors, the EO concentration and the temperature at time t, respectively; k_{T_R} is the rate constant at a finite reference temperature T_R; z is the temperature increase required to reduce the decimal reduction time (D-value) by 90%; n is a model parameter.

The model was validated under the following conditions: 15–90% of RH, 200–1200 mg/L of EO, and z-value of 29.4°C/84.9°F. The same authors also deduced an expression for determining the accumulated lethality of an EO sterilization process (Rodriguez *et al.*, 2001):

$$F_{T_R,C_R,z} = \frac{1}{C_R^n}\int_0^t C(t)^n \, 10^{\frac{T(t)-T_R}{z}} \mathrm{d}t \qquad [4.2]$$

where C_R is a reference EO concentration; F is the exposure time at T_R and C_R that would cause the same lethal effect as the $T(t)$ and $C(t)$ temperature conditions (i.e. equivalent process time).

Mosley *et al.* (2002) deduced an alternative model for equivalent process time prediction:

$$F_{C_R,T_R} = \left(10^{\log t_{T_R}}\right)\frac{C}{C_R} \qquad [4.3]$$

For varying EO concentration and/or temperature conditions, the equation would be:

$$F_{C_R,T_R} = \sum_{i=1}^{m} F_i = \sum_{i=1}^{m} \left[10^{\left[\log t_{T_i} + \frac{1}{z}(T_i - T_R) \right]} \right] \frac{C_i}{C_R} \qquad [4.4]$$

where i is the process step in which the EO concentration and temperature are constant and m is the total number of process steps.

The mathematical models above presented are essential for designing EO sterilization processes. Optimization and validation of the different methodologies are a requirement (Mendes *et al.*, 2007).

4.5.2 Microbiological methods

The three approaches for microbial lethality assessment are: overkill, BI/bioburden and bioburden. By this order, the complexity and the work for cycle definition increases, but usually the process time decreases.

The selection of the method for estimation or calculation of the cycle lethality is also part of the process, using either the fraction-negative or direct enumeration method (also known as survivor curve method). Fraction-negative analysis involves running sterilization cycles in which some, but not all, of the BIs are inactivated. The proportion of viable and non-viable BIs allows D-value calculation by Holcomb-Spearman Karber (HSK), Limited HSK (LSKP) and Stumbo Murphy Cochran (SMC) methods. The enumeration method consists of counting the surviving organisms on each BI, using a serial dilution/plate count method. For both situations, the bioburden recovery and sterility test methods should be validated to ensure recovery of injured organisms (Booth, 2000; ISO 11135-1, 2007; AAMI TIR 17, 2008; AAMI TIR 16, 2009).

Evaluation of product bioburden

An understanding of the viable microbial population on a device (bioburden) is necessary and required to support the validation process. The following methods have been used to estimate the natural bioburden on the product and its resistance compared with the BI:

- When microbial identifications are performed, the D-values can be obtained from the literature. The time required to inactivate the bioburden can be directly compared with that of the BI.
- When microbial identifications are not performed but the bioburden is low (<100), the entire bioburden population would need to have a

D-value 1.5–2 times greater than the BI (in order to present a greater challenge and resistance); this is not supported by the literature.
- When the microbial identifications are not performed and the bioburden is high, the appropriateness of the BI should be determined by exposure to a fractional cycle (and sterility testing), from which BI can be recovered.

The chosen product samples should be representative of the product family having the highest or most resistant bioburden, and several different products can be tested if there is more than one high-bioburden family group (Booth, 2000; ISO 11737-1, 2006).

Overkill method

The overkill approach uses BI data to assess the microbial inactivation rate for a given process. The overkill method is applicable as long as the BI resistance (expressed as D-value) exceeds that of the product bioburden.

Half-cycle approach

Due to its relative ease of use and the robust SAL obtained, the half-cycle approach is the most widely used method to validate MD sterilization by ethylene oxide. In this approach, more than 6 spore log reduction (SLR) of a 10^6 BI (*B. subtilis*) is attained (by achieving sterile BI samples); therefore, if exposure time is doubled, more than 12 SLR (or a 12D process) might occur. The estimation based on equivalent exposure time (U) is recommended, instead of the exposure time (t) to compensate the sterilant injection (including nitrogen blanket, if used) and evacuation time. The following expression can be used for its estimation (Mosley *et al.*, 2002, 2005; Mosley and Houghtling, 2005):

$$U = \frac{t_{\text{injection}}}{2} + t_{\text{nitrogen overlay}} + t + \frac{t_{\text{exhaust}}}{2} \qquad [4.5]$$

assuming a constant rate of pressure increase (in the injection phase) and pressure decrease (in the exhaust phase).

Combined BI/bioburden method

If the product bioburden is routinely tested and if the microbial population is low, then a combined BI/bioburden method can be used for cycle development. This method is based on the assumption that the bioburden is less (or equally) resistant than the BI.

Combined BI/bioburden method defines the treatment extent required to achieve the specified SAL, from knowledge of the BI and of the product bioburden population to be sterilized. The BI/bioburden method is only applicable when there is a high level of confidence that the bioburden data are representative of the 'worst-case' conditions. This method usually results in shorter cycle times and is gaining acceptance as a cycle-optimizing methodology.

Absolute bioburden method

The 'bioburden method' is based on the inactivation of the microbial population in its natural state. The absolute bioburden approach is rarely used, because of the extensive amount of microbiological data required. However, in the following two situations this approach could be the best choice:

- when the natural product bioburden has a higher EO resistance than the BI (high bioburden levels, high intrinsic resistance to the sterilant, the location of the bioburden in/on the device, or a combination of these factors); or
- when the natural bioburden of the product is low and consistent (cycle optimization).

The absolute bioburden approach requires a strong reliance on bioburden levels and resistance to support the continued effectiveness of the sterilization process. This approach requires environmental and process controls to maintain a consistent manufacturing process, a validated bioburden recovery technique and a selection of representative product samples for performing fractional exposure cycles on a regular basis (quarterly is recommended). The absolute bioburden method is not recommended for parametric release (Ernest, 1973; Booth, 2000; Pflug, 2003; Rogers, 2005; ISO 11138-1, 2006; ISO 11138-2, 2006; ISO 11135-1, 2007; ISO/TS 11135-2, 2008; AAMI TIR 16, 2009; ISO 14937, 2009).

4.6 Process optimization and the process challenge device

4.6.1 Process optimization

Cycle design studies play a crucial role in the optimization of the sterilization process, particularly in minimizing the turnaround time required to get the product to market. Mathematical modeling of sterilization and aeration processes allows controlling each phase and, consequently, it is possible to attain the reduction of the overall process time. Additionally, one should

consider the equipment used and the product being sterilized, since these factors dictate the process variables definition.

Traditionally, the process time of EO sterilization is greatly influenced by two operations: microbiological analysis and/or aeration time. The implementation of parametric release eliminates microbiological analysis from routine, which results in significant time-saving. The validation of sterilization and aeration processes, with consequent assessment of EO residues in compliance with the requirements of ISO 10993-7, also allows processing time reduction (Ernest, 1973; Booth, 2000; Pflug, 2003; Mendes *et al.*, 2007; ISO 11135-1, 2007; ISO/TS 11135-2, 2008; AAMI TIR 16, 2009).

The microbiological qualification approaches previously described should also be considered as a part of process optimization, since the attained cycle is influenced by the methodology considered.

4.6.2 Process equivalence

Process equivalence is a method used to assess sterilization by different equipment, minimizing the number of tests required to qualify the process. The particular requirements that should be followed and the studies involved for assuming process equivalence, and consequently a reduced performance qualification (PQ), are described in AAMI TIR 28 (Booth, 2000; AAMI TIR 28, 2009).

4.6.3 Sterilization load and the process challenge device

The sterilization load with the highest density (and with the lowest thermal diffusivity) represents a sterilization challenge (AAMI TIR 16, 2009) and these products are usually elected for EO processing. It is important to analyze the challenge (in terms of lethality) that the devices under consideration present to the sterilization process (Booth, 2000). Similar devices can be grouped into product families. After product families are defined, the most difficult-to-sterilize product in the family, which represents all devices in the group (family representative), should be identified. The master process challenge device (PCD) will be the worst-case product, or representative member, of the multiple-product families and it should be selected to challenge the sterilization process. The PCD packs may be a user-assembled test pack or a commercially available, pre-assembled test pack. Its selection can be done by a sterilization specialist evaluation (considering it estimated resistance to EO sterilization) or after some testing, if it is not clear, which of several products is more difficult-to-sterilize. This testing usually includes a thermodynamic (temperature and humidity response of the load) and a comparative microbial resistance study that shall comprise at least one fractional cycle run.

By placing the BI (e.g. strip, a dot or thread) within the most interior location of the PCD, the sterilization conditions can be defined. This is called the establishment of an EO processing group, because it results from the collection of EO product families (probably with dissimilar devices) that are qualified in a common sterilization process (by the same PCD). All products within this EO processing group should present an equivalent (or lower) challenge to the sterilization process, when compared with the PCD. The product design and complexity, its composition, its microbial load, resistance of product and packaging to the sterilant gas diffusion, pallet density of the product (due to the temperature and absorption characteristics) and the desired SAL must be evaluated.

The same procedure should be followed when adopting a new or altered device (and/or packaging) into an existing validated sterilization process. The currently validated product or PCD would then be used as the basis for comparison with any candidate product. If the candidate product represents a greater challenge to the sterilization process than the PCD, a PQ should be performed in accordance with AAMI/ISO 11135-1:2007. The AAMI TIR 28 is a useful guide for minimizing the risk of introducing a new or modified product that represents a greater challenge to the sterilization cycle than the one previously validated.

External process challenge device

External process challenge devices (EPCD) are placed in the load but externally to the product, and are often used in routine processing to facilitate retrieval from the load after sterilization. An EPCDs resistance should be considered against the product bioburden that is being sterilized and the internal PCD (IPCD) because it should represent the most difficult-to-sterilize product within the load.

The EPCD selection can be performed during cycle development and/or validation because it serves as a surrogate for the IPCD by demonstration during fractional exposures of a resistance greater than or equal to that of the IPCD (Booth, 2000; Rogers, 2005; ISO 11135-1, 2007; ANSI/AAMI ST 41, 2008; ISO/TS 11135-2, 2008; AAMI TIR 28, 2009; AAMI TIR 16, 2009).

4.7 Qualification of ethylene oxide (EO) sterilization

Specific guidelines for validation of the sterilization processes, which includes physical and microbiological PQ, are developed and published by AAMI in conjunction with ISO. The validation of EO sterilization processes is described in detail in ISO 11135 (2007).

4.7.1 Protocol

A protocol, which outlines the overall validation requirements, must be prepared. The protocol should describe the MD and should specify the procedures to be followed during process validation and acceptance criteria (Booth, 2000; Rogers, 2005; ISO 14161, 2009).

4.7.2 Final report

A final report should compile all data, process conditions and test results that support process assessment.

4.7.3 Installation qualification and operational qualification

This topic will not be covered since the basis for its development is analogous to other similar processes.

4.7.4 Performance qualification

The performance qualification (PQ) consists of rigorous microbiological and physical testing to demonstrate the efficacy and reproducibility of the sterilization process. The microbiological performance qualification (MPQ) assures that the required lethality for the product/load combination in the sterilizer is achievable. The physical performance qualification (PPQ) is useful in defining reproducibility criteria while assuring product or package integrity. The PQ should be performed in the production chamber, setting one or more process variables (temperature, humidity and EO concentration) at or below the minimum production routine levels, reducing the time in the preconditioning area, increasing the chamber loading time and the cycle starting time. This procedure assures safety of the sterilization cycle (Booth, 2000; ISO 11135-1, 2007; AAMI TIR 16, 2009).

The ISO 11135-1 (2007) provides recommendations for preparing, placement and handling of PCDs or worst-case products, test samples and temperature and humidity sensors, and their minimum number (depending on the vessel size). In addition, the minimum number of PCDs depends on the MPQ method chosen. The minimum number of cycle runs is also described in ISO 11135, for each specific method.

One should consider the representative product locations/sites through the load that challenge the sterilization process (i.e. the most difficult-to-sterilize locations) to ensure that a required SAL is attained (ISO 11135-1, 2007; AAMI TIR 16, 2009).

4.7.5 Routine monitoring and control

After validation of the sterilization process, adequate procedures to be routinely followed must be defined. Specifications must describe the sterilization process aspects necessary to assure conformance with the validated cycle and to be maintained with an established change control procedure. The conformity with the specified process parameters must be attained; otherwise, product cannot be released as sterile (ISO 11135-1, 2007).

The conventional traditional release method requires that the process parameters are within the validated tolerance and that the BIs exposed to the sterilization process are inactivated. The parametric release is exclusively based on the recording and evaluation of the process parameters, since the equipment potentialities are enough to evaluate the impact of process parameters on microbiological inactivation (AAMI TIR 20, 2001). The physical monitoring provides real-time assessment of the sterilization cycle parameters and it is essential to detect the eventual malfunctions early, so that appropriate corrective actions can be taken (ANSI/AAMI ST 41, 2008).

4.7.6 Parametric release

Parametric release is the assessment of sterilization adequacy based on physical parameters measurement. If a sterilization cycle operating within specified tolerances has been shown to be both effective and reproducible, confirmation that the process parameters were within tolerance is taken as evidence of cycle reliability. The requirements for validation and routine release are more stringent for parametric release. These requirements are outlined in ISO 11135 (2007) and guidance can be found in AAMI TIR 20 (2001). The direct analysis of humidity during conditioning and EO concentration during sterilant exposure time are key parameters (Booth, 2000; AAMI TIR 20, 2001; ISO 11135-1, 2007). This procedure enhances operational efficiency and is also of economical interest for the healthcare market, since it decreases the running costs.

4.7.7 Maintaining process effectiveness and requalification

A periodic requalification study is recommended at least every two years and, preferentially, every year. If no substantial changes occurred in the process or materials, a documented evaluation review may be sufficient to verify that nothing that would affect the process has changed. Some specialists recommend a confirming cycle (in addition to the paperwork) to increase

the reliability of this evaluation. This review should also demonstrate that the resistance of the product bioburden has not increased to a level that would invalidate the use of the PCD or compromise the SAL claim of the process.

For parametric release, revalidation must be performed annually. Additionally, requalification should be conducted after relocation, any major redesign of the sterilizer, sterilizer malfunctions and major repairs (Booth, 2000; ISO 11135-1, 2007; ANSI/AAMI ST 41, 2008; AAMI TIR 16, 2009).

4.8 Contract sterilization

Contract sterilization continues to grow, mainly due to the increased requirements related to EO sterilization. The responsibility for sterility is shared by the MD manufacturer and the contract sterilization facility. Therefore, it is essential that the division of responsibilities is clearly defined and understood by both parties to ensure a well-controlled sterilization process.

AAMI TIR 14 provides additional guidance on this topic and, in particular, gives guidelines for manufacturers' selection of a sterilization facility and for the written agreement that must be established between product manufacturer and contract sterilizer. This written agreement should define the responsibilities of each part related to the sterilization process and should establish the handling procedures to be adopted (AAMI TIR 14, 2009).

4.9 Acknowledgments

The authors acknowledge Bastos Viegas, SA, for supporting research on ethylene oxide sterilization processes.

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5

Non-traditional sterilization techniques for biomaterials and medical devices

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Abstract: The toxicity of ethylene oxide (EO) and the increasing use of heat-sensitive materials in medical devices has led to the development, within the past 15 years, of a number of low-temperature sterilization alternatives. These non-traditional sterilization methods will be the subject of the present chapter. Methods include hydrogen peroxide gas plasma, low-temperature steam formaldehyde, ozone and immersion in peracetic acid solutions and are already currently used in healthcare centers. Other alternative methods such as X-rays, chlorine dioxide, or high-intensity light are briefly described at the end of the chapter.

Key words: low-temperature sterilization, hydrogen peroxide gas plasma sterilization, ozone, steam formaldehyde.

5.1 Introduction

5.1.1 The limits of conventional sterilization methods

The preceding chapters have described the traditional sterilization methods – that is, methods that have a long history of safe and effective use as demonstrated by ample literature, clearances and validation. They include moist and dry heat, radiation (e.g. gamma, electron beam) and ethylene oxide (EO) gas sterilization.

As detailed in these chapters, none is an ideal sterilization technique. While steam sterilization combines many advantages, it is unsuitable for many polymers, due to high temperatures and humidity rates attained during the process. More generally, medical devices are getting smaller and more fragile, with complex geometries, and often include polymeric compounds (such as coatings or adhesives) that require low-heat sterilization processes. Radiation sterilization is not suitable for healthcare centers. Moreover, damage to polymers rapidly increases with radiation dose, thus preventing the use of this method for reprocessing medical devices. EO gas has been used since the 1950s for heat- and moisture-sensitive medical devices. However, several constraints have led to the development of alternative technologies for low-temperature sterilization in the healthcare setting. First, the use

of chlorofluorocarbon (CFC) as stabilizing agent in EO sterilization was banned under the Clean Air Act in 1995, forcing the development of 100% EO or EO combined with a different stabilizing gas such as HCFC and CO_2. These have been already discussed in Chapter 4.

More generally, EO sterilization has, despite its advantages, some major limitations. The first is EO high toxicity, which induces risks for staff workers as well as for patients. The Occupational Safety and Health Administration (OSHA) regulates the acceptable vapor levels of EO (i.e. 1 ppm averaged over 8 h) due to concerns that EO exposure represents an occupational hazard (Weber and Rutala, 1998). More recently, some countries such as Canada and a few states in the USA (e.g. California, New York, Michigan) require the use of EO abatement technology to reduce the amount of EO being released into ambient air. In addition, EO is absorbed by polymeric materials and requires a long aeration, making the cycle long and not appropriate for some devices such as endoscopes.

Altogether, the toxicity of EO and the increasing use of heat-sensitive materials in medical devices has led to the development, within the past 15 years, of a number of low-temperature sterilization alternatives. These include hydrogen peroxide gas plasma (HPGP), low-temperature steam formaldehyde, ozone (O_3), immersion in peracetic acid or formaldehyde, chlorine dioxide, X-rays, etc. These non-traditional sterilization methods will be the object of the present chapter.

Among these methods, some of them have already a relatively long history. This is the case of HPGP, ozone and immersion in peracetic acid, which have been cleared by the FDA. Low-temperature steam formaldehyde is also commonly used in Europe (but not in North America). Other technologies are more recent, such as vaporized hydrogen peroxide, vapor phase peracetic acid, gaseous chlorine dioxide, X-rays or pulsed light (Rutala and Weber, 2001a; Rutala, 2008).

5.1.2 FDA classification in non-traditional and novel non-traditional sterilization methods

In the USA, the FDA recently proposed to separate non-traditional sterilization methods in two different categories (CDRH, 2008). This separation comes from the fact that, in recent years, the FDA has received an increased number of 510(k)s for devices labeled as sterile that use non-traditional sterilization methods in their manufacture. While the FDA has experience with some types of non-traditional methods of sterilization, it is considered that others are still very new and unknown and that they carry a substantial risk of inadequate sterility assurance. Failure to assure sterility, of course, presents a serious risk to human health because of the risk of infection. Therefore, the FDA intends to inspect the manufacturing facility before clearing a 510(k) for a device that is sterilized by a novel non-traditional sterilization process.

The FDA recognizes three categories of sterilization methods currently used to sterilize medical devices in manufacturing settings – traditional, non-traditional and novel non-traditional. These latter two categories are defined as (CRDH, 2008):

> Non-traditional sterilization methods: methods that do not have a long history of safe and effective use and for which there are no FDA-recognized standards, but for which published information on validation of these methods exists and for which FDA has previously evaluated data as part of a QS evaluation and determined the methods to be adequate. This category is limited to:
>
> a. Hydrogen peroxide gas plasma
> b. Ozone.
>
> Novel non-traditional sterilization methods: newly developed methods for which there are no FDA-recognized standards, there is no FDA inspectional history, or there is little or no published information on validation, and for which there is no history of comprehensive FDA evaluation of sterilization validation data. A novel non-traditional sterilization method is also a method that has not been evaluated by FDA as part of a QS evaluation and that employs sterilization methods that FDA has not reviewed and determined to be adequate to provide reasonable assurance of safe and effective use. Novel non-traditional methods include:
>
> a. Chlorine dioxide (ClO_2)
> b. Ethylene oxide-in-a-bag (EO-in-a-bag, diffusion method, or injection method). This method differs from traditional EO methods in that EO-in-a-bag specifies a volume of EO instead of a concentration (e.g. 7.2 grains instead of 500–600 mg/L), uses an EO cartridge or capsule, uses humidichips, or uses a long gas dwell time (e.g. greater than 8 h)
> c. High-intensity light or pulse light
> d. Microwave radiation
> e. Sound waves
> f. Ultraviolet light
> g. Vaporized chemical sterilant systems (e.g. hydrogen peroxide or peracetic acid). (CDRH, 2008)

In the present chapter, we will also differentiate between non-traditional techniques which are relatively widely used and well known (presented in Section 5.2) and novel non-traditional methods, for which only a quick overview will be given in Section 5.3.

5.2 Non-traditional sterilization methods

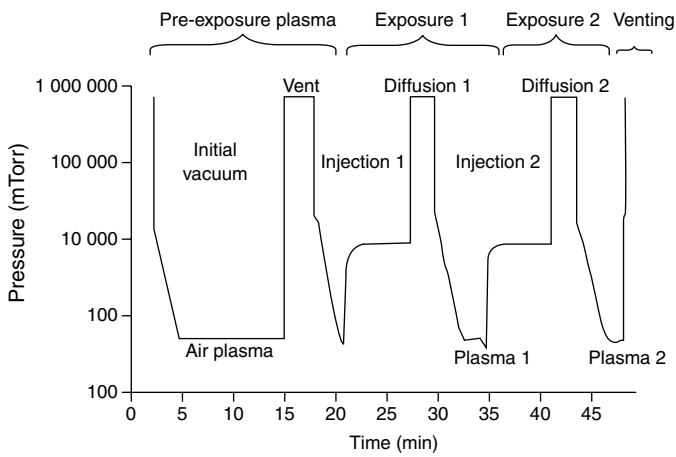
Although the practice varies among countries, the following sterilization technologies can be considered as non-traditional techniques: low-temperature hydrogen peroxide gas plasma, ozone, low-temperature steam formaldehyde and some liquid chemicals. These are detailed below.

5.2.1 Low-temperature hydrogen peroxide gas plasma

Hydrogen peroxide (H_2O_2) gas plasma (HPGP), also called low-temperature hydrogen peroxide gas plasma sterilization (LTHPGP) and marked under the trade name Sterrad® (Advanced Sterilization Products (ASP), Johnson & Johnson, Irvine, CA) is the most widespread novel technology, at least in North America. It is used as an industrial sterilization but also, and more importantly, has encountered much success in healthcare centers, with several thousand hospitals equipped around the world. This process combines an oxidative chemical phase (vaporized hydrogen peroxide, a strong antimicrobial agent), followed by low-temperature plasma, as described below.

Principle

The five phases of the LTHPGP sterilization process consist of vacuum, H_2O_2 injection, diffusion, plasma and vent (Fig. 5.1) (Rutala *et al.*, 1999). The hydrogen peroxide is provided in multi-dose cassettes containing ten single doses of liquid (nominal) 59% hydrogen peroxide which becomes vapor when injected under vacuum. After vacuum and diffusion of H_2O_2 for a certain period of time (which varies between the available systems), an electromagnetic field is created in which the hydrogen peroxide vapor breaks apart, producing a low-temperature plasma cloud. Very succinctly, a cold plasma (sometimes called the fourth state of matter) is a partially ionized gas at reduced pressure, comprising ions, electrons, ultraviolet light and reactive neutrals such as radicals, excited and ground-state molecules. H_2O_2 produces reactive species, such as hydroxyl and atomic oxygen radicals,



5.1 Sterrad® 100S sterilization cycle. (*Source*: Figure published with permission of Advanced Sterilization Products, a Division of Ethicon Inc., a Johnson & Johnson company.)

ultraviolet light, etc., which attack the cell membrane, DNA and enzymes (Holler *et al*., 1993; Crow and Smith, 1995).

Following the reaction, the activated components lose their high energy and recombine to form oxygen and water. The measured efficacy of the Sterrad®100 process was reported to be above all due to hydrogen peroxide, the chemical agent that is left to diffuse during 50 min, prior to its destruction by the action of plasma exposure. However, gas plasma can further kill microorganisms and helps in eliminating H_2O_2 residues (Lerouge *et al*., 2002). The first Sterrad® system was cleared in 1993 as an alternative to EO and has known wide success, with several thousand systems in clinical facilities. In several countries it has become the most common low-temperature alternative to EO.

Compared to the first generation (Sterrad® 100), present Sterrad® systems (Sterrad® 100S) now use shorter but repeated cycles, which enable the reduction of total processing time to 52 min compared with 75 min initially (Rutala and Weber, 2001b). Moreover, several systems of different load size are now available: Sterrad® 100NX, Sterrad® 100S, Sterrad® 200 and Sterrad® NX, with various size and cycle duration depending on the need. Cycle duration is about 38 min for the smallest chamber (Sterrad® NX, 30L, conceived for operating rooms and small facilities, see Fig. 5.2) to 75 min for the largest chamber (150 L) (Sterrad® 200) (Rutala, 2008).

Sterrad sterilization can be used on metals, elastomers, silicone and most polymers (Lerouge *et al*., 2000a, 2002). Liquids, oils, powders, cellulose and cotton (or other materials which strongly absorb H_2O_2) and most biological tissues can, however, not be proceeded with this technique.

Advantages and limitations

Hydrogen peroxide gas plasma sterilization is an interesting technology in that it is a fast, low-temperature process. It leaves no toxic residues or by-products. Indeed H_2O_2 is eliminated during the plasma phase. In addition, plasma chemical active species disappear almost immediately after the plasma power is turned off; by-products are water vapor and oxygen. It is, therefore, potentially safer for healthcare workers, patients and for the environment, and this eliminates the need for aeration or ventilation. The complete sterilization cycle is thus much shorter than EO, enabling quick instrument turnaround which decreases the need for duplicate inventories and associated costs in hospital centers. Moreover, in the recent studies conducted and published on the inactivation of prions, the latest technology (Sterrad® 100NX™ and Sterrad® NX™) has been proven to be more effective than steam at the 134°C/18-min cycle (Rogez-Kreuz *et al*., 2009). Although this needs further confirmation, this would represent significant advancement in the fight against an incurable disease, Creutzfeld-Jakob

5.2 Sterrad® NX small chamber for the operating room. (*Source*: Figure published with permission of Advanced Sterilization Products, a Division of Ethicon Inc., a Johnson & Johnson company.)

Disease (CJD), and its latest variant (vCJD) sometimes referred to as the human version of Mad Cow Disease.

The limited penetrability of vaporized and plasma-reactive species into the innermost areas, lumens and packaging materials is one main limitation of this technology. This limits the size of each sterilization load, and impairs the efficiency for some devices, especially those with small diameter/length ratio of lumens.

Penetration of H_2O_2 into long or narrow lumens was first addressed outside the USA by the use of a diffusion enhancer – that is, a small, breakable glass ampoule of concentrated H_2O_2 that is inserted into the device lumen and crushed immediately before sterilization (Alfa, 1997). In Canada, Sterrad®100 and 100S were approved without restriction (Alfa *et al.*, 1996). The diffusion enhancer was, however, not FDA cleared and sterilization of flexible endoscopes was not allowed (Crow and Smith, 1995; Rutala, 2008). However, much progress has been made in this regards since the first generation. For example, the Sterrad® 100NX (152 L) can now process endoscopes, including delicate da Vinci 3-D endoscopes and also single-channel flexible endoscopes. One specific cycle permits sterilization of up to two

single-channel flexible endoscopes, as long as the lumen is made in stainless steel, Teflon® or polyethylene and that the length/diameter ratio stays below a certain limit specified by the manufacturer (about 1 for 500 mm length). Other limitations include the relative high cost of Sterrad® sterilization systems and operation (due to the cost of H_2O_2 ampoules). There is no significant difference with EO on this point.

Finally, Sterrad® is incompatible with several materials (including cellulose and cotton, as discussed above) and material damage, especially of polymers, can be induced by oxidative species (Lerouge *et al.*, 2000a). Little is known about this issue compared with the numerous data acquired regarding alterations by radiation or heat sterilization. Polyacetal and nylons may have limited life. Paper, cellulose and linen absorb H_2O_2 and cannot be processed by this technique. Despite these limitations, Sterrad® systems' efficiency and safety have been tested on thousands of biomedical devices.

Yet, care must be taken since some surface modification of biomedical devices sterilized by Sterrad® is practically assured. Hydrogen peroxide is known to be a strong oxidizer. Moreover, gas plasma is well known for its ability to modify solid surfaces, via etching, deposition and surface modification reactions which depend on the design and plasma parameters. Ion bombardment is probably limited in Sterrad® systems, but oxygen-containing plasmas are known to oxidize polymeric surfaces, and enhance their wettability and adhesion properties. Surface modifications are especially of concern when instruments are reprocessed, since alterations are generally cumulative. Nowadays, there is an increasing need to reuse biomedical devices for economic reasons: in many countries, reuse of 'single-use' devices (e.g. catheters) is frequently practiced in hospitals as a cost-saving measure, even though these polymeric devices are a priori quite sensitive to sterilization and cleaning procedures. It is, therefore, of major importance to be aware of possible material alterations induced by any given sterilization technique.

Plasma sterilization

Hydrogen peroxide gas plasma sterilization should not be confused with plasma sterilization. Indeed, as stated above, Sterrad® efficiency to destroy microorganisms relies much more on the H_2O_2 phase. In the 1990s, another sterilizer using plasma technology (combined with peracetic acid) was commercialized, namely the Plazlyte™ Sterilization System (AbTox Inc., Mundelein, IL). It was, however, removed from the marketplace because of reports of corneal damage to patients when ophthalmic surgery instruments had been processed in the sterilizer (Duffy *et al.*, 2000).

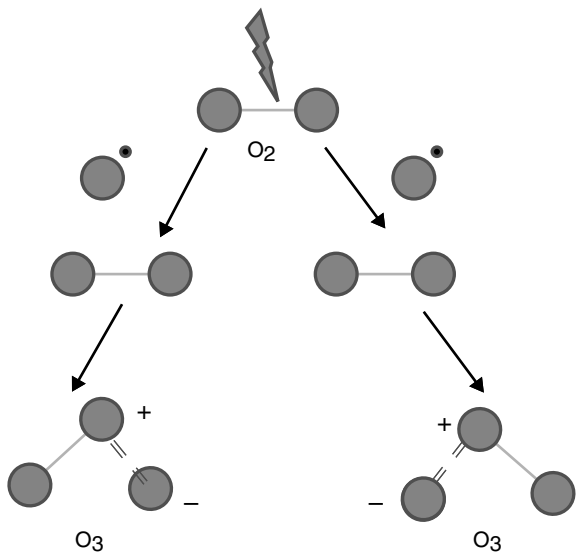
However, more generally, low-pressure plasmas have been able to kill microorganisms, very rapidly (Lerouge *et al.*, 2000b; Moisan *et al.*, 2001; Kylian *et al.*, 2006). It is a promising technology in that it acts rapidly, does

not leave toxic residuals on processed parts or in the exhaust gas, and the temperature of a substrate usually does not exceed 60°C. This field is rapidly moving forward, for sterilization as well as for cleaning processes (Baxter *et al.*, 2005; Kylian *et al.*, 2006; Halfmann *et al.*, 2007). However, the complexity of the process and the relatively low penetration of plasma-active species are also important limitations. Chapter 6 is devoted to plasma sterilization.

5.2.2 Ozone sterilization

Another interesting alternative to EO is ozone (O_3) sterilization. Ozone is a strong oxidative gas, with a characteristic pungent odor, that chemically alters and inactivates numerous chemical contaminants and pathogens (Kim *et al.*, 1999). Ozone is produced when O_2 is energized and split into two monatomic molecules. The monatomic oxygen molecules then collide with O_2 molecules to form ozone, which is O_3 (Fig. 5.3). Its oxidation potential (E_H^0 = 2.07 V) is greater than that of hypochlorite acid (–1.49 V) or chlorine (–1.36 V).

O_3 can be artificially produced by the action of high-voltage discharge in air or oxygen. It has been used for decades for water decontamination (Murphy, 2006; Dufresne *et al.*, 2008), as well as for air and food sterilization. An ozone sterilizer for medical devices, the STERIZONE 125L system marketed by Technologies of Sterilization with Ozone Inc. (TSO_3, Québec, Canada) has

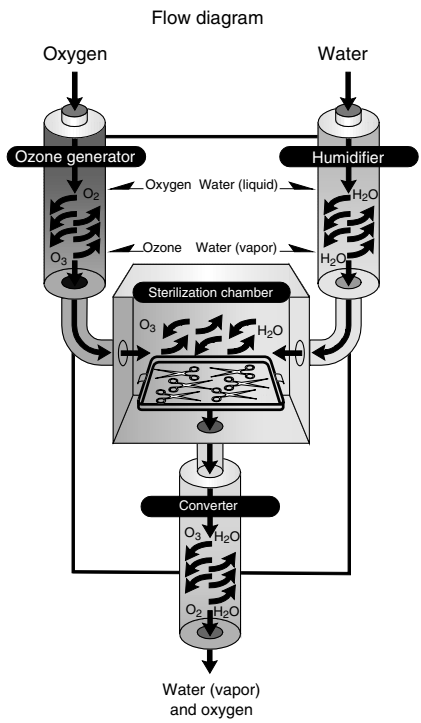


5.3 Schematic representation of O_3 production from O_2.

been licensed by Canada in 2002 and by the FDA in 2003. Ozone is created in an enclosed ozone generator by passing oxygen through an electrical field which converts O_2 into O_3 (Fig. 5.4). In the 125L system, the sterilization cycle lasts about 4.5 h and is divided into two identical half-sterilization periods, where a vacuum is created, followed by humidification of devices and generation of ozone. The ozone produced is measured by an in-line ozone monitor, ensuring proper sterilant quality. After exposure to two ozone cycles, ventilation is carried out to remove O_3 from the chamber and wrappings.

Ozone safety

Excessive exposure to any sterilant can be a health and safety hazard. OSHA has established a short-term exposure limit of no greater than 0.3 parts per million (ppm) over a 15 min period, and an exposure limit of no greater than 0.1 ppm as an 8 h time-weighted average. However, safety issues with ozone are much reduced compared with EO. Indeed, the human nose can detect ozone at levels of approximately 0.003 ppm, so that technical staff would be aware of ozone in the environment long before a hazard exists. Because the ozone sterilizer produces its own sterilizing agent, it avoids transportation



5.4 Principle of ozone sterilizer. (*Source*: Figure published with permission of TSO_3 Inc., Quebec, Canada.)

or physical contact of the sterilant with technicians. Moreover, ozone has a short half-life and tends to recombine easily in the form of O_2. Residues on medical devices are therefore not an issue. In addition, because the sterilizer creates a negative pressure chamber during the processing cycle, any leaks would enter the chamber and the ozone would be diluted before entering the environment. The manufacturer, however, recommends that the sterilizer unit be placed in a room with at least ten air exchanges per hour.

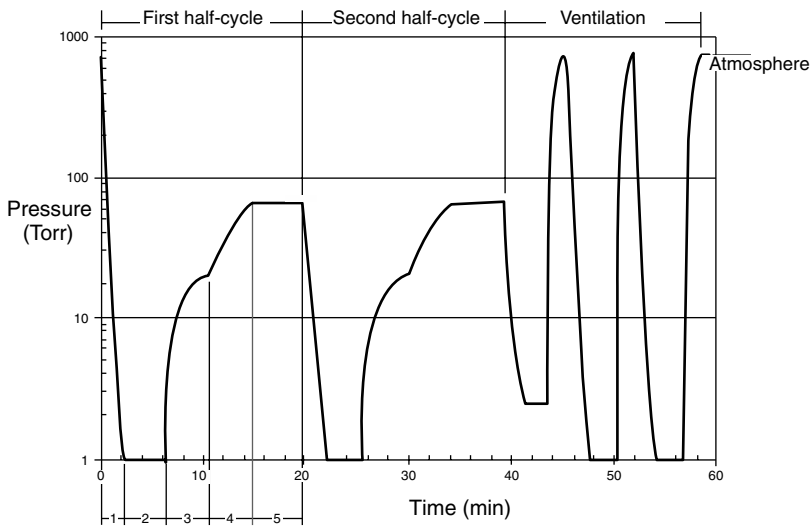
Advantages and limitations

Main advantages of ozone sterilization are low-temperature (temperature range of 30–36°C), safe and easy use and absence of toxic by-products or residues so that it is safe for patients and environment. Moreover, since operation requires only water, oxygen and electricity, which are readily available in hospitals, operation costs are very low, compared to EO or H_2O_2 gas plasma. Ozone required for one sterilization cycle costs a few cents, compared with EO sterilant (about US$10 each) and H_2O_2 for Sterrad® (US$12 each). Another economic advantage compared with EO arises from the ability to release and use instruments as soon as they complete the sterilization process, requiring a smaller instrument inventory. However, the sterilization cycle of the STERIZONE is significantly longer than that of Sterrad® (4.5 h and < 1 h, respectively).

According to preliminary testing by the National Research Council Canada in Winnipeg, the ozone sterilization process from TSO_3 Inc. (Technologies of Sterilization with Ozone Inc. – TSX: TOS, TOS.WT) shows potential for prion inactivation. The technology has also undergone testing in Canada against the non-human TSE agent called 'scrapie'. This effect has not been confirmed yet on a model directly relevant to vCJD in humans, although testing has been carried out since 2009. Ozone has the potential to completely destroy prions because it destroys the proteins and prions are proteins. Recent study on the efficiency of a UV-ozone cleaner concluded that this process can degrade pathogenic prion protein and inactivate prions, even when the agent is associated with surfaces. However, a small amount of infectivity persisted despite UV-ozone treatment (Johnson *et al*., 2009).

The main disadvantage of ozone sterilization is the limited number of devices for which this sterilization process has been cleared to date, partly because of its recent development and partly inherent to the process itself. O_3 is a very strong oxidizer and the humidity within the sterilization chamber is high. These factors may cause natural gum products and some plastics to degrade, and metals to corrode. Items that should not be sterilized by ozone include natural rubber, latex, textile fabrics, but also metals such as copper, brass, bronze, zinc or nickel. Other materials can be sterilized but may be altered by ozone, especially after several sterilization cycles.

Easy recombination of O_3 in O_2 on materials surfaces limits its efficiency in complex geometries and long lumens. The TSO_3 ozone sterilizer has gained FDA clearance for stainless steel devices with rigid lumens of specified diameter and length, but the system is not intended for use in processing any flexible endoscopes. Glass or plastic ampoules or liquids are also not appropriate for ozone sterilization. Finally, to date, it has not been cleared for implants or devices intended to be in contact with human body for more than 24 h. For these reasons, pure ozone sterilizers are not likely to replace EO and the STERIZONE 125L is not under production anymore. However, to overcome these limitations, a new system combining H_2O_2 and ozone has been developed by TSO_3 and commercialized by 3M™ in Europe and Canada under the name of 3M™Optreoz™125-Z low-temperature sterilization system. It is presently under evaluation by the FDA for homologation. H_2O_2 is first introduced in the chamber, then ozone is added (Fig. 5.5). As claimed by the company, a synergistic effect between the ozone and hydrogen peroxide would facilitate sterilant penetration into challenging devices such as long, narrow lumens (Wallace, 2010) and enables use of a significantly lower concentration of ozone compared with STERIZONE; Optreoz process also has a significantly shorter cycle time. The Optreoz sterilizer allows the user to select one of the three



5.5 Sterilization cycle of Optreoz sterilizer, for instruments with rigid channels and rigid channel endoscopes. Complete cycle duration is 56 min. Shorter (46 min) and longer duration cycles (100 min) are also available for general instrumentation and for complex instruments/long multichannel flexible endoscopes, respectively. (*Source*: Adapted with the permission of TSO_3 Inc., Quebec, Canada.)

pre-programmed cycles, ranging from 46 to 100 min, based on the devices to be sterilized. The sterilizer requires no outside venting.

Altogether, this is quite promising since it may result in a process that is more compatible with materials. However, ozone and H_2O_2 may also have a synergistic effect on material degradation. More generally, care must be taken since data on efficiency and safety are not available in peer review publications yet and the system is still under evaluation by the FDA.

Ozone can also be generated by corona discharge, cold plasma and ultraviolet (UV)-ozone devices. In the case of UV-ozone generators, ultraviolet light at two wavelengths contributes to ozone generation (185 nm photons which dissociate O_2 to form O_3 via a radical reaction) simultaneously with contaminant removal from surfaces (254 nm photons) (Vig, 1985). Since UV-based systems produce much less ozone and require substantially longer exposure times than other generators, it is not used for sterilization, but it has been successfully employed for surface cleaning (i.e. to remove carbon from Si microchip surfaces, X-ray optics and samples being prepared for elemental analyses (e.g. spectromicroscopy)) (Johnson *et al.*, 2009).

5.2.3 Low-temperature steam formaldehyde (LTSF)

Low-temperature steam formaldehyde is another low-temperature alternative to EO, which is used in hospitals in several European countries, such as Germany, Holland, UK and Sweden (Rogers, 2006). This physico-chemical process, first proposed by Alder *et al.* (1966) in England, combines a high concentration of formaldehyde gas (about 8–16 mg/L) with sub-atmospheric steam (relative humidity of 75–100%) at lower temperatures than steam (70–80°C, and sometimes even as low as 65°C). The process consists of four stages: (a) pre-treatment (consisting of repeated evacuations and steam flushes to remove air and humidity from the devices); (b) injection of formaldehyde in the form of formalin solution evaporated in the chamber. Steam is then added. The admission is repeated several times to enhance the penetration into lumens and cavities. Dynamic pulsing is required to improve penetration; (c) exposition while maintaining the specified temperature, sterilant concentration, pressure and humidity; (d) post-treatment involving repeated vacuum and steam flushes to remove residual formaldehyde.

Since the temperature stays below 85°C, steam formaldehyde was suggested as a solution to the incompatibility of many materials with high temperatures required during steam sterilization (121°C or 134°C). Formaldehyde is, however, a toxic, irritating, mutagenic and allergenic gas and is classified as a suspected carcinogen in humans. Its immediately dangerous to life or health (IDLH) is of 20 ppm and in USA the permissible exposure limit for formaldehyde in work areas is 0.75 ppm measured as a 8 h time weighted average (TWA),

as regulated by the OSHA. Airborne concentrations above 0.1 ppm can cause irritation of the eyes, nose and throat. This extremely irritating smell of formaldehyde at very low levels tends to prevent exposure to higher concentrations. In any case, the toxicity of formaldehyde may explain why formaldehyde steam sterilization system has not been FDA cleared for use in healthcare facilities and is not used in North America, and more especially why it may not be considered as an ideal alternative to EO. Its use is declining in Europe.

This system has, however, some advantages over EO: the cycle time for formaldehyde gas is faster than that for EO and the cost per cycle is relatively low. Moreover, formaldehyde is readily removed from materials without prolonged aeration, in contrast to EO. However, EO is more penetrating and operates at lower temperatures than steam/formaldehyde sterilizers.

In addition to low-temperature steam formaldehyde, gaseous formaldehyde has been around for many years. Its use is yet even more limited by formaldehyde toxicity, odor and carcinogenicity since, without steam, formaldehyde concentration must be higher. Gas formaldehyde is still used in several countries, mainly in Asia.

5.2.4 Liquid chemicals

Liquid sterilization consists of immersing devices in a chemical sterilant. However, it should be generally considered as a high-level disinfection owing to its efficiency limits and its inability to conserve sterility of packaged products after sterilization (Rutala *et al*., 1998). Indeed, the contact times that would be required to achieve real sterilization (SAL of 10^6) are high, ranging from 3 to 12 h depending on the product. Generally, a shorter processing time is used and only high-level disinfection is achieved. The data indicate that the survival curves for liquid chemical sterilants may not exhibit log-linear kinetics and the shape of the survivor curve may vary depending on the formulation, chemical nature and stability of the liquid chemical sterilant (Favero, 2001).

Another difference between thermal and liquid chemical processes for sterilization of devices is the accessibility of microorganisms to the sterilant since liquids cannot easily penetrate barriers such as biofilm and blood, or penetrate into narrow lumens, for instance. Moreover, with some exceptions, liquid processes can generally not be monitored using a biological indicator to verify sterility (Bond, 1993). Another limitation relates to the post-processing environment of the device. Devices cannot be wrapped or adequately contained during processing in a liquid chemical sterilant to maintain sterility following processing and during storage. Due to these inherent limitations,

the use of liquid chemical sterilants is limited to the reprocessing of critical devices that are heat-sensitive and incompatible with other sterilization methods. The FDA maintains a list of cleared liquid chemical sterilants and high-level disinfectants that can be used to reprocess heat-sensitive medical devices, such as flexible endoscopes (http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/ReprocessingofSingle-UseDevices/UCM133514). These include, among others, solutions and mixtures of glutaraldehyde, peracetic acid, hydrogen peroxide and *ortho*-phthalaldehyde (OPA). A few systems using liquid chemicals have been commercialized and FDA cleared. One of these is the SYSTEM 1 Sterile Processing System (Steris Corporation), which uses a liquid peracetic acid solution (35%) with a proprietary anti-corrosion formulation to sterilize medical, surgical and dental instruments chemically, in particular flexible and rigid scopes. This automated machine was introduced in North America in the late 1980s. In brief, the concentrated peracetic acid is diluted to 0.2% with filtered water at about 50°C, and then circulated within the chamber and pumped through the channels of endoscopes for 12 min, decontaminating exterior surfaces, lumens and accessories. Then, peracetic acid is discarded and the instrument rinsed four times with filtered water, followed by clean filtered air to remove excess water. The conservation of sterility after removal of the machine is, however, still a problem. Moreover, several concerns persist owing to the efficacy of the system, in particular the ability of the filtered water to maintain sterility during rinsing (Muscarella, 2002). Furthermore, care must be taken to connect lumened endoscopes to an appropriate channel connector to ensure that the sterilant has direct contact with the contaminated lumen (Rutala, 2008).

5.3 Novel non-traditional sterilization methods

Other sterilization techniques for healthcare centers are in development and classified as novel non-traditional sterilization methods by the FDA. These will be described only briefly below.

5.3.1 X-rays

X-ray sterilization is emerging as a result of recent increases in the beam power ratings of industrial electron accelerators, which can generate intense X-ray beams (exceeding by far those of common medical X-ray equipment). X-rays are produced by interposing a metal target between the electron beam and the product to be treated. X-rays are more penetrating than electron beam, but less penetrating than gamma rays. They are more costly than gamma processes but will decrease exposure times, require less shielding and shorten turnaround times. The feasibility of radiation processing with

high-energy X-rays has been demonstrated in various industrial facilities in several countries. In Europe, Japan and North America several installations are already equipped for both electron beam and X-ray sterilization. More details on the advantage and limitation of X-rays sterilization can be found in Chapter 3, which covers radiation sterilization techniques.

5.3.2 Ultraviolet (UV) radiation

The wavelength of UV radiation ranges from 328 to 210 nm. Its maximum bactericidal effect occurs at 240–280 nm. Mercury lamps, which emit at 254 nm, are therefore commonly used. However, in contrast to other radiation-based technologies, UV light radiation has very low penetrability. It is limited to the treatment of water (Hall *et al.*, 2003) and surfaces. UV irradiation by germicidal lamps is routinely used to sterilize the interiors of biological safety cabinets between uses, but is ineffective in shaded areas, including areas under dirt.

5.3.3 Chlorine dioxide gas

Another alternative for chemical sterilization is chlorine dioxide gas (ClO_2), an oxidative gas, which is most efficient at temperatures ranging from 25°C to 30°C (Kowalski and Morrissey, 2004). Chlorine dioxide possesses the bactericidal, virucidal and sporicidal properties of chlorine, but, unlike chlorine, does not lead to the formation of trihalomethanes or combine with ammonia to form chlorinated organic products (chloramines). It is also not mutagenic or carcinogenic in humans. It is commonly used for decontaminating surfaces and equipment. The use concentration is usually between 10 and 30 mg/L. The process has been shown to be effective for the sterilization of medical products, is relatively rapid (1.5–3 h) in duration and there is little or no need for post-sterilization. However, this strong oxidative gas also requires pre-humidification and may corrode some materials. Although this technology was first developed in the late 1980s (Jeng and Woodworth, 1990), it has still not been FDA cleared yet (Rutala, 2008), raising questions regarding its efficacy or safety.

5.3.4 Vaporized chemical sterilant systems

To overcome the limitations of liquid sterilization, sterilizers using vaporized hydrogen peroxide (VHP) were proposed in the mid-1980s, using various technologies to transform liquid H_2O_2 (around 30–35% concentration) into vapor and delivering it in the chamber. One method uses a deep vacuum to pull liquid hydrogen peroxide from a disposable cartridge through a heated

vaporizer and then, following vaporization, into the sterilization chamber. In another approach, VHP is brought into the sterilization chamber by a carrier gas such as air using either a slight negative pressure (vacuum) or slight positive pressure. Applications of this technology include vacuum systems for industrial sterilization of medical devices and atmospheric systems for decontamination of large and small areas (French *et al.*, 2004). VHP has several advantages: rapid cycle time (e.g. 30–45 min), low temperature, environmentally safe by-products (only water and oxygen), relatively good material compatibility and ease of operation, installation and monitoring. However, it also has limitations, mainly lower penetration capabilities when compared with EO. It also shares Sterrad® incompatibility with cellulose and nylon. In fact, these systems are very close to Sterrad® systems, without the advantage of elimination of H_2O_2 by plasma, but with the advantage of larger chambers that enables to process more devices at the same time. Further investigation of this method is required to demonstrate both safety and effectiveness. VHP has not yet been cleared by FDA for sterilization of medical devices in healthcare facilities (Rutala, 2008). Similarly, vaporized peracetic acid has also been recently proposed, but is not yet cleared.

5.3.5 Microwaves

Increasing interest in the use of the microwave ovens as a sterilization method has been observed. It has been suggested as a practical physical sterilization method, and its low cost, speed and simplicity have encouraged research to be conducted in several areas. However, there are few publications yet on this subject, and a number of parameters have been shown to influence the efficacy of the process (e.g. presence of water, microwave power). When tested as an alternative for the sterilization of dental instruments, it was concluded that dry heat was the method that least affected the cutting capacity of the carbide burs and that microwave sterilization was not better than traditional sterilization methods (Fais *et al.*, 2009). The use of microwaves is not still recognized by any standards as a sterilization method (Rutala, 2008) and there is no warranty that this type of method will get clearance in the future. However, a new microwave technique has been recently approved for food processing in the USA (http://www.foodproductiondaily.com/Processing/Microwave-sterilization-system-may-revolutionise-food-processing).

5.3.6 Pulsed high-intensity light

Finally, pulsed high-intensity light sterilization (also called white light (PWL), or pulsed light) has been the subject of a number of patents. This technology

involves the pulsing of a high-power lamp (xenon, for instance) (Dunn *et al.*, 1997; Rowan *et al.*, 1999; Wekhof, 2000). Its mechanism is unclear as yet, but UV radiation plays an important role, and this technology seems to encounter the same limitations as UV sterilization, namely the lack of penetration which severely limits the interest in this technology for sterilization of medical devices. However, several papers have shown its potential for surface decontamination, in particular in the field of food products (Demirci and Panico, 2008; Oms-Oliu *et al.*, 2012).

5.4 Conclusions

There is no singular sterilization method that is compatible with all healthcare products including drugs, polymers, devices and materials. Medical devices are getting smaller and more fragile, with complex geometries and polymeric or biological compounds that require low-heat sterilization processes. The last two decades have shown a multiplication of low-temperature processes proposed to replace EO. They all have their own limitations and no ideal sterilization technique presently exists. This means that more attention than ever is required when choosing a sterilization method. The parameters and effects of different sterilization methods must thus be evaluated and reviewed before selecting the proper method. The very new methods may be risky, since all limitations are not well understood to date. In addition, little is known about the effect of these new technologies on biomaterials. To avoid risks, it is recommended, if possible, when designing a new device, to choose materials that are compatible with steam autoclave or radiation. Manufacturers should take advantage of the novel medical-grade materials compatible with high temperatures and radiation.

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6

Sterilization and decontamination of surfaces by plasma discharges

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Abstract: This chapter discusses the possible application of non-equilibrium plasma discharges for the sterilization and decontamination of surfaces. First, the basic properties of electrical discharges are reviewed and a survey of different methods of plasma generation both at atmospheric pressure and reduced pressures is provided. The chapter subsequently discusses the interactions of plasma with diverse biological systems such as bacteria and bacterial spores, endotoxins and proteins.

Key words: sterilization, decontamination, non-equilibrium plasma.

6.1 Introduction

The infections that can be acquired during invasive medical procedures can be attributed to various microorganisms such as *Staphylococcus aureus*, which can be present on the surface of surgical tools and can come into contact with sterile tissues during surgery. Several studies have demonstrated that some of these micro-organisms are becoming increasingly resistant to antibiotics and have a serious impact on the health of the patient (Elixhauser and Steiner, 2007); this underlines the need for improved sterilization procedures. However, recent UK studies (Lipscomb *et al.*, 2006, 2008) have shown that significant quantities of residues composed of salts, proteins and organic matter are left on surgical instruments even after complete cleaning and sterilization in sterile service departments (SSDs). These observations raise serious public health concerns, as other pathogens can also be transferred to the patient by these residues. These pathogens include the prion protein, suspected to be the principal agent for the transmission of Creutzfeld Jakob Disease (CJD); this could therefore potentially be transferred during a surgical intervention carried out with contaminated instruments. The most sensitive operations, in which the risk of transmission is greatest, include brain, spinal and retro-ocular surgeries.

Another concern is related to pyrogens (fever-inducing substances) deriving from gram-negative bacteria (lipopolysaccharides (LPS)) or

gram-positive bacteria (lipoteichoic acid (LTA), peptidoglycan (PGN)). These pyrogens, or bacterial endotoxins, can cause sepsis when in contact with the bloodstream and provoke septic shock, a major cause of death among hospitalized patients. Pyrogens are extremely resistant to temperature (Tsuji and Harrison, 1978) and are not removed by conventional sterilization procedures such as autoclaving. No report on the effect of other common sterilization techniques has yet been published to the best of our knowledge.

The above observations indicate that SSDs should treat surgical instruments not only to sterilize them (i.e. by destruction of living microorganisms), but also to decontaminate them (i.e. by removal of inert residues which may provoke adverse reactions in the host organism). We will see in the following sections that these two aspects, although not based on the same mechanisms and treatments, are nevertheless intimately linked.

Sterilization operations must follow strict rules (ISO EN 15883 or NICE guidelines IP196). For instance, the normal procedure followed in SSDs consists of successive treatments in a mechanical washing/disinfection device: they are, first, pre-washed at room temperature to prevent blood coagulation and adhesion of proteins. The instruments are then cleaned and sonicated with an alkaline enzymatic detergent and, finally, washed at high temperature and rinsed. They are then visually inspected after drying and packaged for sterilization, which in most cases is achieved by autoclaving. As mentioned above, these operations are not efficient in completely removing organic residues, and other sterilization methods are also unable to resolve the issue. Moreover, the usual sterilization techniques have several drawbacks, including the potential degradation of fragile instruments (autoclave) by high temperatures; very long operation time due to degassing or toxicity of the reagent used (ethanol, hydrogen peroxide); and very high investment and maintenance costs not adapted to reusable medical devices (γ or electron-beam (e-beam) sterilization). Potential alternatives are thus under investigation, including the use of non-equilibrium plasma discharges: we will see that plasma processes are able to kill bacteria, but are also highly efficient in the removal of organic material, while working at low temperature and using gases with little or no toxicity. Tests carried out in different laboratories showed that many pathogens can be destroyed by non-equilibrium plasma discharges (Lerouge *et al.*, 2001; Moisan *et al.*, 2001; Rossi *et al.*, 2006) and the nature of plasma interactions with microorganisms or biomolecules has been extensively studied. The mechanisms of interaction are very different from those used by the Sterrad® process from Johnson & Johnson, in which the active principle is hydrogen peroxide, the plasma discharge being used only to remove H_2O_2 from the surface more rapidly. In this chapter we present the basic principles of atmospheric- and low-pressure plasma discharges and the different equipment used for their

production, identify the active species produced and discuss their interactions with organic materials, pathogens (protein, pyrogens) and bacterial spores, showing how these mechanisms could be used for sterilization and decontamination of surfaces. The plasma processes for sterilization and decontamination of medical devices are now in an advanced stage of development, and the first decontamination/sterilization industrial reactors based on plasma were installed in pharmaceutical packaging plants in 2010.

6.2 Overview of plasma generation

The word *plasma* denotes in physics quasi-neutral ionized gas – that is, a gas in which a certain fraction of particles is charged. The presence of charged species turns a plasma into a highly conductive gas that responds readily to electromagnetic fields. As a consequence of this, a plasma presents unique properties as compared with solids, liquids or gases and is therefore often referred to as the fourth state of matter.

A plasma is normally generated by supplying sufficient energy to a neutral gas to induce the formation of charged species – electrons and ions. This process proceeds by means of inelastic collisions between energetic species with neutral atoms or molecules or perhaps in collisions with walls surrounding the gas. There are various potential methods of providing a gas with the necessary energy to ionize it. One possible way is based on thermal heating: a typical example of this is a flame, where the energy is produced by exothermic chemical reactions in the molecules. In this case, all the ions, electrons and neutral species constituting the plasma are in a thermodynamic equilibrium (they have equal temperature), and plasmas created in this way are called *thermal plasmas*. However, the temperatures needed to create thermal plasmas are extremely high (e.g. the energy needed to ionize argon is 15.8 eV, which is equivalent to a temperature of approximately 180 000 K), which limits their practical use in many technological applications.

Another way to produce a plasma for technological use is based on the application of an external electric field. The basic properties of such plasmas, which are denoted as *electrical discharges*, as well as different methods for creating them, will be described in subsequent sections.

6.2.1 Basic properties of electrical discharges

Any neutral gas contains a certain amount of charge carriers created, for instance, by the interactions of cosmic rays with the gas. These charged particles are accelerated in an external electric field by the Lorentz force up to kinetic energies sufficient for ionization of atoms or molecules in the gas volume, which happens principally through electron-impact ionization:

$$e + A \rightarrow A^{+} + 2e \qquad [6.1]$$

It can also occur through the emission of new charged particles from electrodes induced by the impact of energetic species. Continuous production of new charged carriers can balance their recombination losses and a steady state can be reached. However, due to the significant differences in masses of electrons and atomic or molecular ions, the electrons reach considerably higher kinetic energy compared with heavier atomic or molecular ions, whose kinetic energy (temperature) remains relatively low and close to the temperature of neutral species. Since the temperatures of electrons and other neutral species (atoms, molecules, radicals) are different, such plasmas are called *non-equilibrium plasmas* or *nonthermal plasmas*.

Moreover, collisions between particles present in a plasma do not lead solely to their ionization. In fact, a significant portion of energy supplied to the plasma is used for excitation of atoms and molecules. The presence of excited species has two important consequences. First, excited species, and particularly long-living metastables, can act as energy carriers: their internal energy can be released when they impact on the surfaces of objects placed into the plasma, which can lead to physical sputtering of their surface, or can contribute to volume ionization by a process known as Penning ionization:

$$A^* + B \rightarrow A^+ + B + e \qquad [6.2]$$

Second, excited species can be the source of intense light emission, which is connected with their radiative transition to lower energetic levels:

$$A^* \rightarrow A + hv \qquad [6.3]$$

Depending on the difference between energy levels of a particular atom or molecule, the radiation can be emitted in the visible (e.g. in the case of nitrogen molecules), UV (e.g. bands of NO radicals) or even VUV (e.g. spectral lines of argon) spectral range.

Furthermore, inelastic collisions between electrons and molecules can also cause their dissociation:

$$e + XY \rightarrow X + Y + e \qquad [6.4]$$

or dissociative ionization:

$$e + XY \rightarrow X^+ + Y + 2e \qquad [6.5]$$

This triggers subsequent chemical reactions leading to the presence of species initially not present in a working gas. Typical examples related to the topic of this chapter are the production of atomic oxygen in plasma sustained in O_2 gas, or of OH radicals in discharges containing water vapor.

Species created in this way can chemically react with the surfaces of objects placed into the plasma, which can cause modifications of their physical or chemical properties, or etching of their surfaces.

Although the processes described above by no means provide a complete description of a plasma (readers interested in further details of electrical discharges are directed to the excellent books by Von Engel, 1964; Raizer, 1991; Lieberman and Lichtenberg, 1994; and the review articles by Braithwaite, 2000; Conrads and Schmidt, 2000; Kogelschatz *et al.*, 1997), they illustrate the key features of plasma discharges that make them highly interesting for a wide range of technological applications in general and for the sterilization and decontamination of surfaces, in particular. They can be summarized as follows:

1. A significant fraction of species in plasma discharges is charged. Such charged species can be further accelerated by an additional electric field to reach energies sufficient for physical sputtering of the treated objects.
2. Plasma discharges can be operated at moderate temperatures (and in some cases even at room temperature) allowing the treatment of heat-degradable materials.
3. A plasma is a potent source of radiation, comprising germicidal UV and VUV photons.
4. In plasma discharges, species not present in the initial working gas can be created. Such atoms, molecules and radicals (i.e. species with unpaired electrons and thus highly chemically reactive) can interact with treated objects, resulting in modification of their properties or in some case in their volatilization.
5. The interactions between an immersed object and the plasma surrounding it are limited to a thin surface layer of the treated object and thus do not induce significant modifications of its bulk properties.

6.3 Plasma generation at low and atmospheric pressures

After the basic characteristics of electrical discharges have been discussed, an overview of the different methods for their production will be provided. Prior to this, however, it is important to stress that the summary given is not intended to be comprehensive, but rather a basic survey of the concepts already successfully used for sterilization and decontamination of surfaces. Furthermore, although the electrical discharges can be operated at both atmospheric pressure and reduced pressures, low-pressure and atmospheric-pressure discharges are substantially different from the technological point of view. The production methods are thus presented separately.

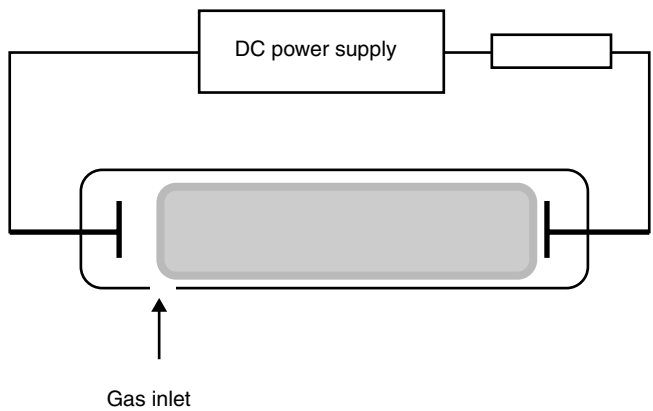
6.3.1 Electrical discharges operated at low pressures

DC discharges

DC discharges are the simplest kind of electrical discharges sustained at low pressures (i.e. fractions of atmospheric pressure). They are typically produced in a closed vessel using a pair of internal electrodes as depicted in Fig. 6.1. In this type of configuration, positive ions created in the plasma volume by electron-impact ionization are accelerated towards the cathode surface in an area known as the cathode fall region. Collisions of these accelerated ions with the cathode lead to the emission of secondary electrons from the metallic electrode, which counterbalance losses of electrons on the vessel walls or on the anode. The operation of DC glow discharge requires a resistor connected in series with the discharge to prevent high currents and thus transition to an arc. Although the possibility of using this kind of electrical discharge for the sterilization of bacterial spores has already been proven (e.g. Soloshenko *et al.,* 2000), it is not often used. There are several reasons for this: relatively high process temperature, high voltage, difficult scalability or the potential deposition of material sputtered from the cathode onto the surfaces of treated objects, which can compromise their properties and functions.

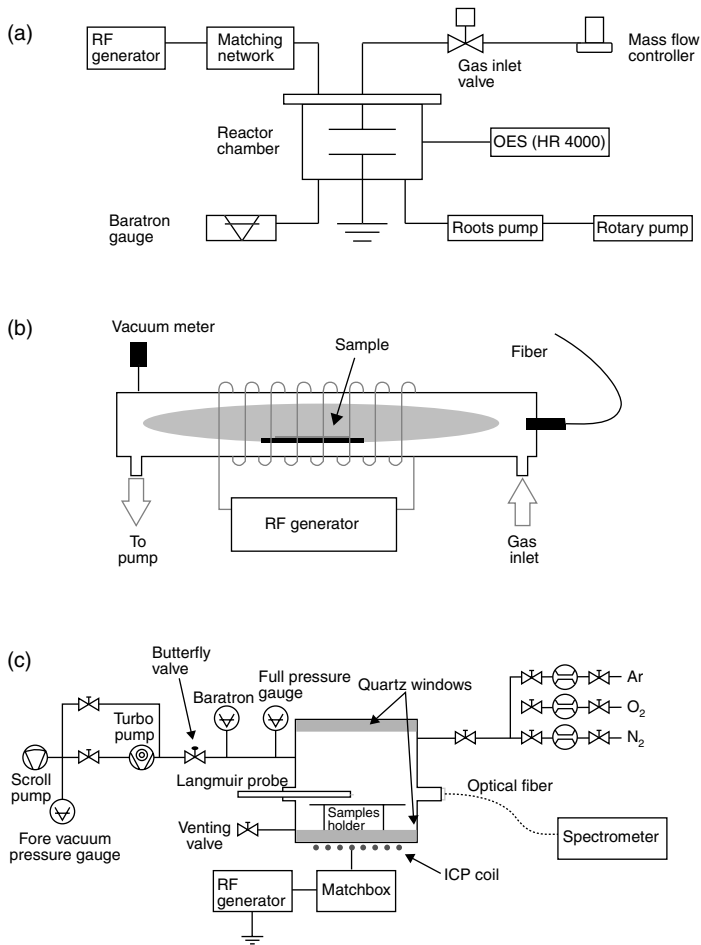
Radio frequency (RF) discharges

The most common low-pressure electrical discharges used for technological applications are radio frequency (RF) discharges that usually operate in the frequency range of 1–100 MHz. The power of an external field can be coupled to the plasma in two different ways: either capacitively or inductively. In the capacitive method, the discharge is sustained between two electrodes that are powered by an RF electrical field as schematically



6.1 DC glow discharge.

depicted in Fig. 6.2a. The electrons are accelerated by this oscillating electrical field, and generate secondary electrons in inelastic collisions with neutral gas. In the inductive mode, the electric current is passed through a coil that can have the shape of a helix (Fig. 6.2b), a spiral or a loop placed into the plasma reactor or outside the plasma reactor (Fig. 6.2c). The RF current creates a time-varying magnetic field around the coil, which in turn induces an electric field in which electrons are accelerated, leading to breakdown and formation of plasma. Independently of the method of power coupling,



6.2 Examples of low-pressure RF plasma reactors: (a) capacitively coupled plasma (Sureshkumar and Neogi, 2009), (b) inductively coupled plasma reactor using helix configuration (Vujošević *et al.*, 2007) and (c) inductively coupled plasma reactor using spiral coil outside the plasma reactor (Stapelmann *et al.*, 2008). OES, optical emission spectrometry; ICP, inductively coupled plasma.

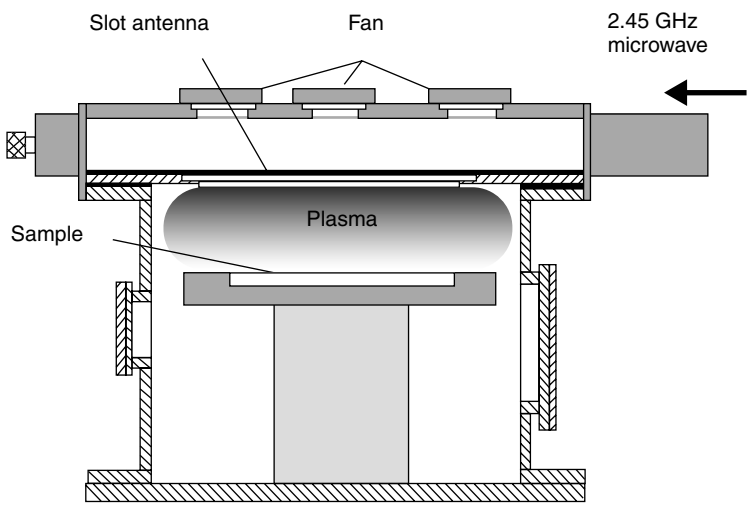
RF discharges require a corresponding network to match the impedance of the power circuit to that of the plasma.

Although capacitively coupled discharges were successfully applied for the decontamination of surfaces (e.g. Whittaker *et al.*, 2004 or Sureshkumar and Neogi, 2009), most studies use inductively coupled discharges (e.g. Kylián *et al.*, 2006a; Halfmann *et al.*, 2007a; Vujošević *et al.*,2007; Liu *et al.*, 2008) since these offer markedly higher plasma densities (i.e. densities of charged particles) and thus also higher treatment efficiency (e.g. Bol'shakov *et al.*, 2004; Rossi *et al.*, 2008).

Microwave (MW) discharges

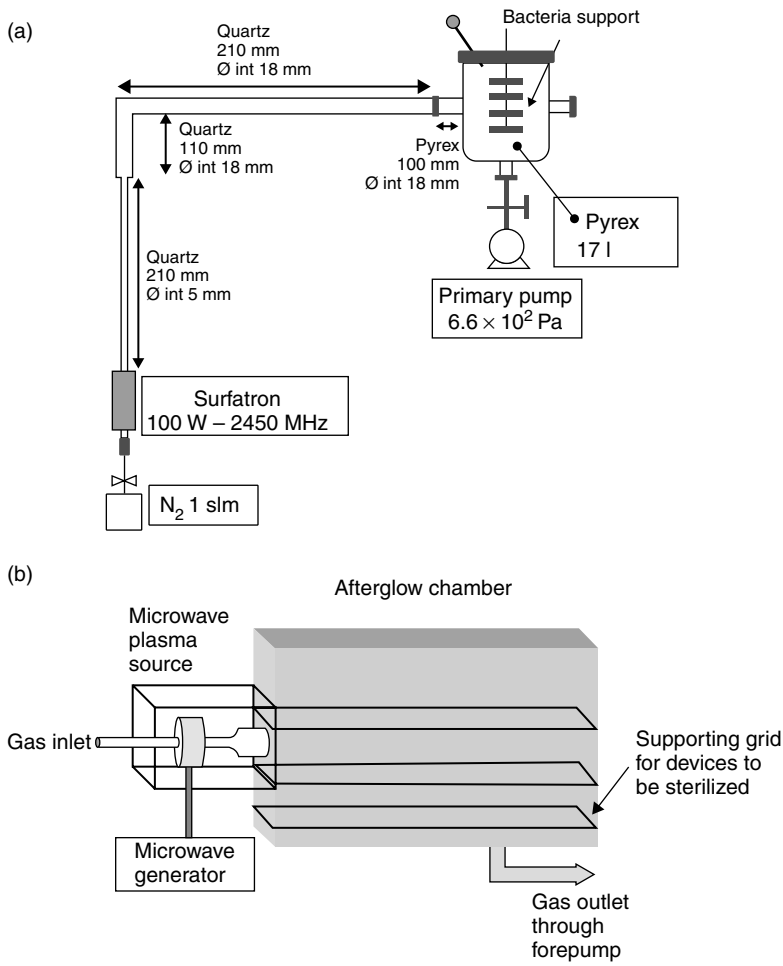
Another type of electrical discharge widely used for the treatment of biological pathogens is a microwave discharge. The main characteristic of this discharge is the short period of its exciting field, which typically has a frequency of 2.45 GHz. Moreover, microwave (MW) plasma and RF plasma produce different electron energy distributions: in discharges operated at otherwise identical parameters, there is a higher population of high-energy electrons in the case of MW discharges, which leads to more effective production of reactive species. A typical example of a microwave plasma reactor used for the sterilization of biological pathogens is provided in Fig. 6.3.

In addition to their application in the direct treatment of surfaces, microwave discharges are also often used as sources of so-called *afterglows*. When the plasma is ignited in a flowing gas, some species produced in the discharge zone can be transported by the flowing gas to a zone, where the external electromagnetic field is not sufficient to sustain a discharge. This



6.3 Example of microwave plasma reactor (Nagatsu *et al.*, 2003).

zone is called *afterglow of plasma* or simply *afterglow*. The region can be still rich in radicals or excited atoms or molecules, but contains relatively few charged particles. The absence of charged species limits their potential adverse impacts on the treated objects. Moreover, afterglows are typically much colder than plasma in the active zone, which reduces the thermal load on the treated objects. These principal advantages of afterglows are counterbalanced, however, by their lower treatment efficiency: the treatment times needed to assure sterility of surfaces exposed to afterglow are thus markedly higher as compared with treatments performed in the active plasma zone (Rossi *et al.*, 2009). Typical set-ups using afterglow plasma are presented in Fig. 6.4.



6.4 Examples of microwave post-discharge plasma reactors: (a) Cousty *et al.*, 2006, (b) Moisan *et al.*, 2002.

6.3.2 Electrical discharges operated at atmospheric pressures

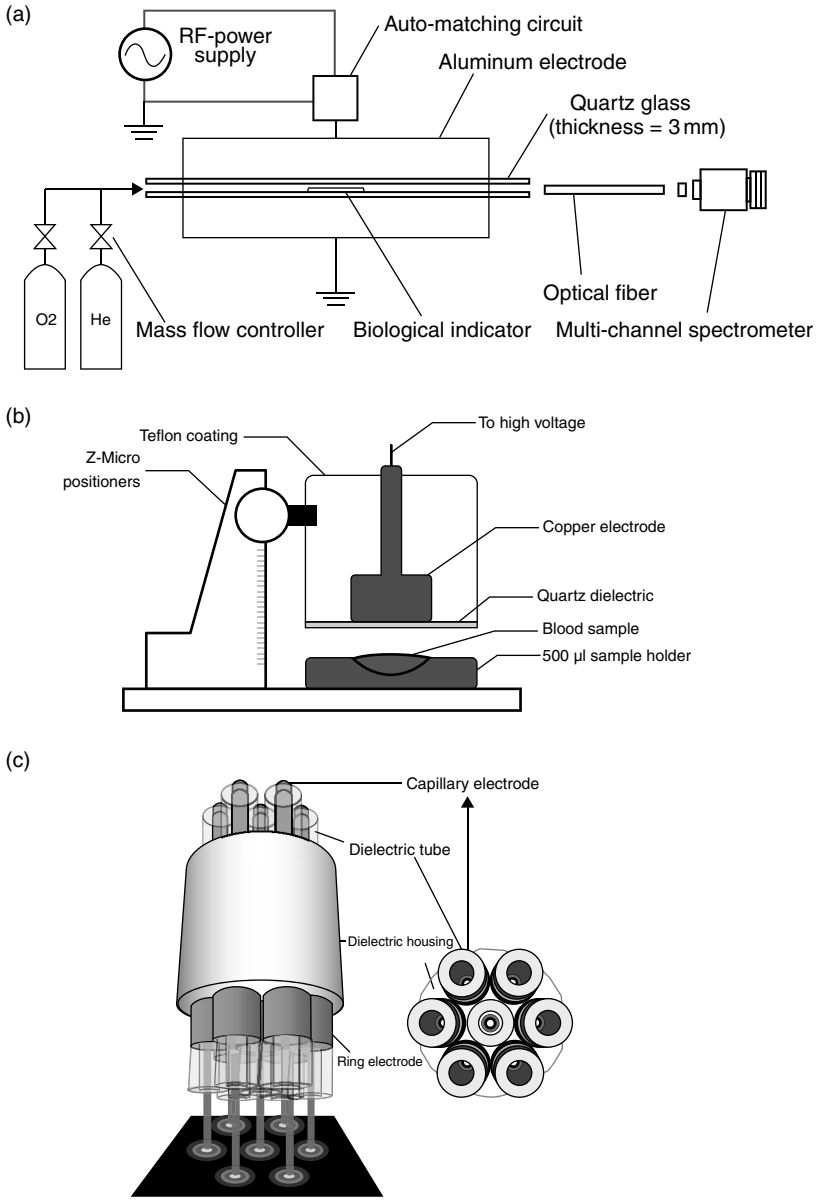
Up to this point, we have only considered electrical discharge operated at low pressures. Nevertheless, recent developments in the field of discharges operated at atmospheric pressures have opened up the possibility of using these discharges for the sterilization and decontamination of surfaces. This is currently attracting increasing attention, due to the fact that electrical discharges at atmospheric pressures offer several operational advantages: they do not require vacuum equipment and there is no idle time connected with the pumping down to operational pressure. Moreover, the mild conditions produced by atmospheric discharge could allow them to be used for the treatment of living tissues. Here we present three basic concepts already extensively used either for sterilization of bacterial spores or for removal of protein films.

Dielectric barrier discharges

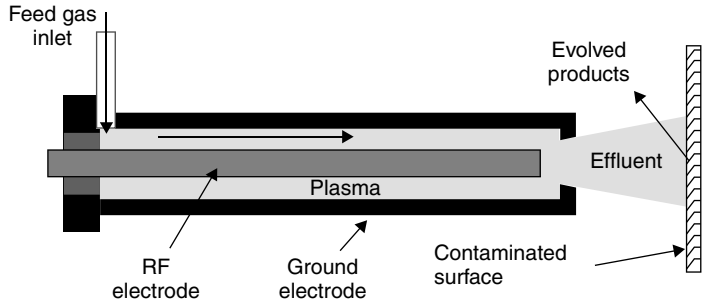
Dielectric barrier discharges (DBD) can be considered as a special type of RF discharge with frequencies in the range of 50 Hz–1 MHz. They are generated between two electrodes placed close to one another (the typical distance between the electrodes is a few millimeters), of which at least one is covered by a dielectric material. After the plasma ignition, charged particles collect on the surface of the dielectric. This accumulated charge compensates the external field, thus limiting the discharge current and subsequently quenching the discharge. This arrangement is therefore self-limiting in current and inhibits glow-to-arc transition. In the second half of the cycle, when the applied voltage increases again, the discharge reignites. The plasma ignition and quenching is then periodically repeated. Different configurations of DBD discharges exist; some of the most important for the topic of this chapter are presented in Fig. 6.5. Two principal modifications of the DBD discharge should also be mentioned. First, the dielectric material covering one of the electrodes can be replaced by highly resistive material (these discharges are known as resistive barrier discharges (RDB)) (Laroussi *et al.*, 2002), which allows operation even in a DC mode. The second modification is based on the direct replacement of the grounded electrode by the living tissue (this kind of discharge is known as a flowing electrode dielectric barrier discharge (FE-DBD)) (Fridman *et al.*, 2006). Moreover, trials have recently been reported that have tested the possibility of using several plasma discharges in an array in order to allow treatment of larger areas (Kong *et al.*, 2009). An example of a seven jet array is presented in Fig. 6.5c.

Atmospheric pressure plasma jet

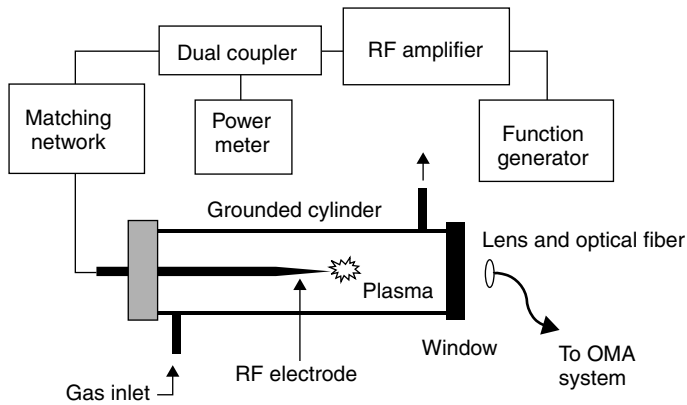
Another type of electrical discharge operated at atmospheric pressure is the atmospheric pressure plasma jet, which is schematically depicted in Fig. 6.6.



6.5 Dielectric barrier discharges: (a) DBD (Akitsu *et al.*, 2005), (b) FE-DBD (Fridman *et al.*, 2006) and (c) multi-jet system (Nie *et al.*, 2009).



6.6 Atmospheric pressure plasma jet (Herrmann *et al.*, 1999).



6.7 Plasma needle (Stoffels *et al.*, 2002). OMA, optical multi-channel analyser.

It is, in fact, a capacitively coupled device composed of two coaxial electrodes. The inner electrode is excited by RF power, whereas the outer electrode is grounded. In between the electrodes, a high flow rate gas produces the plasma, and the chemically active species as well as excited atoms and molecules are transported from the nozzle to the substrate at high velocity.

Plasma needle

The concept of a plasma needle (Fig. 6.7) was introduced by Stoffels (Stoffels *et al.*, 2002). The plasma needle consists of a thin metallic wire, whose tip is sharpened. This wire, which serves as the powered electrode, is placed into a grounded metallic cylinder. Upon application of an RF power to the tip, the plasma, which is generated in a flowing gas, is typically 0.1–1 mm in size. The plasma needle has been used to treat locally living tissues, the zone affected being limited to a few mm^2. It was found that the treatment

could lead to the death or apoptosis of the cells depending on the conditions (Stoffels *et al.*, 2008).

6.3.3 Summary

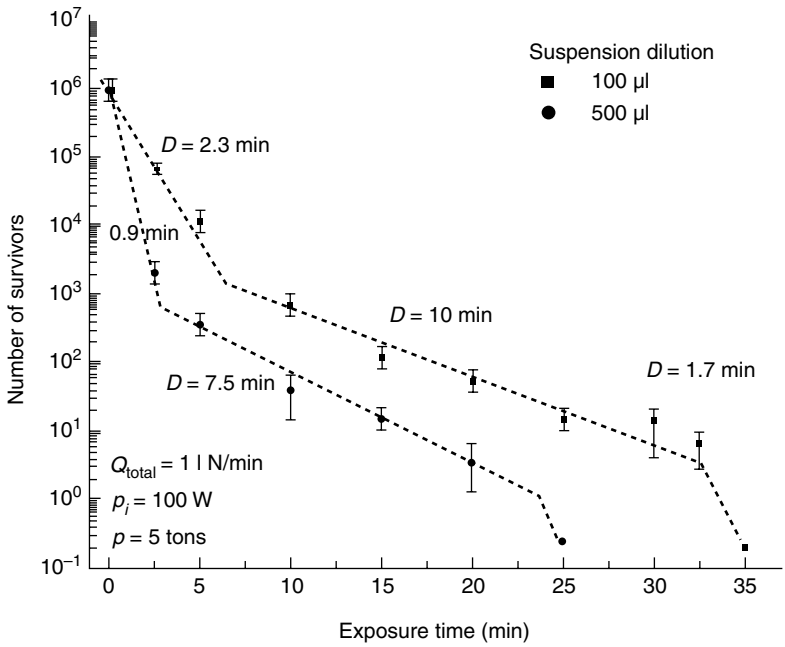
It has been shown that there are different ways to produce electrical discharges for sterilization and decontamination of surfaces. These methods differ in the operational pressure range (from fractions of Pascal up to atmospheric pressure), driving frequencies (from direct current, RF up to microwaves), gas flow rates (a few cm^3/min in the case of low-pressure discharges up to several L/min in most of the plasmas operated at atmospheric pressure) and geometries. Moreover, the properties of plasmas are closely linked to the composition of the working gas mixture and the deposited power. All of these factors dramatically influence the efficiency of the plasma treatment of biological pathogens, which can lead to apparently contradictory conclusions regarding the main biocidal agents. It is important, however, to stress that the nature of the interactions between plasma and biological samples remains the same: the selection of a particular discharge type and operational parameters in fact only accelerates some processes and reduces the importance of others.

6.4 Interaction of plasma with biological pathogens: bacteria and bacterial spores

In the following two sections, the effects of non-equilibrium plasma on bacteria and bacterial spores are discussed. Due to differences in action of plasma operated at low and atmospheric pressures, the different mechanisms are going to be discussed separately.

6.4.1 Sterilization in low-pressure discharges

The use of low-pressure plasma discharge as a sterilization tool was first studied on bacteria and bacterial spores more than 15 years ago; since then it has been the subject of extensive reviews and further development (e.g. Moisan *et al.*, 2001, 2002; Rossi *et al.*, 2006; von Keudell *et al.,* 2010). In particular, Moisan and other groups, working in N_2/O_2 post discharge, observed two- or three-phase kinetics in the spore deactivation process (see Fig. 6.8). By varying the nitrogen/oxygen ratio, it was found that sterilization was maximized in mixtures that produced intense UV emission (Moisan *et al.*, 2001, 2002; Philip *et al.*, 2002). Further work confirmed that spore inactivation or destruction by low-pressure plasma near and post discharges can be



6.8 Typical survival curve of bacterial spores exposed to MW plasma afterglow (Moisan *et al.,* 2002).

attributed to several different mechanisms that will be briefly summarized in this section.

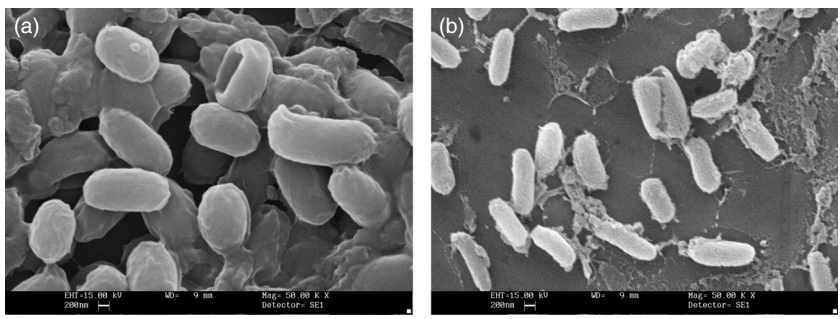
DNA damage by UV or VUV photons

UV/VUV photons emitted by the discharge are capable of inducing DNA damage and of causing the alteration of DNA repair mechanisms (Munakata, 1974; Setlow, 2006). The effect observed can be attributed to the production of chemical bonds between adjacent thymines of the DNA strands, preventing repair mechanisms. Moreover, Munakata *et al.* (1986, 1991) studied the interaction of UV photons produced by a synchrotron source (wavelength below 300 nm) with different types of wild and mutant *B. subtilis*, and found that the sensitivity of spores to VUV/UV radiation was at its maximum in the range of 150–170 nm and 220–270 nm. The results were corroborated for different types of plasma discharges with different mixtures (e.g. Lerouge *et al.*, 2000; Feichtinger *et al.*, 2003; Boudam *et al.*, 2006; Halfmann *et al.*, 2007b). It was found that UV photons were typically most efficient at wavelengths in the range of 200–300 nm for N_2/O_2 (Xu *et al.*, 2007) and $Ar/N_2/O_2$ (Halfmann *et al.*, 2007b) plasmas, while maximum efficiency was observed at 112–180 nm for Ar (Pollak *et al.*, 2008) and O_2

(Zhao *et al.*, 2010) plasma discharges. It was also found that complete sterilization is observed beyond a minimum dose of irradiation (Phillip *et al.*, 2002) and that UV fluence at 254 nm (obtained from laser and lamp sources with values varying by eight orders of magnitude) did not change spore viability (Rice and Ewel, 2001). Interesting results were obtained when a filter was placed in between the plasma and the treated spores (Singh *et al.*, 2009). This study showed that the kinetics of spore sterilization depends not only on UV/VUV radiation, but also on the presence of a gas in the proximity of the spores: when the spores were placed in a high vacuum and irradiated by UV/VUV emitted by plasma, the kinetics of their sterilization was markedly slower compared with when the spores were surrounded by a N_2/O_2 mixture at reduced pressure.

Erosion of the spore walls

This effect has been observed by many authors using various working gases or gas mixtures and has been connected to the chemical active species produced by the plasma discharge (e.g. atomic oxygen, OH radicals or fluorine atoms) (e.g. Lerouge *et al.*, 2000; Nagatsu *et al.*, 2005; Cousty *et al.*, 2006; Hayashi *et al.*, 2006; Kylián *et al.*, 2006a; Vujošević *et al.*, 2007; Vicoveanu *et al.*, 2008; Vratnica *et al.*, 2008) that are able to etch the spores. An example of etched spores is given in Fig. 6.9. This effect is even more pronounced in the active discharge where the mechanism of chemical sputtering has been demonstrated (Opretzka *et al.,* 2007; Raballand *et al.*, 2008; von Keudell *et al.*, 2010). The erosion leads to the direct killing of spores through the destruction of their membrane and leakage of cellular content. This route is particularly important when spores are stacked or embedded in an organic matrix, which shields the spores from the action of UV and limits the efficiency of UV action on DNA.



6.9 Bacterial spores: (a) before and (b) after plasma treatment.

Action of heat

It has already been established that wet heat, as produced by autoclaves, is an efficient bactericide, particularly at temperatures higher than 120°C. The mechanism of action of wet heat on spores is not clear, but is definitely not related to DNA damage: it is influenced by the water and core mineral ion content of the spores, and the intrinsic stability of core proteins, as well as acid-soluble spore proteins, which protect the DNA from heat damage. In contrast, the action of dry heat is accompanied by DNA damage, which is mutagenic (Setlow, 2006). Nevertheless, it is important to stress that there is a general requirement to reduce the process temperatures to below 70°C, in order to allow the treatment of heat-degradable materials.

Depending on the discharge conditions (pressure, gas composition, power applied, direct or post-discharge), one or more of the above processes may act simultaneously and contribute to sterilization. The rates of the different mechanisms are often markedly different, and are strongly dependent on both the plasma parameters and on the position relative to the discharge: when the samples are placed in the active plasma zone, they are submitted to ion bombardment, which favors spores erosion; however, when the samples are placed in the afterglow, mild conditions prevail and the contribution of UV radiation increases. The relative contributions of UV and erosion, as well as spore stacking, lead to the final sterilization kinetics observed: in particular, when the spores are stacked, the blocking of UV by the top layers produces the two-phase kinetics reported in the literature (Moisan *et al.*, 2001, 2002; Boudam *et al.*, 2006; Rossi *et al.*, 2006, 2007; Vicoveanu *et al.*, 2008; von Keudell *et al.*, 2010) and illustrated in Fig. 6.8.

Moreover, the relative contribution of the different mechanisms has been studied using O_2 RF plasma in direct discharge (Vicoveanu *et al.*, 2008). In these conditions, the temperature of the sample increases rapidly in the first minutes of the treatment, and reaches a quasi-steady state from then on. At the same time, the inactivation kinetics observed has two phases: a fast phase in the first minutes, followed by a slower phase, both increasing with plasma power. Using masks and filters to block plasma particles and UV radiation, respectively, the authors confirm that the actions of the following three processes simultaneously contribute to spore deactivation: (i) temperature increase, (ii) interaction of plasma particles with the sample and (iii) UV emission. They show that UV emission is the fastest mechanism contributing to spore inactivation in the first phase of the treatment, while the action of the charged particles is predominant in the second phase. Contrary to many authors, they attribute the two-phase kinetics not to spore stacking and shielding, but to the intrinsic modification of the kinetics due to the increase in temperature during treatment. However, these experiments were carried out without temperature control, and the formalism

used led to the various mechanisms having an intrinsic additivity. On the contrary, the synergetic effect (i.e. non-additivity) of heat and UV photons on the deactivation rate of *B. atrophaeus*, with a thermal activation energy of 12–14 kJ/mol, was very clearly demonstrated with a N_2/O_2 flowing plasma afterglow (Boudam and Moisan, 2010). At this point, it is important to note that there are at least two different mechanisms leading to the heating of the treated surfaces. The first is the transfer of the kinetic energy from the species forming the plasma to the surface. The second is connected with the surface reassociation of principally atomic species. The latter process is strongly material dependent, which can lead to significant variations in the sterilization efficiency with substrate materials, as noted in the literature (Cvelbar *et al.*, 2006).

Similarly, it must be borne in mind that another synergetic effect has been demonstrated between ion bombardment and reaction of radicals during plasma treatment in direct discharge, referred to as 'chemical sputtering' (Opretzka *et al.,* 2007; Raballand *et al.,* 2008; Rauscher *et al.*, 2010; von Keudell *et al.,* 2010). This effect can account for the very wide range of inactivation rates observed in the literature, depending on temperature, UV emission and ion bombardment, which can be active separately or simultaneously and synergetically during treatment.

6.4.2 Sterilization using atmospheric-pressure plasma discharges

The use of atmospheric plasma for sterilization of different types of spores or bacteria has also been the subject of a great deal of research. Different types of discharge were used, and the results were summarized in several review articles (e.g. Montie *et al.*, 2000; Laroussi, 2002, 2005; Gaunt *et al.*, 2006; Moreau *et al.*, 2008).

However, the conditions and geometry vary to a large extent, which makes the comparison of the results difficult. Moreover, determining the main processes leading to the sterilization of bacteria or bacterial spores is more difficult in this case than with low-pressure discharges, due to the presence of additional obstacles. First, the plasma diagnostic of discharges generated at atmospheric pressure is rather complex and thus only a limited number of studies provide sufficient details regarding the properties of the plasma. Second, contamination by air backflow in the discharge occurs in the majority of cases unless particular precautions are explicitly taken. In other words, the working gas mixture is not well defined, since it generally contains a non-negligible fraction of impurities, such as, for instance, water vapor or air, which may markedly alter the interactions between plasma and biological pathogens.

Nevertheless, the role of UV (e.g. Trompeter *et al.*, 2002; Park *et al.*, 2003; Heise *et al.*, 2004; Lee *et al.*, 2005),[1] heat and erosion of spores were shown to be similar to what was observed for low-pressure plasmas. In addition, further two mechanisms that were not observed with low-pressure plasma discharges are active in this case.

Electrostatic disruption

This effect is attributed to the accumulation of surface charge on spore membranes, which results in a build-up of electrostatic forces. Such forces could exceed the total tensile force on the membrane and cause it to be disrupted (Laroussi *et al.*, 2003; Yu *et al.*, 2005). This mechanism has been observed with a resistive barrier discharge (RBD) on yeast (Yu *et al.*, 2005), on gram-negative bacteria (Laroussi *et al.*, 2003) and at lower extent also on gram-positive bacteria (Deng *et al.*, 2006).

Interaction with reactive oxygen and nitrogen species

Reactive oxygen (ROS) and reactive nitrogen (RNS) species such as hydroxyl radicals (OH), hydrogen peroxide (H_2O_2), the superoxide anion (O_2^-) and nitric oxide (NO) can interact with various classes of biomolecules presented in cells and bacteria, leading to a complex chain of events that can cause cell death. The molecular targets for ROS are DNA, lipids and proteins (Farr and Kogoma, 1991). In the case of lipids, ROS attack unsaturated fatty acids in the cell membrane, thus initiating lipid peroxidation. As a consequence, the structural integrity of the membrane is compromised and osmotic imbalance occurs, which may ultimately lead to cell lysis. The reaction of ROS with proteins has also serious implications for the function of cells, since the accumulation of damaged proteins can significantly disrupt the cell metabolism. Finally, ROS and, in particular, OH radicals can cause a DNA strand to break. Nevertheless, OH radicals cannot diffuse freely through the cell to reach DNA due to their high reactivity. Therefore, it is assumed that OH radicals are created in the vicinity of DNA from hydroxide peroxide by the Fenton reaction:

$$O_2^- + H_2O_2 \xrightarrow{Fe/Cu} 2OH + O_2 \qquad [6.6]$$

6.5 Interaction of plasma with biological pathogens: pyrogens and proteins

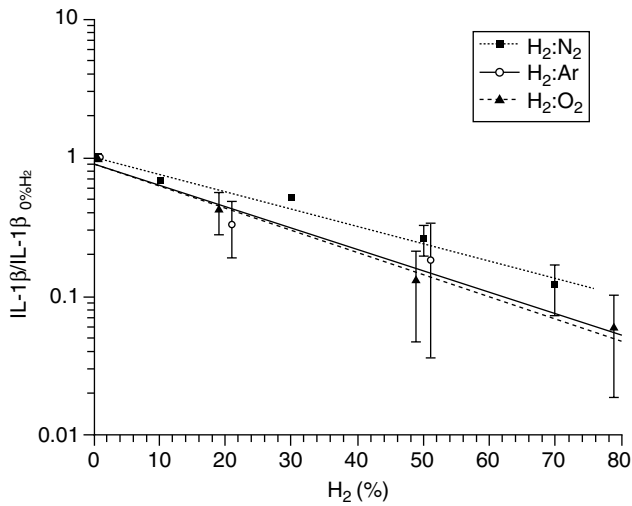
Unlike bacteria and bacterial spores, pathogenic biomolecules are not living organisms. As a consequence, different strategies for their removal/inactivation have to be followed. The main results related to the application of

[1] Although the range of gas mixture composition leading to UV emission is extremely narrow (Boudam *et al.*, 2006) as compared with low-pressure discharges.

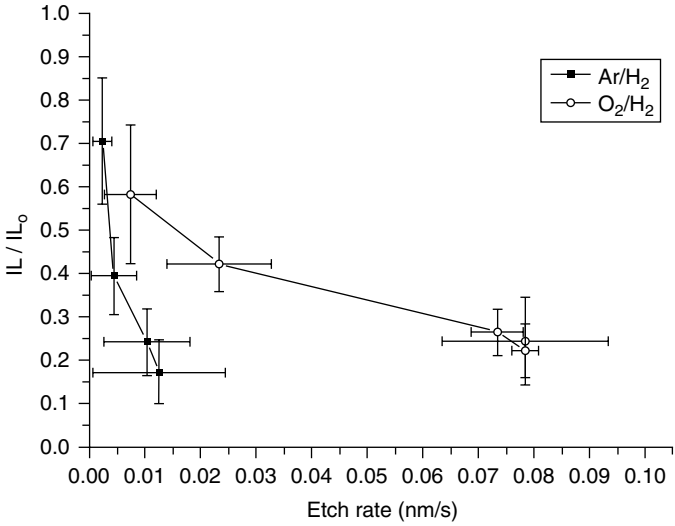
plasma for treatment of pyrogens and proteins are summarized in following two sections emphasizing the effect of reactive species.

6.5.1 Effect on pyrogens

Pyrogens are another common surface contaminant that is generally not addressed in the hospital sterilization processes. The outer coatings of spores and bacteria contain endotoxins (e.g. lipopolysaccharides (LPS), peptidoglycans (PGN) sor lipoteichoic acids), which are potent modulators of the human immune system. Their presence in the blood stream leads to physiological events such as fever, swelling or sepsis, and at higher doses to death (Beutler *et al.*, 2003). Endotoxins are extremely resistant to temperature and difficult to remove by conventional methods; their elimination by low-pressure plasma has been studied extensively in our group. We have shown that UV radiation in the 200–300 nm range, while having sufficient intensity to sterilize bacterial spores, does not decrease the biological activity of pyrogens (Kylián *et al.*, 2006c). Moreover, we showed that a MW low-pressure post-discharge plasma treatment is able to inactivate different kinds of pyrogenic substances at low temperatures (Kylián *et al.*, 2006b; Rossi *et al.*, 2006; Hasiwa *et al.*, 2008) through etching and chemical reactions with plasma radicals. These experiments were performed on different types of pyrogens, namely LPS, zymozan, Lipid A and PGN. In particular,



6.10 Bioactivity after plasma treatment normalized to the bioactivity in discharge in pure oxygen (applied microwave power 1000 W, pressure 13.3 Pa, total gas flow 100 sccm, treatment time 60 s, LPS dilution 1 ng/mL). (*Source*: After Kylián *et al.*, 2006b.)

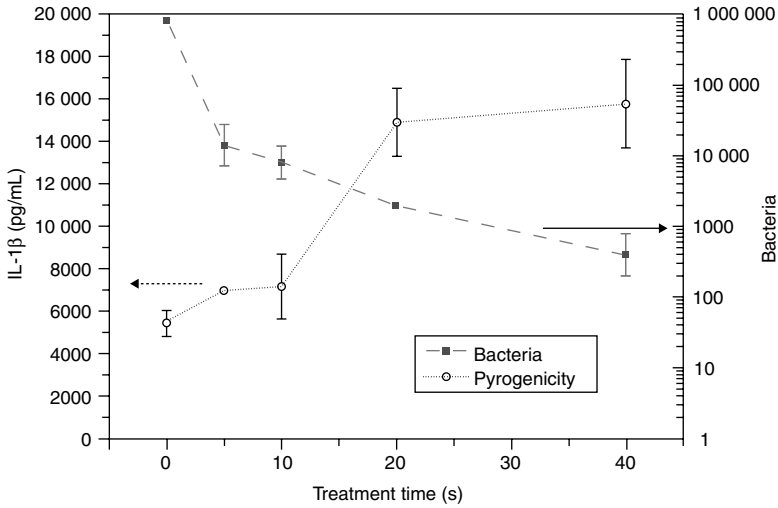


6.11 Relative change of the IL-1β response of a LPS contaminated surface treated by H_2/O_2 and Ar/H_2 microwave post discharges. By varying the composition of the discharge, different etching rates are observed, not correlated to the activity of the surface.

it was found that mixtures containing O_2 and H_2 are the most efficient (see Fig. 6.10), leading to two different deactivation mechanisms, as depicted in Fig. 6.11. Analysis of the mechanisms led to the conclusion that one of these mechanisms is based on the removal of the pyrogen film by etching (oxygen containing mixtures), whereas the other proceeds by means of chemical changes in the structure of the pyrogenic molecule (Rossi *et al.*, 2006; Kylián *et al.*, 2008a; Rossi *et al.*, 2009). While depyrogenation in the case of O_2/H_2 can obviously be attributed to the physical removal of contamination by etching, the loss of pyrogenic activity in the case of Ar/H_2 is related to the volatilization of the fatty acid chains (e.g. $C_{12}H_{23}O_2$ and $C_{14}H_{27}O_2$) as well as the alteration of phosphoryl groups (PO, PO_2, PO_3 and PH_2O_4) (Kylián *et al.*, 2008a; Rossi *et al.*, 2009; von Keudell *et al.*, 2010), which are the different components governing the bioactivity of Lipid A (Brandenburg *et al.*, 2000; Erridge *et al.*, 2002). It was also found that PGN could not be deactivated by post-discharge treatment, for any of the different gas mixtures that we tested (von Keudell *et al.*, 2010).

Other tests were performed in direct discharge. Since a chemical sputtering mechanism can be expected on other organic substances by oxidation of their carbon backbone, we tested different O_2-based plasma discharges (Rossi *et al.*, 2008) and found that all the compounds tested, including PGN, could be etched by an O_2-based mixture (Rossi *et al.*, 2008, 2009).

An important point to underline is that the sterilization of bacteria may lead to an increase in the pyrogenic character of the surface. In the



6.12 Relationship between decrease in bacteria population and increase in pyrogenicity (IL-1β release). *Staphylococcus aureus* numbers, 10^6. ICP treatment 100 W, 13.3 Pa.

experiment presented in Fig. 6.12, 10^6 bacteria (*Staphylococcus aureus*) were sprayed into two-well chamber slides (tested to be pyrogen-free beforehand) and were allowed to dry. After plasma treatment, the CFU was evaluated together with the inflammatory mediator release (IL-1β), an indicator measuring the pyrogenic activity of the surface. The results clearly show that the destruction of bacteria corresponds to a net increase in the pyrogenic activity of the surface; this is the result of plasma etching of the bacterial spore membrane, which releases pyrogens during the first stages of the treatment. This result underlines the connection between sterilization and decontamination of surfaces: a limited sterilization treatment may potentially lead to a contamination of the surface if the etching of the organic material is only partially carried out. To our knowledge, the action of atmospheric plasma discharge on pyrogens has not been reported in the literature to date.

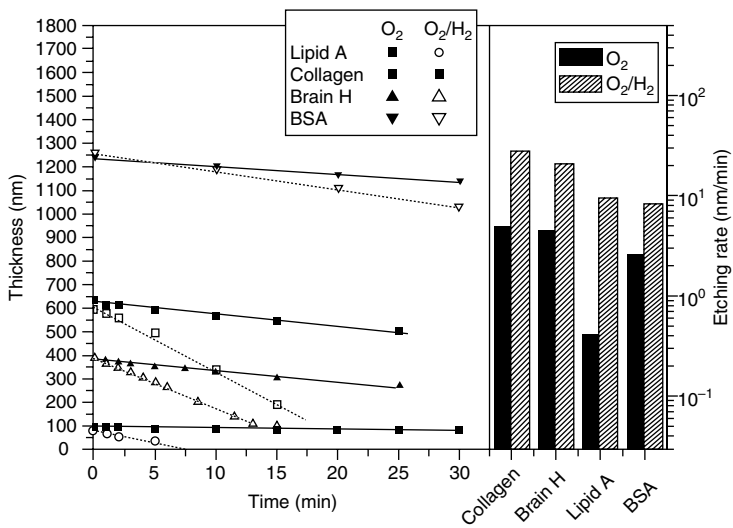
6.5.2 Effect on proteins

Protein residues remaining on the surface of instruments after surgical operations constitute another group of possible contaminants. These residues might contain pathogens, and in particular prions, since this protein has been found in the peripheral and skeletal tissues of patients with the different forms of CJD. So CJD could possibly be transmitted by contact with contaminated instruments. Unlike bacterial spores, proteins in general and prions in particular do not contain genetic material, and UV radiation

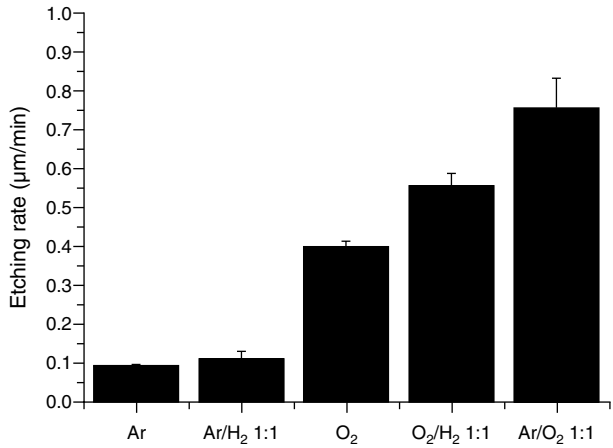
does not cause their destruction/deactivation. Moreover, prions have been found to be extremely resistant to conventional sterilization and decontamination techniques (e.g. Taylor, 1999; Lipscomb *et al.*, 2006) because of their unique and stable secondary and ternary structure that cannot be easily altered.

Although the possibility of removing prions by means of non-equilibrium plasma discharges has already been demonstrated (Baxter *et al.*, 2005), research into the mechanism of their removal is limited. The effects of non-equilibrium plasma discharges have been studied on non-pathogenic models of proteins (e.g. Mogul *et al.*, 2003; Bernard *et al.*, 2006; Rossi *et al.*, 2006; Kylián *et al.*, 2008b) in order to identify general mechanisms and guidelines useful for prion elimination. The results of these studies reveal the possibility of removing proteins by oxygen containing discharges, which induce their fragmentation and volatilization after oxidation. By using a microwave post-discharge (local plasma densities of the order of 10^9 cm^{-3}), we found that the etching rate of proteins is strongly dependent on the gas mixture used and is of the order of 1–20 nm/min (Fig. 6.13) (Rossi *et al.*, 2009).

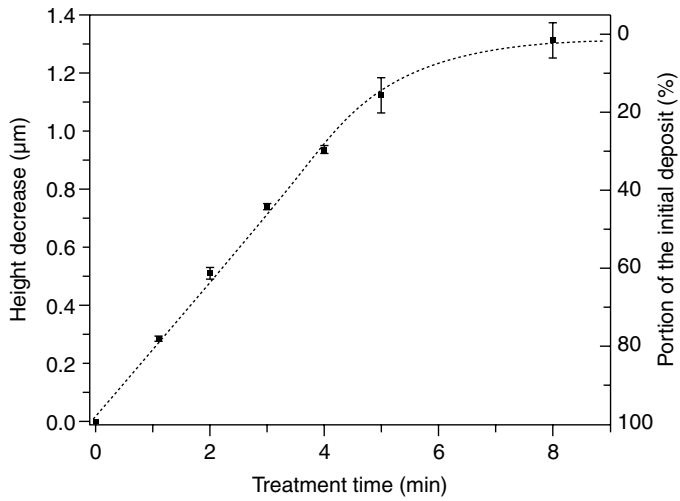
In direct inductively coupled plasma discharges (plasma densities of the order of 10^{10} cm^{-3}), the etching rates increase by one order of magnitude (Rossi *et al.*, 2009), the most efficient mixtures being Ar/O_2 (Fig. 6.14) (Kylián *et al.*, 2008b). The protein removal follows a two-phase kinetics, composed of an initial fast etching followed by a second slow phase, as shown in Fig. 6.15. The first phase was attributed to the fast volatilization of the organic phase



6.13 Thickness evolution of brain homogenate, collagen, Lipid A and bovine serum albumin (BSA) with treatment time in a MW post discharge (left panel) and corresponding etching rates (right panel) (1000 W, 16 Pa, 100 sccm).

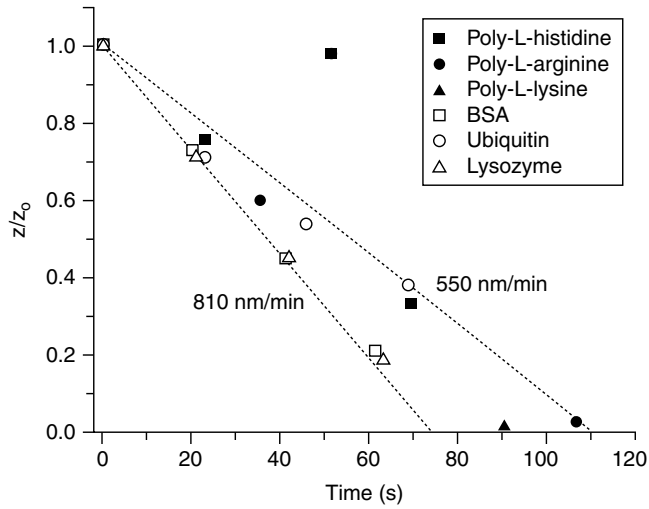


6.14 BSA removal rates measured in the ICP discharge (13.3 Pa, 10 sccm, 500 W).



6.15 Stylus profilometry measurement of thickness of the protein film as a function of treatment time. (*Source*: After Rossi *et al.,* 2008.)

of the proteins, while the second phase was related to the enrichment of the surface in non-volatile elements (based, for instance, on Na, Ca, F), which are part of the initial protein composition (Rossi *et al.*, 2008). It was also found that the same mechanism of chemical sputtering observed for bacterial treatment is also operative for proteins, in close correlation with the measured etching rates between post and direct discharges (Kylián *et al.*,



6.16 Comparison of removal kinetics of different proteins and polypeptides (plasma treatment Ar/O_2 20:1 mixture, 10 Pa, 200 W). (*Source*: From von Keudell *et al.*, 2010.)

2009a; Rauscher *et al.*, 2010). Here again, synergy was observed between ion bombardment and radicals such as atomic H or O, as well as O_2. Moreover, it was also found that the initial etching rate did not depend on the structure of the polypeptides contained in the proteins (Kylián *et al.*, 2009b), leading to the conclusion that most proteins could be volatilized with the similar efficiency, regardless of their structure (see Fig. 6.16). The most important parameters found were the radical content of the discharge and the plasma density (Rossi *et al.*, 2009), both of which are responsible for the synergy observed during chemical sputtering.

The treatment of protein by atmospheric plasma discharge has also been the subject of several investigations (Deng *et al.*, 2007a, 2007b; Bayliss *et al.*, 2009). By using an atmospheric-pressure glow discharge with pure He and He/O_2 mixtures, the authors showed that proteins could be destroyed at low temperatures, the main agents being excited atomic oxygen and excited nitric oxide, with a possible synergetic effect between the two species. Electrophoresis of the protein films before and after plasma treatment was used to show that the proteins were heavily degraded and fragmented by the treatment, and that the plasma action could be summarized as chemical degradation and volatilization. By using fluorescent proteins detected by LIF, they also identified a two-phase kinetics, which they found to be related to differences in thickness in their deposits and not to changes in the surface composition of the film during etching. However, no chemical analysis of the surface after treatment is provided in this work, and a comparison of the removal rate with low-pressure plasma is impossible.

6.6 Further issues with the use of plasma for sterilization purposes

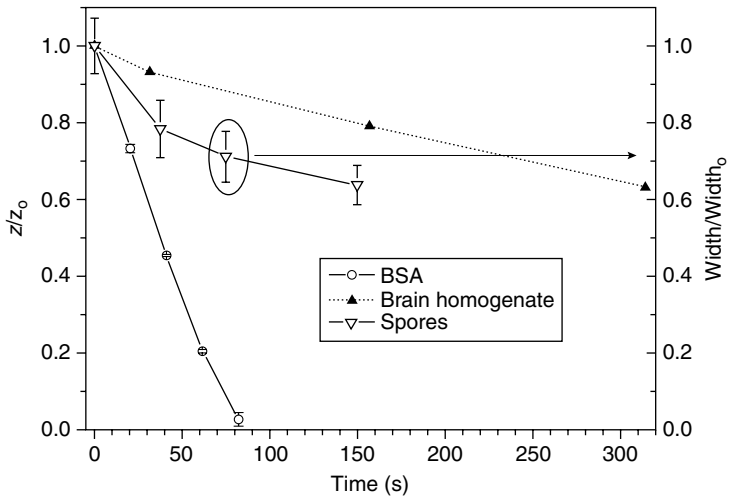
Although there was enormous progress in the field of plasma-based sterilization/decontamination in the last decade, there are still some open questions that have to be answered. The aim of this section is to mention briefly some of them as well as to draw further perspectives of this sterilization/decontamination technique.

6.6.1 Optimization of the plasma sterilization/decontamination process

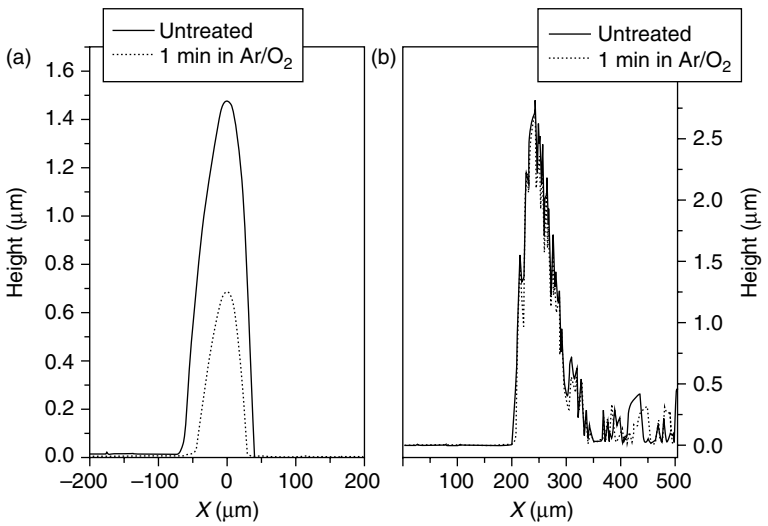
The results from the literature clearly show that atmospheric- and low-pressure plasma can be used to sterilize and decontaminate surfaces. As demonstrated above, different mechanisms have been clearly identified, namely heat, UV emission, ion bombardment and reaction with reactive species, the synergetic effects being illustrated by various studies. The different effects lead to different efficiencies and kinetics: for instance, UV leads to very effective sterilization when spores are not stacked or embedded in inorganic substances. At the same time, UV alone is not efficient at removing pyrogens or proteins, when chemical etching or chemical sputtering are the most efficient strategies. Recent work has shown that both conditions (UV emission and etching) are normally not obtained from binary gas mixtures, but can be obtained by ternary mixtures (e.g. $Ar/O_2/N_2$) at low pressures (Stapelmann *et al.*, 2008; Kylián and Rossi, 2009). This provides a general strategy for determining a process able to simultaneously treat different types of contaminant.

6.6.2. Matrix effect

Most of the studies mentioned in this review have examined single or pure species. However, in real cases of contamination, the deposit to be eliminated can be a complex mixture of proteins, lipids, sugars and an organic matrix, which completely changes the behavior of the deposit. The significance of this difference in behavior is illustrated by two examples. For instance, Fig. 6.17 presents the results of etching of bovine serum albumin (BSA) and brain homogenate, together with the etching rate of spores in a low-pressure inductively coupled plasma reactor working in direct discharge mode (Rossi *et al.*, 2009; von Keudell *et al.*, 2010). It can be seen that the etching rate observed is very different in these three cases. Figure 6.18 shows the profile of BSA spots after 1 min of etching in an Ar/O_2 plasma. When BSA is diluted in pure water, a fast etching can be observed, while when the dilution contains phosphate buffered saline (PBS) (containing salts), etching is strongly reduced due to the accumulation of non-volatile compounds on the surface,



6.17 Etching behavior of BSA and brain homogenate evaluated by profilometry. Comparison with spores etching rate evaluated from dimension measurements (plasma treatment Ar/O_2 20:1 mixture, 200 W, 10 Pa). (*Source*: After von Keudell *et al.*, 2010.)



6.18 Profile of a BSA spot after etching in Ar/O_2 plasma: BSA diluted in pure water (a) and in PBS (b).

which blocks the reaction leading to volatilization. These two results very clearly indicate that washing of the instruments prior to decontamination/sterilization is of the utmost importance, and that plasma sterilization must form part of a whole chain of treatment, including washing.

6.6.3 Practical limitations of plasma processes

Apart from the problems related to the matrix effect, another practical difficulty in the use of plasma discharges is related to their directionality (atmospheric plasma discharge) and their limited penetration in high aspect ratio holes and trenches (low-pressure plasma discharges). The former requires special handling of the equipment during treatment, which complicates the operation. The latter requires the procedure to be carried out at intermediate pressures: this limits the efficiency of the chemical sputtering effect, which is particularly important for decontamination. A further aspect to be taken into account is the selectivity of treatment when dealing with polymeric material, as the decontamination treatment may possibly lead to etching the substrate material.

Finally, plasma treatments are not very compatible with pre-packaged instruments, as the packaging may block the reactive species created by the plasma discharge. This is particularly crucial for atmospheric plasma treatment, since at low pressure, the plasma discharge could in principle be created inside the package.

6.6.4 Treatment of biological pathogens in an aqueous environment

Another important issue is related to the possibility of treating biological pathogens in an aqueous environment by means of atmospheric pressure plasma discharges. It has been shown that bacteria can be effectively sterilized not only in a 'moist' environment – that is, when a minute amount of non-liquid water is present (Dobrynin *et al*., 2009) – but also when completely suspended in liquids (Liu *et al*., 2010; Oehmigen *et al*., 2010). This effect was attributed to a gradual acidification of the water caused by the reactions of NO_x produced in plasma with water and the subsequent oxidation of the bacterial fatty acids by per hydroxil radicals at low pH (Liu *et al*., 2009). Another explanation assumes that there is synergy between reactive oxygen and nitrogen species, particularly NO and H_2O_2 (Nosenko *et al.*, 2009). In this reaction scheme, NO releases iron ions from intracellular metalloproteins; the higher Fe^{2+} content then catalyses the reduction of H_2O_2 to the highly toxic OH radicals.

6.6.5 Treatments *in vivo*

The possibility of using atmospheric pressure plasma discharges for the treatment of living tissue is currently the subject of a great deal of research. It has been demonstrated that different plasma sources are capable of

effectively killing bacteria, but are non-destructive to human tissues (e.g. Sladek *et al.*, 2004; Fridman *et al.*, 2008; Nosenko *et al.*, 2009). Although the reason for this selectivity is still not well understood, three possible explanations have recently been proposed:

1. Mammalian cells have a defense mechanism against oxidative stress. For instance, the presence of NO induces cellular synthesis of antioxidative enzymes in cells (Nosenko *et al.*, 2009). This mechanism, which is absent or considerably lower in bacteria, then counterbalances the increased production of Fe^{2+} ions responsible for the formation of toxic OH radicals.
2. There is a 'size' effect. The bacteria are much smaller than mammalian cells and thus the dose of toxic compound needed for their inactivation is lower (e.g. Dobrynin *et al.*, 2009). Moreover, electrostatic forces that can eventually lead to the rupture of membranes are considerably lower in the case of cells as compared with bacteria since the charging is in the first approximation inversely dependent on the diameter of the treated object (Morfill *et al.*, 2009).
3. There is a 'complexity' factor. The mammalian cells in tissue communicate with each other, which may lead to a lower toxicity effect than that observed on single cell bacteria (e.g. Dobrynin *et al.*, 2009).

Moreover, it has been found that, in addition to its ability to inactivate bacteria, plasma treatment of living tissues also has therapeutic effects in some cases. For instance, it can be used for wound healing, tissue regeneration, blood coagulation (e.g. Fridman *et al.*, 2006, 2008), or even for killing of cancer cells (Vandamme *et al.*, 2010). This opens new and exciting perspectives for the use of plasma discharges not only as a sterilization or decontamination device, but also as an important tool in a number of medical fields – for instance, dentistry, dermatology, cosmetics or minor surgery.

6.7 Conclusions and future trends

This review has summarized the work carried out to date on low-pressure and atmospheric-pressure sterilization and decontamination. The main mechanisms of plasma action on various biological pathogens have been identified and it has been shown that plasma treatment is a viable process for sterilization and decontamination at low temperatures. However, several important points still need to be addressed before plasma technologies can be applied on a large scale. Apart from general process engineering problems, the first issue is linked to the variability in efficiency of the different decontamination mechanisms, which leads to difficulties

in treating large loads with different shapes and sizes homogeneously. The second issue is linked to the matrix effect, which underlines the need to integrate plasma technology in a complete washing/cleaning process. Finally, the economics of the whole process, including plasma discharge, will have to be studied and optimized so that large-scale application can be considered.

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7

Sterilisation techniques for polymers

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Abstract: The effect of terminal sterilisation techniques on medical polymers is diverse. Few sterilisation agents are without adverse effects on polymers: heat (steam and dry heat), radiation, ethylene oxide, ozone, hydrogen peroxide. Heat can alter, damage, degrade, distort and expand heat-sensitive polymers. Ethylene oxide is toxic and can leave toxic residues. Hydrogen peroxide and ozone can absorb, affect the gloss of, discolour and oxidise some polymers. Radiation can degrade, destroy and change molecular structures of some polymers. This chapter considers not only chemical and physical effects of sterilisation techniques, but also some effects on applications, biocompatibility and device compatibility.

Key words: biocompatibility, comparative efficiencies, polymers and sterilisation.

7.1 Introduction

Sterilisation techniques are fundamental to the preparation of polymers into sterile medical devices and hospital products. Polymers are the most widely used class of materials in biomedical devices, but they may be sensitive to various sterilisation techniques. Finding the correct polymers for medical devices or biomaterials requires serious consideration regarding design, processing and performance, including biocompatibility, functionality and the effect of sterilisation. The effect of sterilisation on polymers is a key factor in device design.

For example, device designs with thick and dense absorbing polymers may absorb larger quantities of toxic ethylene oxide (EO) residues or hydrogen peroxide, and limit the penetration of hydrogen peroxide, steam and less penetrable electronic-beam (e-beam). Unfortunately, there is no singular sterilisation technique panacea for 'all' polymers and implantables. Consequently, polymer compatibility to sterilisation technique is a major concern, and is the focus of this chapter. As part of the manufacture process for a device, the impact of the sterilisation technique on the final biocompatibility and functionality of the device must be evaluated. Both the product biocompatibility and physical and functionality properties provided must be validated through the intended shelf-life of the device.

Manufacturers should be selective in their choice of polymers for components and devices. They should also be aware of how polymers may interact with various tissues, particularly during long-term implantation. Concern for polymer compatibility will ultimately offer longer life cycles and better cost-effectiveness for the user.

7.1.1 Sterilisation techniques and their effects on polymers

There are few techniques capable of sterilising polymer products. Factors to consider when selecting a technique include the fact that steam or dry-heat sterilisation could degrade or melt some plastics. EO leaves toxic residues; hydrogen peroxide and oxidising agents can oxidise or damage some materials. Radiation can alter the molecular structure of many polymers (by cross-linking or scission), cause odours and discolouration, embrittle and degrade some materials, affect bond strengths and cause changes to shelf-life. Consequently, the effect of sterilisation on medical materials and polymers provides reasons why one technique is employed and why another is not considered. However, being suited to more than one sterilisation technique will improve the accessibility of a device. What techniques are acceptable and available for medical polymers and devices will determine the sterilisation method(s) of choice.

Most sterilisation techniques, except for EO, involve harsh treatment that results in adverse physical and chemical effects, including molecular changes that may not be visible, affecting mechanical properties, functionality, safety and toxicity.

7.2 Dry-heat sterilisation

Dry-heat sterilisation may be as simple as baking but is often more complex. It is used for sterilising oils, petroleum jellies, surgical catguts and instruments, glassware, including vials for pharmaceutical drugs, and silicone prosthesis and other medical devices. It is also used in sterilising dental instruments to minimise corrosion of sharp items and in laboratories for depyrogenation of glassware, where other techniques are not able to destroy pyrogens. Dry heat is frequently used as part of aseptic processing in the pharmaceutical industry. It is also the method of choice for spacecraft sterilisation, and for sterilising electronics boards, ceramics and other moist heat-sensitive materials and products.

Dry heat can sterilise most heat-resistant polymers, as can steam sterilisation, but it can also sterilise moist heat-sensitive polymers. Steam and dry heat have many similarities, including ease of control and monitoring, low

cost and the absence of toxic residues or wastes, as may occur with EO or radiation. Steam and dry heat are less expensive than EO and radiation processing.

Some other dry heat applications include the following:

- Silicone implants that are sterilisable. They are cross-linked by radiation, impermeable to steam or absorb peroxides and EO.
- Sterilisation of dry chemical-containing devices that would otherwise be destroyed by moist heat, EO, radiation or oxidising agents.
- Sterilisation of electronics components which are damaged by steam, high humidity, EO/formaldehyde or irradiation. Radiation-sensitive materials, such as acetal, polypropylene (PP), silicone and Teflons®, are good candidates for dry heat.
- Polyurethanes (PUs), which are hydrolytically attacked by steam or degraded by radiation, are good candidates at low temperatures.
- Sterilisation of contrast medium at extremely high temperatures (e.g. 190°C), but with extremely short exposure times (e.g. 6–12 min).

Some temperature–time relationships for dry heat sterilisation are shown in Table 7.1.

A disadvantage of dry-heat sterilisation is the long time required to heat up and cool down. The transfer of (dry) heat is relatively slow and, particularly for polymers, requires removing significant moisture and sterilisation of contact areas at elevated temperatures for extended exposure times.

Table 7.1 Temperature–time relationships for dry heat sterilisation

Temperature	Time (overkill)
330°C	1.15 s
190°C	6 min*
180°C	30 min†
170°C	60 min†
160°C	120 min†
150°C	180 min (3 h)
105–135°C	Overnight (e.g. >8 h) or longer†
88°C‡	4–5 days

Notes: Time chosen depends on load size, mass and configuration, time to penetrate and the degree of overkill. Lesser times may be chosen based on bioburden control, resistance and improved heating methods.

* A Cox steriliser uses forced heat air at 2500 ft/min to heat. Infrared irradiation can heat materials more quickly.

† Exposure times vary with equipment, circulation, loading and cool down.

‡ Dry heat below 100°C may be possible but requires additional moisture removal from microbes, dehydration (desiccation) and low bioburden to be effective.

Overcoming stratification of temperature and difficult to penetrate areas (e.g. joints and mated surfaces) is critically important. Heat is an effective process for heat-tolerant polymer materials and devices, many of which have been designed to be resterilised. Implantables are not typically resterilised in practice, although a few may be.

Dry heat is recommended only for those materials, such as certain glass containers, oil, powders, some polymers (e.g. acetals, silicone, Teflons®), where it is undesirable to use steam.

7.2.1 Effects of dry heat on polymers

Dry heat can distort, melt, soften or expand many polymers. It requires higher temperatures for the same cycle or exposure time as steam sterilisation, or longer exposure times at steam temperatures. With expertise, the longer dry cycle or exposure times may be significantly reduced. Under such circumstances, dry heat may be more compatible with more heat-tolerant or moisture-sensitive polymers than steam.

Polymers can only be sterilised by dry heat below their melting, transition or degrading temperatures. Polymers and materials compatible with dry heat sterilisation (low and high temperatures) are diverse (see Tables 7.2 and 7.3). Heat sterilisation can be harsh on polymers, requiring elevated temperatures for complete inactivation of heat-resistant spores and particularly for prion inactivation (e.g. >300°C). However, good control of bioburden can allow for lower dry-heat inactivation temperatures (e.g. 105–135°C).

Dry heat cannot sterilise aqueous liquids, only non-aqueous substances (e.g. oils) per se. Consequently, steam is a better technique when it comes to sterilising aqueous solutions within heat-tolerant polymers. However, dry heat may be applied as part of the drying and cooling phase of the steam process. Compared with steam, dry heat does not involve limited penetration (silicone is non-hydroscopic), moisture sensitivity of some polymers and post-sterilisation wetting problems. It can sterilise acetals, PP up to 120°C and Teflons® (e.g. FEP, PCTFE) up to 170°C; irradiation would damage and embrittle these polymers.

Heat sterilisation, whether by dry heat or by steam, can cause thermal degradation of polymers and this may be due to oxidation. Thermal degradation of polymers involves molecular deterioration as a result of overheating. At high temperatures, the components of the long-chain backbone of the polymer begin to separate (molecular scission) and react to change its properties. Thermal degradation provides an upper limit to the service temperature of plastics, as does the possibility of mechanical property loss. Indeed, unless correctly prevented, significant thermal degradation can occur at temperatures much lower than those at which mechanical failure is likely to occur. Consequently, plastics or polymers selected for heat

Table 7.2 Polymer compatibility to dry heat and steam sterilisation techniques

Polymer	Comments (vary – consult authors or suppliers)
Acrylonitrile butadiene styrene (ABS)	Very unlikely, but some may be poor to possible, depending upon grade, filler Run low temperature process
Fluoropolymers	
Polytetrafluoroethylene (PTFE)	Compatible up to 170°C or higher Certain grades may allow for several cycles or long service; however, although PTFE has great thermal stability, once the activation energy for the rupture of the C-C bonds in the chain has been exceeded, it can unzip quantitatively releasing a potentially toxic monomer
Perfluoroalkoxy copolymer (PFA)	Working temperatures up to 204°C or higher Long term up to 170°C
Poly chlorotrifluorethylene (PCTFE)	Up to 150°C continuous
Polyvinyl fluoride (PVF)	Heat deflection temperature up to 134°C; limited use
Polyvinylidene fluoride (PVDF)	Per use temperature is 150°C (302°F); however, some grades may only go to 125°C Multiple, maximum operating temperature of 275°F/130°C
Ethylenechlorotrifluorethylene	Compatible to 266°F (131°C); melt at 412°F (211°C)
Ethylene tetrafluoroethylene (ETFE)	Up to 150°C
Fluorinated ethylene propylene (FEP)	Up to 170°C or 200°C (392°F)
Polyoxymethylene (e.g. polyacetal)	Up to 121°C or higher; may degas May use up to 100 cycles at 121°C, but it may begin to degrade, emitting formaldehyde
Polyacrylic (e.g. PMMA)	Poor to fair; some highly resistant grades
Polyamide (e.g. Nylon)	Poor to excellent Absorbs moisture, some films will allow moisture to diffuse through
Polycarbonate	There are grades that can be sterilised at 134°C Some formulations only allow a few cycles; other formulations allow up to 200 repeat cycles

(*Continued*)

Table 7.2 Continued

Polymer	Comments (vary – consult authors, or suppliers)
Polyester	Possible to excellent; depends upon type, grade, form and function Some good PET films at 240°F, PEN good Mylar resistant but will not allow steam penetration Aliphatic polyesters sensitive are to hydrolysis, while aromatic polyester (e.g. PET) may be less susceptible
Polyethylene (PE) – various densities; LDPE, LLDPE, HDPE, spun polyolefin®	Poor to fair HDPE fair and spun polyolefin fair High density better than low density Reinforcement of HDPE improves its temperature
Polyimides (PI)	Possible to excellent; depends upon grade, form and function PEI withstand up to 4000 cycles 1000–2500 at 5 min @134°C
Polymethylpentene (PMP)	Excellent up to 235°C; PMP withstands repeated autoclaving, up to 150°C
Poly (ether) Ketone	High temperature resistance; PEEK has heat resistance Good up to 2000 h of steam Typically long service
Polypropylene	Depends upon grade, form and formula Use heat-resistant gradewith heat stabiliser for multiple cycles
Polypropylene copolymer (PPCO)	It is autoclavable; provides properties of polypropylene and polyethylene PPO replaces polyallomer
Polystyrene	Standard polystyrene not autoclavable; but syndiotactic polystyrene (S-SPS) is excellent, as is styrene
Polyphenyloxides (PPO)	Good, 215°C; can be mixed with styrene
Polysulfones	Typically all types are excellent; however, polyether sulfone (PES) is less resistant Repeated autoclave cycle – PS up to 1500 cycles; but not PES
Polyurethane	Poor/possible, but some grades may be fair/good
Polyvinylacetates	Depends upon form, function, formulation and co-polymerisation. Heat-stable PVA hot-melt adhesives used
Polyvinylchloride	Rigid PVCNL, but possible with modifiers; plasticised PVC good depending on form, formulation and function

Styrene acrylonitrile copolymer (SAN)	Possible to fair; depends upon grade
Silicone	Has tremendous heat resistance, but is not a barrier to moisture vapour; dry heat may be better in some applications. If exposed to repeated steam sterilisation will eventually relax silicone and will become gummy
	Silicone is hydrophobic, it will resist moisture
	Diffusion, unless nano-channels exist
Thermoset polymers	
Epoxy reinforced plastics	Numerous types of reinforced epoxies
	Physical properties can vary. Heat distortion temperatures of up to 470°F
Phenolics	Autoclaving can lead to phenolic degradation and extractable into fluids
Polyester, unsaturated	There are a variety of unsaturated polyesters (e.g. vinyl esters). C better cross-linked.
	Possible to good
	Isophthalic acid-based polyester
	High-temperature resistance
Polyimides (e.g. BI maleimides (BMI) and acetylene terminated polyimide (ACTP))	BMIs and ACTP have use-service temperatures of 127–232°C and 316°C
Polyurethane (PU)	Typically possible; depends upon grade, form and function. There are heat-resistant cross-linked polyurethanes
Aliphatic	Radiation cross-linking increases its resistance
Aromatic	Aromatic thermoset PUR does not form 4,4′-methylenedianiline (MDA) in polyurethane
Adhesives	
Acrylic	Can tolerate autoclaving; depending upon grade and formulation, fair to good
	There is an acrylic adhesive film in a tape up to 280°F
Epoxy	Depending upon grade and formulation, deflection temperature from 200 to 500°F
	Some can lose retention of initial strength on only five cycles
Fluoroepox(y)ies	Epoxy adhesives; depending upon cure and formulation, good to excellent
	Epoxy adhesives cured with heat are more heat resistant than those cured at room temperatures

(*Continued*)

Table 7.2 Continued

Polymer	Comments (vary – consult authors, or suppliers)
Silicone adhesives	Typically good; depends upon form, formulation and function, good to excellent Some may be good for only up to 6–8 cycles
Elastomers	
Butyl	Good,depending upon type and grade Resistant to water and up to 120°C Multiple use – a halobutyl(halogenated poly (isobutylene))
Ethylene propylene diene monomer (EPDM)	Good up to 125°C in water; up to 134–150°C in air; continuous-use operation; temperature of 105°C
Natural rubber-latex (synthetic cis 1–4 polyisoprene)	Possible to fair There are autoclavable grades Plastomers enhance thermal stability. Possible to fair Hardens with use Withstands repeated autoclaving at 250°F for 20 min
Nitrile rubber (acrylonitrile butadiene)	Good resistance to moisture and water Tolerate temperatures of up to 120°C at lower processing conditions, below 230°F Better if hydrogenated nitrile rubber
Polyacrylic	Polyacrylate: it is a heat resistant rubber; water resistance can be improved but with decrease in heat.Typically, resistance to water is poor.
Polychloroprene	Fair resistance to moisture, up to 230°F; intermittent to 250°F Fair to very good It is possible to resterilise at below 230°F
Silicone elastomer	There are some representative polymers for steam sterilisation technique
Styrene block copolymers, SBR	Depends upon grade, type, form and formulation Possible to fair It is possible to resterilise up to 100°C

Thermal-based polyisocyanate, urethane (polyether/polyester)	There are some heat-resistant grades; depends upon type, form and formulation With silicone there is increased heat resistance Not likely to be multiple sterilisations
Thermoplastic elastomer (TPE)	Polyolefin that can be moulded into autoclavable parts
Urethane elastomer aliphatic	Typically possible; some up to 135°C. Steam autoclaving possible with selected grades
Urethane elastomer aromatic	Formation of 4,4′-methylenedianiline (MDA) with steam

Sources: AAMI TIR 17[1] and references 2, 3 and 5.

sterilisation should be reviewed for their transition temperatures as well as melting temperatures. The chemical reactions involved in thermal degradation may lead to physical and optical property changes relative to initially specified properties. Thermal degradation generally involves changes to the molecular weight (MW) and MW distribution of the polymer and property changes include reduced ductility and embrittlement, chalking, colour changes, cracking and a general reduction in desirable physical properties. (Note: Radiation also involves changes to MW and MW distribution, but oxidative radiation can also cause cross-linking.)

7.2.2 Adaption of dry heat to minimise effects on polymers

The main disadvantage of dry heat is its elevated temperatures, which are not compatible with many materials and polymers, but it becomes more effective at lower temperatures (e.g. 121°C vs 170°C). Where there is a need for rapid sterilisation, dry heat is often lacking, having a long cycle or exposure time. However, with the addition of chemical or physical agents the heating time of polymers and products may be drastically reduced. Common time–temperature relationships for sterilisation with dry heat are shown in Table 7.3. To enable spacecraft sterilisation, lower dry-heat sterilising temperatures were established. Spacecraft sterilisation can be performed in the range 105–35°C. The possibility of sterilisation below 100°C (e.g. 66–88°C), but at extremely long exposure times under dry/desiccated conditions, was also discovered.

At these lower temperatures and longer exposure times, more polymers can be adequately sterilised with fewer degrading effects on their properties than with steam sterilisation. Dry heat sterilises polymers without the hydrating, moisturising and wetting of steam (which may cause hydrolysis of some bonds, etc.). At lower temperatures, dry heat may sterilise as many, if not more polymers than moist -heat sterilisation, because of corrosion, hydration, hydrolysis or wetting of certain materials. However, the extended heating time for dry heat sterilisation may cause a gradual softening or distortion of certain materials (e.g. polyvinyl chloride, PVC); this may require reducing any load on PVC. A longer cycle, lower temperature and integration of heat lethality during the heating and cooling steps can be used to avoid polymer or product degradation. Knowledge of the rate of polymer degradation or decomposition and the kinetics of bioburden death rate at different temperatures enables optimisation of cycle parameters. Improving heating up and cooling down with dry-heat creates a total cycle time that may be shorter than a total EO cycle with preconditioning and aeration.

Table 7.3 Polymers and materials compatible with dry-heat sterilisation technique (low and high temperatures)

Acetal (ACL), delrin, or polyoxymethylene up to 121°C (dry)
Aluminum up to 190°C (dry)
Cellulose acetate (non-load) up to 120°C
Cellulose acetate butyrate (non-load) up to 130°C
Cotton muslin up to 204°C
Glass >190°C
Grease (depends upon the type of grease) (dry)
Ethylene chlorotrifluoroethylene (ECTFE) up to 150°C
Epoxies (vary up to 177°C)
Ethylene propylene diene monomer (EPDM) up to 149°C
ETFE up to 150°C
Ethylene acrylic 149°C
Fluorocarbon rubber 199°C
Fluorinated ethylene propylene (FEP) up to 170°C
Fluoro silicone 232°C
High-density polyethylene (HDPE) up to 120°C
Hydrogenated nitrile rubber 149°C
Liquid crystal polymer (LCP) up to 275°C
Metals (note some metal temper may occur above 160°C) up to 190°C (dry)
Muslin up to 160°C
Natural rubber 104°C, but low heat ageing resistance
Neoprene/chloroprene rubber 121°C
Nitrile rubber 100°C, and low heat ageing resistance
Nylon 4/6 (polyamide heat-stabilised grades) up to 130°C
Nylon 6 <100°C
Paper (varies depending upon paper) up to 160°C (dry)
Perfluoroalkoxy (PFA) up to 170°C
Petrolatum gauze up to 160°C
Phenolics (vary) up to 150°C
Polyacrylate (ACM) 149°C
Polycarbonate (PC) up to 134°C
Polyetherimide up to 134°C
Polyetherketone (PEI, PEEK, etc.) up to 170°C
Polyethylene (vary per molecular weight (e.g. 80–142°C))
Polyethylene terephthalate copolymer (PETG) up to 170°C
Polyimide 232°C
Poly 4-methyl-pentene-1 (PMP) up to 170°C
Polypropylene (PP) up to 135°C, no stacking
Polyphenylene oxides (PPO) 100–148°C
Polypropylene copolymer (PPCO) up to 120°C
Polysulfone (PSF) up to 160°C
Polytetrafluoroethylene (PTPE) up to 170°C
Polyvinyl chloride tubing (flexible-non-load, varies) up to 120°C
Polyvinylidene fluoride (PVF) up to 125°C
Styrene-butadiene rubber 100°C, but heat ageing resistance
Silicones up to 200/232°C
Teflons® up to 170°C
FEP up to 170°C
PFA up to 170°C

(*Continued*)

Table 7.3 Continued

Select a polymer whose temperature transition or melting temperature is comfortably 'above' the required, selected or chosen dry heat sterilisation operating temperature. Melting and/or deflection/maximum temperature can vary with formulation changes.

Note: Polymer responses may vary with the length of exposure to a temperature.
Sources: AAMI TIR 17[1] and references 2, 3 and 5.

Since dry-heat sterilisation involves not only elevated heat but also removal of moisture or desiccation of microbes, additional means to improve moisture removal and microbe desiccation will significantly shorten required exposure time, but a case by case decision is usually required.

Increased temperatures and rapid microbe dehyrdation will result in shorter inactivation times. However, the time to heat up and cool down will be longer for shorter exposure times. Consequently, the total process time may be an adjustment of heat-up, exposure time and temperature, and cool-down to optimise the process. Since it is easier to achieve lower temperatures, particularly with low heat transfer of polymers, reduced temperature dry-heat processing may be optimal. For *in situ* produce and aseptic assembly sterilisation with minimum or no packaging, efficient loading for heating may improve heat-up time as well as the time to penetrate and sterilise.

Cycle and exposure time depends on load, penetration time and the validation approach used. Load, mass or stress affect a material during dry-heat sterilisation and some materials may soften and flatten as a result of direct contact with other items in the load.

Choosing a polymer involves selecting a material that best fits the dry-heat process and temperature of choice. The number of polymers that can be dry-heat sterilised has increased (see Tables 7.2 and 7.3). As the sterilisation temperature and microbial moisture decrease, the heat-up and cool-down periods of the cycle also decrease.

Polymer selection begins with consideration of heat deflection, glass transition, melting and/or optimum operating temperatures. Heat stability can be enhanced by the addition of heat stabilisers to the formulation. Dry-heat sterilisation is useful for polymers that are adversely affected by moisture, hydration (e.g. ethylene vinyl acetate (EVA)) or cross-linking (silicone). For example, some transparent plastics that absorb small amounts of water vapour and appear cloudy after autoclaving are ideal candidates for dry-heat sterilisation. Conversely, materials are not 'easily' heat sterilisable – for example, acrylonitrile–butadiene–styrene (ABS), acrylics, polystyrene and low-density polyethylene (LDPE) – can be damaged by exposure to high temperatures. Dry-heat sterilisation of silicones is preferable because

radiation causes cross-linking; EO creates too many toxic residues and many techniques fail to sterilise intrinsic oils and other materials, where humidity cannot be diffused.

7.3 Steam (moist heat) sterilisation

Steam sterilisation can be as simple as using a pressure cooker, but is often more complex. It is a traditional method used in hospitals and laboratories where reusable materials and products are frequently resterilised. Pharmaceutical companies use it for sterilising heat-resistant drug solutions and it is also used in decontamination of infectious waste. The method is limited to use with heat-tolerant, moisture-resistant polymers. Compatibility with high temperatures and moisture resistance is necessary for moist steam cycles. Steam is compatible with aqueous liquids and can sterilise most metals, glass and a large number of heat-resistant plastic materials. The number of materials compatible with steam varies considerably with the sterilisation temperature.

Steam sterilisation is often performed at temperatures of 121–34°C. However, processing temperatures of moist-heat sterilisers may range from 105°C to 150°C. In saturated-steam processes, the processing temperature corresponds to a saturated-steam pressure significantly above atmospheric pressure.

Operating and process pressures used in steam applications cover a wide range, depending on the type of process required. Processes might use high vacuum levels to eliminate air, while exposure pressures range from a low of 3 psig for a low-temperature process to as high as 70 psig for air overpressure, water-spray and water-immersion processes. The latter processes are generally used to maintain the integrity of the polymer, shape of the container and compensate for the pressure created by the increase in temperature.

Cycle and exposure times vary with temperature and with heat-up and cool-down times. The rate of product heating should be controlled to minimise the possibility of differential expansion. The cool-down phase of a cycle can be a critical period in which polymers, packaging or containers burst or distort with change in internal pressure versus external pressure, and requires a positive pressure overlay. A longer heat-up and cool-down phase typically reduces the exposure time required. Heat-up time enhances the heating of material. Cooling time reduces heat and eliminates moisture from the steriliser.

Time–temperature relationships[2] for steam sterilisation include:

- 3 min 134°C* pre-vacuum for immediate to use or flash sterilisation;
- 18 min 134°C* pre-vacuum for reduction of prion activity;
- 12–15 min 121°C* pre-vacuum or not for immediate to use sterilisation;
- 30+ min 121°C* for wrapped instruments, trays or liquid bottles;

- 60 min 121°C* for reduction of prion activity;
- 120 min 121°C immersion in 1N NaOH for sterilisation of prions;
- 30–40 min 115°C*.

*Exposures vary depending upon:

- load density,
- heat capacity,
- configuration,
- heat-up,
- cool-down time,
- time to penetrate,
- overkill approach versus bioburden.

Lesser times may result based upon bioburden control and resistance, and integration of time/temperature during heat-up and cool-down steps.[2]

Standard steam sterilisation is carried out at 121°C for 15 min. Processing temperatures can be reduced to 110–15°C, depending on the bioburden, device design and heat resistance of the polymer. With recent emphasis on the environment and toxicity, ease of sterilisation of the cotton mould *Pyronema domesticatum* and sterilisation of prions, there is renewed interest in steam and its compatibility with the environment and health and safety. Immediate use (or flash) steam sterilisation continues to be a process for use with pre-vacuum cycles; however, it can be applied to pre-vacuum, high vacuum and steam-flush-pressure-pulse sterilisers and different cycles (i.e. gravity-displacement and dynamic air removal).

Flash steam processes should not be used for:

- implants, except in a documented emergency situation when no other option is available;
- post-procedure decontamination of instruments used on patients who may have Creutzfeldt-Jakob disease (CJD) or similar prionic disorders;
- devices or loads that have not been validated with the specific cycle employed;
- devices manufactured and sold sterile and intended for single use only.

Note: Flash steam sterility can be improved with appropriate tray covers or other barriers to items being sterilised, which eliminate or reduce contamination by environmental microbes. Flash steam sterilisation achieves a higher degree of inactivation of highly resistant thermophilic biological indicator spores for equivalent process conditions compared with liquid sterilisation. Liquid-sterilised items are more vulnerable to recontamination,

require drying and offer less barrier protection than flash sterilisation. The latter is also a just-in-time (JIT) approach. Speed (i.e. JIT) and aseptic handling both reduce the opportunity for contamination.

7.3.1 Effects of steam sterilisation on polymers

Unlike dry heat, with steam sterilisation not only is there potential thermal degradation and decomposition of a polymer but also the potential for hydrolysis. Some polymers lose structural integrity at temperatures used for autoclaving. Products made from such polymers may need to be supported to prevent distortion. Polymers where the softening temperature is higher than the autoclaving temperature may suffer from the release of moulded-in stresses and subsequent distortion. Where steam sterilisation is to be used, the effect of multiple cycles needs to be considered to prevent cumulative effects when the product is single-use disposable. If the products are packaged before autoclaving then packaging material and method need to be carefully chosen. The suitability of packaging for steam sterilisation will depend on the polymer, the size and wall thickness of the package and the contents, including any sharp corners, which may pierce the package. Polymers suitable for steam sterilisation are listed in Table 7.2.

The number of polymers capable of tolerating moderate temperature and moisture (steam and/or heated water) is more numerous than often considered:

- Natural (isoprene), ethylene propylene diene monomer (EPDM), urethane, nitrile, butyl and styrene-butadiene rubber.
- Fluoro plastics (other than PTFE and FEP) – that is, PVDF, PCTFE, PETFE.
- 'High-end' engineering resins, PEK, PEEK, polyetherimide.
- Nylons (polyamides), especially aromatics, 12, 11, 6/12 and 6/10.
- High-density polyethylene (HDPE) and UHMWPE.
- Polycarbonate (PC) and alloys.
- Polyesters (e.g. PET and PETG), but aliphatic forms are vulnerable to hydrolysis.
- Polysulfone (PSF) and polyphenyl sulfones.
- PVC; flexible and semi-rigid, colour, plasticiser and HCl corrected, where no load is involved.
- Syndiotactic polystyrene (S-PS); SAN can also be heat resistant.
- Some PUs.
- Polypropylene (stabilised), copolymers (PPCO) and polymethylpentene (stabilised).
- Thermosets – epoxies, phenolic, polyimides, PUs, aromatic polyesters.
- Silicones.

For details of the effects of steam sterilisation on these polymers, see Table 7.2. Resistance of polymers will depend on formulation, additives and stabilisers.

Unlike most other methods, steam is compatible with liquids (including drugs which are packaged in polymers) or filters that sterilise drsugs. Plastics transfer heat more slowly than metal and so it may take longer to reach sterilising temperatures in the autoclave. Because of differences in heat transfer characteristics between plastics and inorganic materials, the contents of plastic containers may take longer to reach sterilisation temperature (e.g. 121°C). Therefore, longer autoclaving cycles are necessary for liquids in large-volume plastic containers. Adequate cycles can be determined only by experience with specific liquids and containers.

Improvements in computer controls, monitoring devices, loading, biological and chemical indicators have paved the way for renewed applications of this technology, and the growing need for more compatible materials. Improvements in polymers for steam sterilisation are being made with addition of heat stabilisers, copolymerisation and improved polymerisation with metallocenes, pelletisation and moulding temperatures. Note: A metallocene is a compound – for example, consisting of two cyclopentadienyl anions ($C_5H_5^-$) bound to a metal centre (M (e.g. iron)) in the oxidation state II, with the general formula $(C_5H_5)_2M$. Closely related to the metallocenes are the metallocene derivatives – for example, titanocene dichloride and vanadocene dichloride. Metallocenes generally have high thermal stability.

The thermal and chemical (steam) degradation of polymers are closely inter-connected, as also are biological and chemical mechanisms. Thermal degradation of polymers by steam is similar to that described for dry heat (see Section 7.2.1), but with the addition of hydrolysis mechanisms. Thermal degradation may involve environmental stress, cracking and plasticiser migration and loss. Steam-induced chemical reactions include oxidation and hydrolysis, which result in particular problems.

Steam sterilisation can cause thermal degradation of polymers and this may be due to oxidation. The thermal degradation of polymers has already been described for dry-heat sterilisation. For example, the thermal oxidative degradation of polycarbonate may begin up to 150°C. Thermal decomposition of a polymer is the chemical decomposition caused by heat. The reaction required to break the 'chemical' bonds in the polymer undergoing decomposition is essentially an oxidative process. For example, some polyesters are somewhat resistant to steam sterilisation, but aliphatic forms are more vulnerable to hydrolysis than the aromatic form. Oxidative degradation may occur in PET at temperatures as low as 100°C; however, the ester bonds in aliphatic PET are prone to hydrolysis and the permanent use of the material in steam above 70°C should be avoided. Some PU formulations are very vulnerable to steam sterilisation because of moisture swelling of the material.

7.3.2 Adaption of steam (moist heat) sterilisation to minimise effects on polymers

Sterilisation techniques may significantly affect the properties of polymers, including their suitability for implantation. Under some conditions, a technique thought to be compatible with a polymer will not be suitable when tested. This incompatibility is often due to changes in process parameters, environment or due to additives that reduce corrosion. For example, the high-temperature flash steam process under vacuum may affect a polymer differently than the low-temperature gravity steam method. The lower temperature will be less harsh on the polymer than the high-temperature flash process.

While polymers can be selected based upon melting temperatures that exceed processing temperature, lower steam sterilisation temperatures can also be implemented so that polymers will become more stable over time (heat-resistance ageing). Polymers with lower melting temperatures can be used as possible future considerations include alternative or combination approaches to lower steam sterilisation temperature. For example, dialysers can be steam sterilised in place (SIP) on carousels and released in a JIT fashion through process controls and parametric release. These dialysers can also be sterilised with liquid water at high temperatures. Many pharmaceutical/healthcare plastic containers, such as high-density polyethylene, PVC and Polyallomer (a copolymer of propylene and polyethylene (PE)) filled with liquids can be steam sterilised at temperatures lower than 120°C. Steam sterilisation can be reduced, however, to as low as 105°C, depending on the bioburden, device design and heat resistance of the polymer material. Lower steam temperatures may be considered with use of acids or certain chemical additives.

Combining steam sterilisation with other sterilising or enhancing physical or chemical agents can further reduce sterilisation temperatures suitable for polymers with lower melting temperatures. For example, a steam–formaldehyde sterilisation method operates at 65–85°C. This approach could be applied to steam with EO or propylene oxide (PO), resulting in a preservative by-product such as propylene glycol for PPO that may be beneficial for incorporation in some biomaterials. An acidic medium for steam heat allows for reduced or lower sterilising temperatures below 100°C. High-density materials are typically more resistant than low-density materials (e.g. PE); except when the physical state of steam (vapor) is changed to heated (liquid) water.

Some miscellaneous concerns regarding steam sterilisation are the following:

- Some chemical additives (e.g. anti-rust agents) in steam will attack transparent plastics and cause a permanently glazed surface after autoclaving, or leave toxic residues after drying and removal of steam and moisture.

- Some transparent plastics (e.g. PVC) may absorb minute amounts of water vapour and appear cloudy after autoclaving. The clouding will disappear as the plastic dries. Clearing may be accelerated in a drying oven at 110°C. For PVC tubing, clearing is obtained at below 75°C for upwards of 2 h.
- Use of polypropylene copolymer (PPCO) bottles may be preferred instead of polysulfone with Tween in the autoclave.
- Steam sterilisation of PUs may result in formation of toxic leachable 4,4′-methylenebisphenyldiamine (MDA); however, a mixture of PU and polysilicone may result in acceptable biocompatibility.
- PP mixed with PE may result in an acceptable heat-tolerant copolymer (e.g. polyallomer or polypropylene copolymer).

In most situations, moist heat sterilisation temperatures are too high to allow many low temperature-tolerant polymer and biomaterials to function properly after high-heat sterilisation. However, since temperature is a useful tool for evaluating the shelf-life of many polymers, it is important to monitor potential changes in polymers or product functionally and performance in the life of an implantable. Also, consideration of sterility entity may be a concern with this technique, where it is sometimes more surface (e.g. silicone prosthesis) than penetrable.

7.4 Ethylene oxide (EO) sterilisation

Ethylene oxide (EO) is a traditional method that is able to sterilise many polymers, including heat-sensitive polymers, but not liquids. It may craze some polymers and it can leave toxic residues and by-products if not handled correctly. The EO technique has some penetration capabilities, but requires a long time for the overall process (e.g. preconditioning, sterilising and aeration). EO is an effective and soft sterilant for most reusable medical materials, polymers and devices. It is used in both hospitals and industrial manufacturing applications for manufacture of disposables.

Common limitations of EO sterilisation relate to diffusion barriers, process time and interactions. Diffusion barriers limit the efficacy of EO sterilisation if the EO gas, temperature and humidity necessary cannot penetrate into all locations within a device – for example, into a stopcock, a very long, thin lumen or large, dense product load. Long overall process times can be an economic limitation to the application to EO due to long preconditioning periods, extended exposure times, post-sterilisation aeration times and post-processing biological indicator testing. While parametric release is difficult to achieve uniformly with this method, faster release times can be achieved with the use of rapid biological indicator incubation times.

Hazardous material handling and toxic residues are issues since EO is an explosive, potential human carcinogen and reproductive toxicant. It requires gas mixtures or special handling, robust scrubbers for gas emissions and significant consideration of worker exposure.

7.4.1 Effects of EO sterilisation on polymers

EO sterilisation is compatible with nearly every polymer, except those that may be particularly sensitive to humidity, low temperature and high EO gas concentrations. EO sterilisation is very gentle with most polymers, and used wisely. Some polymers compatible with the EO technique are listed in Table 7.4. EO is compatible with nearly every polymer; if there is a problem with the polymer because of the technique, there often is an expert solution. EO can sterilise many polymers that can not be irradiated or heat sterilised.

Some of the limitations related to EO may be due to a polymer's absorptivity towards accumulating residues, but this will vary significantly with humidity, EO gas concentration, temperature and aeration. There may be some sensitivity to humidity – for example, for hydrophilic coatings – but there are usually solutions to this problem. Users also need to be careful with EO sterilisation when using polymers as carriers for drug delivery. Drugs such as Taxol-based formulations cannot withstand high-temperature and high-humidity EO cycles.

Although EO will sterilise most polymers and materials for medical devices, because it is a potential human carcinogen and reproductive toxicant, its use is limited and controlled. Post-sterilisation evaluation for toxic residues (e.g. ethylene chlorohydrin) must be performed before release or validation of product. Long exposure and post-sterilisation aeration times, as well as post-processing biological indicator testing, may make the process less practical.

Because it is a gentle process, there is virtually no polymer degradation per se with EO. There may be some effects due to humidity and EO gas carriers (e.g. Freons). If temperature, gas, pressure or humidity effects are high, there are ways to alter these parameters to eliminate their effect.

7.4.2 Adaption of EO sterilisation to minimise effects on polymers

A potential way of lowering EO cycle times, as well as reducing toxic residue levels, is to increase the sterilising temperature from 45–60°C to 70–80°C, as used with the steam–formaldehyde process. The higher temperatures drive EO and ethylene chlorohydrin residues towards ethylene glycol, which is not

Table 7.4 Some polymers compatible with the ethylene oxide technique

Thermoplastics	Effects
Acrylic	Good. Some loss in tensile properties, no discolouration reported on multiple cycles with HCFC-124/EO blends There may be some crazing Excellent with low EO/CO_2 concentration gas mixture, except at high sterilising temperature >63°C. Low EO cycle with EO/CO_2 gas mix had low absorbency and very short aeration
Acrylonitirile butadiene styrene copolymer (ABS)	Compatible High absorbence of EO and long aeration for desorption Excellent with low EO/CO_2 concentration Gas mixture with low EO concentration had low absorbance and short aeration
Non-plasticised polyvinyl chloride (PVC)	Compatible EO/CO_2 concentration Gas mixture with low EO concentration had very short aeration
Plasticised polyvinyl chloride (PVC)	Compatible Plasticised EO absorbs more than non-plasticised PVC Excellent with low EO/CO_2 concentration Gas mixture with low EO concentration had very short aeration
Polyacetal	Compatible, no degradation Low EO concentration with EO/CO_2 gas mix had short aeration
Polyamide (Nylon, all classes)	Compatible Increased residuals with high humidities; but low residuals with low EO concentration with EO/CO_2 mix
Polyarylsulfone	Compatible
Polycarbonate	Compatible. Some formulations may be subject to stress cracking and some loss of tensile properties after multiple cycles and an extended time post-processing, no discolouration
Polyether sulfone	Compatible
Polyetheretherketone (PEEK)	Compatible
Polyethylene (PE, UHMWPE, LDPE, LLDPE, HDPE)	Generally compatible. HDPE may lose some tensile properties, no off-gassing Excellent with low EO/CO_2 gas concentration mix; absorbs and desorbs EO well, very short aeration EO is excellent with UHMWPE for hip and knee implantation
Polyethylene terephthalate glycol copolymer (PETG)	Compatible

Polymethyl methacrylate (PMMA)	Compatible, no discolouration; EO acceptable for contact lenses
Polyphenylene oxide	Compatible
Polypropylene (PP)	Compatible. May be some long-term effect on tensile modulus. Excellent with 100% EO. Good with HCFC, no brittleness
	Can sterilise unstablised PP in syringes with no brittleness
	Excellent for 100% (pure) ethylene oxide gas. Good for HCFC-124 blend. Excellent with EO/CO_2 gas mixture
	Absorbs and desorbs EO well
Polystyrene	Typically poor. Some embrittlement and loss of tensile strength for some formulations has been reported
	However, polystyrene petri dishes have been easily sterilised (excellently) with EO/CO_2 gas mixtures and with moderate humidities; many European IV sets with styrene were compatibile with polystyrene parts
	Polystyrene tissue ware will absorb EO and will not desorb well enough
	For cell culture growth, unless low EO concentration in EO/CO_2 gas mix. No crazing and no residuals with low EO concentration with EO/CO_2 gas mix
Polysulfone	Compatible
Polytetrafluoroethylene (PTFE)	Compatible
Polyvinyl chloride	Compatible. Rigid PVC may decrease impact resistance after exposure. Medical-grade plasticised tubing may contain significant residual levels until aerated
	EO/CO_2 gas mixtures had little EO residuals with low EO concentration
	EO and CO_2 have the same molecular weight
Styrene acrylonitrile copolymer (SAN)	Generally OK for one cycle, but may embrittle and lose tensile properties on multiple cycles. May exhibit surface cracking and stress cracking on multiple cycles. Standard EO cycles have high EO absorbency and poor desorption, requiring long aeration
	Compatible with low EO/CO_2 gas concentration. Low EO concentration cycle with EO/CO_2 mix; had low EO absorbency and very short aeration
Styrenic block copolymer	Compatible
Polyester	Compatible
	With low EO concentration, EO/CO_2 mix had low EO absorbency and very short aeration time

(*Continued*)

Table 7.4 Continued

Thermoplastics	Effects
Polyetherimide (PEI)	Depending on formulation and application. Very thin tubing may present compatibility issues. Bulk structural materials are generally compatible
Polyurethane	Performance depends on formulation, cure conditions, material thickness and end use stresses. PU has high affinity for EO but releases with aeration
	Low EO concentration with EO/CO_2 gas mix had short aeration
Silicone (RTV)	Excellent; no cross-linking
Butyl rubber	Butyl is even stable in liquid EO
Ethylene propylene diene (EPDM)	Generally compatible, but changing curing method to sulphur cure from peroxide cure may result in formation of small amounts of polyethylene oxide inside the matrix of the material
Latex	Compatible, but may be limited to the number of repeat cycles
Neoprene®	Compatible
Polyvinylidene fluoride elastomer	Compatible
Silicone elastomer	Compatible; no cross-linking
	High absorbency or EO desorbs well for short aeration with low EO concentration
	EO/CO_2 mix had low EO absorbency, and very short aeration
	Non-elastomer prosthesis requires long aeration at high EO concentrations; but at very low EO concentrations with EO/CO_2 gas mixtures, EO residuals may be much lower
Teflons®	Good to excellent materials.There may be low EO absorbency, but very slow desorption in some types (e.g. PTFE), but not in PVDF. Low EO concentration in EO/CO_2 mix may result in very little EO absorbency

Note: EO residuals will vary between polymer types, polymer designs, thickness, formulation changes, packaging, etc. Typical aerations vary between 2 and 7 days.The above very short aeration was <12 h at ~50°C with initial low EO concentration with EO/CO_2 gas mixture.
Sources: AAMI TIR 17[1] and references 2 and 3.

toxic according to ISO 10993-7. Ethylene glycol is not as significant a residue as EO and ethylene chlorohydrin. Since the higher temperature and moisture at 70–80°C create more ethylene glycol, residues are not such a significant problem. Improvement of plastics with heat stabilisers and copolymerisation enhances the number that can be sterilised at these slightly higher temperatures.

Preconditioning of some polymers (e.g. cuprophane) allows EO sterilisation to be performed without in-vessel humidification of this moisture-sensitive dialysing material. EO sterilisation requires aeration and ventilation of toxic residues to minimum acceptable levels before medical devices or biomaterials are releasable. Heat, gas aeration/exchange, vacuum and time all help to remove EO residues.

EO may be used to sterilise many implantables. However, the complex matrices of many polymeric devices might result in high EO residue levels or the process may alkylate or hydrolyse chemically reactive molecules during implantation. While EO may be excellent for many implantables, the high cost of setting up validating chambers, process monitoring, environmental management, hazardous materials training, protective clothing, risk managements, EO recovery and additional regulatory paperwork, added to the operating costs, making it unattractive. Potential or possible changes in polymer or product functionally and performance over the life of the implantable must be monitored and evaluated.

7.5 Low-temperature hydrogen peroxide with plasma

Hydrogen peroxide (H_2O_2) has excellent microbiocidal properties, but poor penetration, yet is environmentally acceptable when controlled. H_2O_2 is typically used in the vapour phase for medical materials and devices. While compatible with many polymers, there are some materials that are damaged (e.g. acrylics, cellulosics (including paper), natural rubbers and bioadsorbables, such as polyglycolides and polyesters). It does not have the same penetration as pressurised steam, dry heat, EO or irradiation and is principally a surface sterilant.

It can sterilise somewhat short lumens, but cannot sterilise some polymeric materials and devices in their entirety. While its outcome is usually safe, sterilisation begins with a source of very hazardous highly concentrated H_2O_2. Plasma breaks down the H_2O_2 into water and oxygen. Because H_2O_2 has very high vapour or boiling point, very deep vacuums are required that may adversely affect some packaging and materials. Sterilisation is typically achieved in small vessels, not the large chambers or facilities used with dry heat, EO, radiation or steam.

7.5.1 Effects of H_2O_2 sterilisation on polymers

H_2O_2 and oxidising agents can sterilise a multitude of polymers. Some polymers compatible with H_2O_2 with plasma are listed in Table 7.5. The number of polymers is more limited than EO because of the oxidising effect of H_2O_2. However, it is more attractive than EO sterilisation because of its

Table 7.5 Compatabilities of some polymers with hydrogen peroxide (with plasma*)

- ABS (excellent)
- Acetal – significant colour changes or slight material changes after 10–100 cycles. Grade dependent
- Elastomers – silicones (excellent), thermoplastic polymer elastomer (TPE) (styrenic block copolymer compounds (SEBS), thermoplastic elastomer 'Q' polymer (TPQ)), natural (degrade), EPDM (fair to good), urethane (grade dependent), nitrile (good, grade dependent), butyl (excellent), styrene-butadiene (excellent), polyacrylic (good), polychloroprene (excellent)
- Fluoroplastics (PTFE and FEP, PVDF, PCTFE, PETFE) – excellent
- PEK, PEEK, polyetherimide (excellent, no change after 100 cycles)
- Nylons (polyamides), absorb, severe material degradation after 10–100 cycles. Grade dependent
- Polyethylene, LDPE < LLDPE, HDPE, UHMWPE (excellent, no change after 100 cycles)
- Polyesters (PE) and PETG – excellent
- Polycarbonate (PC) and alloys – excellent
- Polysulfone (PSF) – excellent
- Polyvinyl chloride (PVC) flexible and semi-rigid, colour, plasticised (good no resterilisation)
- PVC unplasticised (some colour change or surface changes after 50 cycles)
- Polyurethane (8 chemical varieties) – some colour change or loss of gloss after 100 cycles; however, polyurethane is a peroxide absorber and this can lead to decomposition of the peroxide needed for sterilisation
- Polypropylene (unstabilised) – excellent
- Polypropylenes (stabilised) and copolymers (PPCO) and polymethyl pentene – excellent
- Polystyrene and copolymers, ABS, PS, SAN – excellent
- Polyacrylics (PA, PMA, PAN) – grade dependent; significant material changes or crazing after 10–50 cycles
- Silicone – excellent, no change
- Thermosets – epoxies, phenolics, polyimides, polyurethanes, polyesters (grade dependent)
- Acrylic – fair, resterilisation not likely

Note: *Material compatibility with hydrogen peroxide vapour sterilisation may not be the same as that with low-temperature hydrogen peroxide with plasma.

Sources: AAMI TIR 17[1] and references 1, 3 and 5.

shorter process time and lack of residuals. Its very short processing time and absence of carcinogens make H_2O_2 very accessible. When designing devices, it is best to avoid absorbers, such as PU, nylon, EVA and cellulosic.

Low-temperature H_2O_2 with plasma has less effect on polymers than H_2O_2 vapour without plasma, because plasma destroys more peroxide residues than with aeration. Plasma and oxidising agents are generally applied only to small niche and minimal-sized devices. It is used predominantly in general hospitals, and less so in medical device manufacture. As a surface sterilant it may not be suitable for implantables.

7.5.2 Adaption of H_2O_2 sterilisation to minimise effects on polymers

Variations in sterilisation techniques may significantly affect the properties of polymers, including their suitability for implantation. Additionally, under some conditions H_2O_2 sterilisation, which is generally thought to be compatible with a polymer, may not be suitable when tested. This incompatibility is often due to small changes in process parameters, the environment or due to the formulation/stability of the sterilant or polymer. A reduction in H_2O_2 concentration will improve the compatibility of some polymers, as well as reducing processing temperature.

The highest MW materials (with the narrowest MW distribution) should be used for most applications. H_2O_2 treatment of implantables might require special processing, but it can sterilise UHMWPE used in knees, hips and shoulders. Concerns over oxidation and plasma effects, and its predominant surface nature, mean H_2O_2 with plasma has not been frequently applied to implantables. However, it is worth considering contacting equipment manufacturers for specific applications. Biocompatibility according to appropriate standards should be established for implantables, regardless of sterilisation technique selected.

7.6 Ozone sterilisation

Ozone is a very strong oxidiser, making it an effective and efficient sterilising agent. It is a relatively new technique for medical devices, although it has been used to sterilise water, etc. In vapour form, ozone can be used to sterilise medical products and other materials within a chamber. Because ozone is metastable, it cannot be stored and is therefore produced *in situ*, making the process safe and environmentally acceptable. At the end of the process, the ozone is degraded to oxygen. Because of the strong oxidising nature of ozone, materials must be resistant to oxidation. The main disadvantage of ozone includes its reactivity with certain polymers. It also has some penetration limitations (e.g. through organic matter and non-diffusible polymers).

A number of polymers are now sterilisable using ozone (see Table 7.6). Ozone sterilisation has recently been introduced to healthcare facilities. There are no toxic residues and it is more penetrable than H_2O_2 vapour (with plasma), but not as penetrable of polymers or devices as EO, steam, dry heat or irradiation.

In gaseous low-temperature ozone sterilisation, the process parameters include vacuum, time, temperature, ozone concentration, humidity and pressure. The ozone concentration is typically 85 mg/L for 15 min at 30–36°C. The process temperatures are generally low, making it suitable for temperature-sensitive materials.

Table 7.6 Some representative polymers sterilisable with the ozone technique

- Elastomers – silicones (peroxides and platinum cured), TPE (SEBS, TPO), natural (isoprene), EPDM, urethane, styrene-butadiene, butyl and natural rubber – are not likely materials
- Fluoroplastics, PTFE and FEP, PVDF, PCTFE, PETFE
- 'High-end' engineering resins, PEK, PEEK, polyetherimide
- Nylons (polyamides), especially aromatics, 12,11, 6/12 and 6/10, but some changes may occur after multiple cycles
- Polyacetals – OK, but some colour change and loss of gloss may occur
- Polyethylene, LDPE < LLDPE, HDPE, UHMWPE
- Polyesters (PE) – unsaturated polyesters are excellent
- Polycarbonate (PC) and alloys
- Polysulfone (PSF)
- Polyvinyl chloride (PVC) – flexible and semi-rigid, colour, plasticiser and HCl corrected
- Polyurethane (8 chemical varieties) – may be poor
- Polypropylenes (stabilised) and copolymers (PPCO), and polymethyl pentene that is radiation stabilised; otherwise unknown
- Polystyrene and copolymers, ABS, PS, SAN
- Polyacrylics (PA, PMA, PAN)
- Thermosets – epoxies, phenolics, polyimides, polyurethanes, polyesters – may vary

Sources: AAMI TIR 17[1] and references 1 and 3.

7.6.1 Effects of ozone sterilisation on polymers

During ozone sterilisation, ozone breaks down into reactive species, including hydroxyl radicals and atomic oxygen. Because of the strong oxidising nature of ozone, polymers must be resistant to oxidation. Polymers and medical devices should also be resistant to high relative humidity levels (>80%), which are required for ozone to be effective. Consequently, materials should be resistant to oxidation and moisture. The method cannot be used for fluids or woven textiles. Although many polymers may be satisfactorily used in the manufacture of a device intended for single use, they might not be effective for use with a reusable or refurbished device.

Polymers compatible with low-temperature ozone sterilisation are listed in Table 7.7. The compatibility of some polymers with ozone remains unknown. Woven materials, polystyrene, PU, butyl and natural rubber, and polychloroprene are unlikely to be compatible. Some cellulosics, however, may be compatible. The shape of a device as well as its design may be closely related to its stability and resistance to sterilisation. Device and polymeric parts with wide surface-to-mass ratios (e.g. fibrous materials) can undergo faster oxidative degradation. While such devices and materials are for single use or used in the manufacture of a device that has limited reuse, such a condition might not be satisfactorily used for a device with a longer expiration

Table 7.7 Compatibility of some polymers for ozone sterilisation technique

Thermoplastics	Compatibility	Number of cycles polymer may be compatible
Fluoropolymers		
Polytetrafluoroethylene (PTFE)	Excellent	No change after > 100 cycles
Perfluoro alkoxy (PFA)	Excellent	No change after > 100 cycles
Polychlorotrifluoroethylene (PCTFE)	Excellent	No change after > 100 cycles
Polyvinylidene fluoride (PVDF)	Excellent	No change after > 100 cycles. PVDF is considered a polymer of choice for ozone
Ethylene tetrafluoro-ethylene (ETFE)	Excellent	No change after > 100 cycles
Fluorinated ethylene propylene (FEP)	Excellent	No change after > 100 cycles
Polyacetals	Good	Colour change and loss of gloss. Slight to significant change may occur after > 100 cycles Contact equipment manufacturer
Polyacrylates (e.g. PMMA)	Good	Slight to significant material change may occur after 10–100 cycles Contact equipment manufacturer
Polyamides (e.g. Nylon)	Good	Colour change and loss of gloss. Significant material change after 10–100 cycles
Polycarbonate (PC)	Excellent	Slight surface change and loss of gloss. No significant change after > 100 cycles
Polyesters, saturated	Excellent	
Polyethylene (PE), various densities	Good	Colour change and loss of gloss. Significant material change may occur after 10–100 cycles
Polyimides (e.g. PEI)	Excellent	Slight surface change. No significant change after > 100 cycles
Polyketones (e.g. PEEK)	Excellent	Unfilled PEEK only – avoid sharp edges. Colour change and loss of gloss. No significant change after > 100 cycles
Polypropylene (PP) natural stabilised	Good	Colour change and loss of gloss. Significant material change may occur after 10–100 cycles Polypropylene may not be good for multiple reuse
Polystyrene	Poor	Significant material or surface change < 3 cycles
Polysulfones	Good	Slight surface change and loss of gloss. No significant change after > 100 cycles

(*Continued*)

Table 7.7 Continued

Thermoplastics	Compatibility	Number of cycles polymer may be compatible
Polyurethane (PU)	Not likely	Significant material or surface change < 3 cycles
Polyvinylchloride (PVC) rigid	Excellent	Colour change and loss of gloss. No significant change after > 100 cycles
Polyvinylchloride (PVC) plasticised	Good	Surface change may occur after 5–25 cycles
Thermosets		
Epoxies	Variable	Significant material change may occur after 10–100 cycles, check reliability and stability
Phenolics	Excellent	Loss of gloss. No significant change after > 100 cycles
Polyester, unsaturated	Excellent	
Polyurethanes	Not likely	Significant material or surface change < 3 cycles; not good
Adhesives		
Acrylic	Good	Application specific. Contact equipment manufacturer
Epoxy	Variable	Application specific. Contact equipment manufacturer
Fluoroepoxy	Good	Application specific. Contact equipment manufacturer
Silicone	Good	Application specific. Contact equipment manufacturer
Elastomer		
Natural rubber	Not likely	Significant material or surface change < 3 cycles
Butyl rubber	Not likely	
Ethylene propylene dienemonomer (EPDM)	Fair	Significant material or surface change with < 3 cycles
Silicone	Excellent	Slight material change after > 100 cycles
Styrenic block copolymers	Not likely	Significant material or surface change < 3 cycles
Polychloroprene	Poor	While in an ozone normal environment, it is OK, but under sterilisation significant material or surface change may occur with < 3 cycles
Urethane	Not likely	Significant material or surface change < 3 cycles

Sources: AAMI TIR 17[1] and reference 13.

period. Ozone and oxidising agents are generally applied only to small niche and minimal-sized devices. They are predominantly used in hospitals, but less so in industry. While ozone and H_2O_2 are both oxidising agents, their effects are different.

Ozone may sterilise some cellulosics better than H_2O_2, but H_2O_2 sterilises butyl rubber, urethanes and natural rubber better than ozone. Silicones may be sterilised better by ozone than H_2O_2. Ozone should have the capacity to diffuse and penetrate deeper than peroxide, but less so than EO, dry heat, steam or irradiation.

7.6.2 Adaption of ozone sterilisation to minimise effects on polymers

Under some conditions, a sterilisation technique such as ozone, which is thought to be compatible with a polymer, will not be suitable when tested. This incompatibility is often due to changes in process parameters, environment or due to the formulation or stability of the sterilant. Ozone sterilisation is compatible with a wide range of commonly used materials, including polymers such as PVC, Teflon®, silicone, PP, PE and acrylics. Ozone consists of O_2 with a loosely bonded third oxygen atom, which is available to oxidise other molecules. A very short half-life means that high concentrations and new ozone generation is required to inactivate microbes at 30–5°C. By reducing temperatures to below ambient, yet above freezing, less ozone is required for sterilisation because it is more stable at lower temperatures; however, lowering the temperature may increase exposure time. Reductions in ozone concentration and temperature will reduce its effect on polymers.

7.7 Radiation sterilisation

Radiation sterilisation has excellent penetration capabilities and is a relatively rapid process. Sterilisation is typically achieved with ionising isotopes (e.g. ^{60}Co) in high-voltage accelerators. It is effective for many single-use medical materials and devices, but not for reusables. Initial capital costs are high, so it is not often used in hospitals, but mainly in the manufacture of disposable devices. Radiation is an inherently fast process, requiring only one dose (e.g. 15–45 kGy), resulting in ease of application. Polymer compatibility is the major limitation of this method and must be ensured before application. Radiation can initiate deep molecular changes in polymers, which require shelf-life testing to demonstrate that no continued damage results. Multiple resterilisations by this technique are not commonly practised.

7.7.1 Effects of radiation sterilisation on polymers

Irradiation can cause changes in polymers that other methods will not, such as bonds scission, crosslinking or a combination of both. Radiation may cause odours, discolouration, embrittlement and degradation, or affect bond strengths, which may cause changes over the life of a polymer implantation. Polymers particularly sensitive to radiation include unstabilised PP, acetals, some Teflons® (e.g. PTFE, PFA, FEP), polyglycolic acid (PGA) and polylactide sutures, polymethylpentene, polyvinylidene fluoride, polymethyl metharylate (PMMA), some acrylic adhesives, butyl rubber, some cellulose esters, natural liquid crystal polymer and (via cross-linking) silicones.

The effects of radiation on polymers may be influenced by:

- chemical composition and formulation of the polymer,
- polymer morphology (crystallinity, MW and density),
- radiation dose and dose rate,
- temperature.

An understanding of radiation chemistry helps to assess why a particular plastic is affected in a certain way. When a plastic is exposed to gamma radiation (from ^{60}Co at energies of 1.17–1.33 MeV), molecular bonds are broken. The polymer either recombines into its original configuration or, if scission occurs, the MW is reduced and the polymer is weakened. Conversely, where cross-linking occurs, a large three-dimensional matrix is formed and the polymer is strengthened. The effects of radiation may also be influenced by the age and environment of the polymer. Higher bond energies result in molecules that are more stable under irradiation, and polymers with a benzene ring are generally very stable. Examples of radiation-stable plastics are listed in Table 7.8.

7.7.2 Polymer degradation by radiation

All plastics are affected by irradiation to some extent. Some effects are favourable or negligible, while others are not. Post-irradiation effects, attributed to trapped free radicals, the presence of peroxides and possibly trapped gases, explain why a PP component acceptable today will shatter in 2 years' time.

- PE is predominantly cross-linked; slight odours may result. HDPE is more resistant than LDPE.
- PP (unstabilised, natural) and polymethylpentene undergo both cross-linking and scission. Embrittlement, breakage and discolouration can occur at higher sterilising doses.

Table 7.8 Compatibilities of some polymers for irradiation

- ABS (excellent)
- Elastomers – silicones (peroxides and platinum cured), TPE (SEBS, TPO), natural (Isoprene), EPDM, urethane, nitrile, butyl, styrene-butadiene
- Fluoroplastics (other than PTFE an FEP) – PVDF, PCTFE, PETFE

PTFE and FEP may be adversely affected by irradiation

- 'High-end' engineering resins, PEK, PEEK, Polyetherimide
- Acetal is adversely affected by irradiation
- Nylons (polyamides), especially aromatics, 12,11, 6/12 and 6/10

Nylon may degrade oxidatively in applications that have large surface-to-mass ratios (e.g.films, fibres, adhesives)

- Polyethylene, LDPE < LLDPE, HDPE, UHMWPE

(high-density PE is more radiation-resistant than low-density PE)

- Polyesters (PE) and PETG
- Polycarbonate (PC) and alloys
- Polysulfone (PSF)
- Polyvinyl chloride (PVC) flexible and semi-rigid, colour, plasticiser and HCl corrected
- Polyurethane (8 chemical varieties). Aromatics may discolour some
- Polypropylenes (stabilised) and Copolymers (PPCO) and polymethyl pentene – radiation-stabilised are good

(natural polypropylene (unstabilised) has little tolerance to irradiation)

- Polystyrene and copolymers, ABS, PS, SAN
- Polyacrylics (PA, PMA, PAN)
- Thermosets – epoxies, phenolics, polyimides, polyurethanes, polyesters

Sources: AAMI TIR 17[1] and references 1, 3, 4 and 5.

- Polystyrene is very stable to radiation because of its benzene ring, although it may begin to yellow above 50 kGy.
- ABS is much less resistant to radiation than polystyrene, but it may be suitable for single-dose irradiation.
- PVC can discolour with irradiation, and it may produce HCl and leach plasticiser.
- Acetal or polyformaldehyde (POM) copolymers are sensitive to radiation and their chains are easily broken (embrittlement); the material often changes from solid to dust, colour from yellow to green.
- Polyamides (nylons) are sensitive to cross-linking, but many are suitable for a single dose; some multiple dose.

A polymer with high radical yields (e.g. G-values) after irradiation is less stable. Oxidation, caused by the presence of oxygen in the gamma-radiation process, can decrease cross-linking and increase degradation, or produce a tendency for chain scission to occur. Oxidation also causes peroxide,

carbonyl and hydroxyl groups to be formed. Post-irradiation effects explain why PVC tubing may not be compatible with certain drugs.

A few plastics are adversely affected by radiation doses of 25–40 kGy; others can be sterilised at lower doses (11–30 kGy). Many polymers are compatible with sterilisation doses of about 25–40 kGy; however, they deteriorate at higher doses or after multiple sterilisations (see TIR 17[1]). PTFE and PFA degrade at low doses, POM above 25 kGy and butyl rubber at slightly higher doses, depending on the grade. Table 7.9 below shows compatibility variations for sterilisation between 25 and 40 kGy.

While the resistance and degradation of the above polymers may vary, other factors may determine their biocompatibility and compatibility with radiation sterilisation.

- Phenolic antioxidants contained in most polymers are responsible for discolouration.
- The elastic modulus of a polymer may be affected by more than one dose of radiation.
- Fillers and reinforcing materials improve the radiation stability of adhesives, coatings and potting compounds. Adhesives, films, fibres, coatings and encapsulates react much the same way to irradiation as the materials from which they are derived.
- Nucleation may increase embrittlement.
- Electronic boards and circuits are not always compatible.

ABS and polycarbonate are generally compatible with one dose of radiation, but may not be sterilised up to 100 Mrad. Both may discolour, with ABS discolouring the most. ABS/polycarbonate blends lose physical properties linearly with an increase in radiation dose. Acrylic polymers are sensitive to radiation as a result of scission of the ester chain. Polymethyl methacrylate (PMMA) has been used in dosimeters because it is sensitive to radiation doses. Radiation-compatible acrylics, however, are available, but not typically for implantable or ophthalmic devices; optical clarity of PMMA may be affected.

7.7.3 Adaption of radiation sterilisation to minimise effects on polymers

With irradiation, there are frequently trade-offs to be considered to minimise effects on polymer properties. The effects of radiation on a polymer may be modified by:

- changing its chemical composition and formulation,
- modifying its morphology (crystallinity, MW and density).

Table 7.9 Compatibility variations for sterilisation between 25 kGy and 40 kGy

Polymer type	Resistant types	Evaluate or check	Degradation
Thermosets	PUR, phenolics, etc. polyimides, epoxies	Polyurethane	
Polyolefins	PP, PE, HDPE	PP, HDPE	Unstabilised or natural PPHDPE not as stable as low molecular weight
Technical thermoplastics	EVA, EVOH, ABS, ABS/PC, PA 6, PA 66, PET, PBT, PVC, Acrylics	PMMA, PC, PVC	POM (Acetal); aliphatic nylon degrades oxidatively when used in applications that have large surface-to-mass ratios (e.g. films, fibres, adhesives) PMMA too affected and discoloured for some applications (e.g. contact lenses)
Fluorocarbons	Perchlorotrifluoroethylene (PCTFE), polyvinylidene fluoride (PVDF), ethylenetetrafluoroethylene (ETFE)	Fluorinated ethylene propylene (FEP)	Perfluoroalkoxies (PFA), polytetrafluoroethylene (PTFE)
High-performance thermoplastics	UHMWPE, PA 46, PA 11, PA 12, PPA, PAA, PPS, PPO, PSU, PPSU, PI, PAI, PEI, PEEK	UHMWPE	Oxidation embrittles UHMWPE, and will continue to occur during *in vivo* use
Thermoplastic elastomers (TPE)	SBS, SEBS	PP/EPDM, PEBA, TPU, COPE (ether–ester copolymer)	
Elastomers	NR, NBR, HNBR, SBR, silicone	Chlorobutyl rubber, neoprene, EPDM, EPR	Butyl silicones can cross-link and become too stiff for some applications (e.g. prostheses)
High-performance elastomers	Fluoroelastomers, fluorosilicones	Silicones, AEM/ACM	Silicone can cross-link
Liquid crystal polymer (LCP)	Commercial, natural	Commercial LCP	Natural LCPs are not stable

Source: AAMITIR 17.[1]

Note: Polymers listed under the column 'Degradation' may be applicable with caution under certain unique situations, formulation changes, nitrogen inert gas, or low dosage.

If PP is modified with additives and stabilisers, it may become more resistant to irradiation. However, conventionally stabilised PPs may not be suitable for sterilisation by high-energy radiation doses (e.g. >30 kGy) because of the severe embrittlement and discolouration that occur immediately in the plastic. There are, however, several alternatives in the design of propylene polymers and formulations that solve these problems and yield resins suitable for irradiation at dosages up to 50 kGy. Early radiation-tolerant PPs were homopolymers stabilised with small quantities of phenolic antioxidants and large amounts of sulphide diester secondary antioxidants; however, these additives can discolour slightly after irradiation, depending upon the dose applied.

Modern resins that can withstand irradiation exhibit reduced crystallinity, narrow MW distribution and are formulated with hindered-amine light stabilisers, thus containing no discolouring phenolic antioxidants. Ethylene-containing random copolymers are also useful substrates for building radiation-tolerant formulations, as are homopolymers with low isotacticity or to which hydrocarbon oils or greases have been added. The hindered amines are, by themselves, non-colouring in PP, but they can interact with phenolic antioxidants to produce extremely deep yellow colours after irradiation. Therefore, when hindered amines are used in a PP formulation, phenolic antioxidants must be not be used.

Reducing the irradiation dose (e.g. from 25 to 15 kGy) also results in enhanced stability of polymer properties. The use of nitrogen in place of air helps to reduce the effect of oxidation of some polymers. Reducing the temperature down to 10°C or lower (e.g. dry ice), or even that of liquid nitrogen depending upon the material, also allows sterilisation of very sensitive biomaterials.

The use of antioxidants in irradiated polymers is important. For example, vitamin E improves the oxidative resistance of irradiated PE, but the mechanism of action is unknown. The use of other antioxidants may have synergistic effects on the wear and mechanical properties of irradiated PE. The application of electron beams instead of gamma irradiation also enhances the properties of a number of polymers, because of the speed of irradiation and lack of oxidation/ozone effects produced from gamma irradiation. This may be also true with X-rays. X-rays will result in less temperature generation compared with the impact of electrons on materials.

Aromatic materials are more resistant than aliphatic materials (e.g. PU); aliphatic PU may break down to relative toxic compounds (e.g. 4,4′-methylenebisphenyldiamine or methylenedianiline (MDA)).

- The use of non-phenolic additives will usually eliminate discolouration problems caused by phenolic antioxidants.
- Although natural PP and polytetrafluoroethylene (PTFE, Teflon®) are typically unstable when irradiated, alternatives and solutions are available that make radiation more suitable.

- PVC and PP should contain heat stabilisers to improve radiation compatibility.
- High levels of antioxidants improve radiation stability, so, in general, levels should be increased if the product is to be radiation sterilised.
- Within a given polymer class, the lower the density the greater the radiation stability.
- If copolymerisation of a sensitive material is possible, it should be attempted.

Some electronic boards or circuits are compatible with low irradiation doses. Premature ageing of plastics may occur due to the oxidative effects of irradiation; consequently, it is always prudent to evaluate accelerated ageing of plastics to assure that this is not a problem under real-life conditions. Some Teflons®, despite their high heat resistance, are degraded by radiation, although some thin films/coatings and certain types of Teflons® have been shown to be radiation-compatible at low doses.

- PE, which is predominantly cross-linked, is compatible with radiation by sterilising in nitrogen rather than in air (with oxygen). Slight odours can be reduced through modification of the formulation.
- Breakage of PP syringe tips has been used for blood-borne disease procedures in disposure of needles, with the needles on end of the tips.
- Radiation-stabilised propylene polymers are available, using high MWs, copolymerisation and alloying with PE containing additional stabilisers. Use of electron beams at high irradiation dose rates may further reduce the oxidative degradation of PP.
- Polymethylpentene is similar to PP, but can be irradiated at low doses.
- High-impact grades of ABS are less radiation-resistant than standard grades.
- PVC can be compatible with radiation, but release of HCl, discolouration and plasticiser leaching must be prevented. Addition of antioxidants and heat stabilisers helps, as does changing the plasticiser (DEHP or DOP) to one that is less toxic and non-carcinogenic.
- Resterilisation using radiation is not normal, although plasticised PVC may be resterilised.
- Among the polyamides (nylons), nylon 10, 11, 12 and 6–6 are more stable than nylon 6. Nylon films and fibres are less resistant to radiation.

Some general considerations when selecting plastics for irradiation:

- Use aromatic polymers (e.g. benzene rings are more stable than aliphatic polymers).

- Material degradation may be reduced by effective device design and material selection – that is, the use of materials with appropriate additives and modifications in the polymer chains.
- Although electronic components are typically not compatible, an increasing number is compatible with irradiation.
- Another means of overcoming compatibility issues in some cases is through the reduction of sterilisation dose required to achieve the desired sterility level. Also, it is important to note that the compatibility of materials is a strong function of the application, and the related material stresses. For example, in some cases it is possible to utilise Teflon® with radiation sterilisation despite it not being generally acceptable.

Additional information about radiation sterilisation material compatibility is provided in AAMI TIR 17.[1] Biocompatibility and functionality need to be evaluated depending on the end use of the polymer and conditions under which it will be used. Radiation is increasingly used for sterilisation of many polymers in numerous medical devices by means of additives and modifications to the polymer chain.

7.8 Sterilisation and polymer efficiency

The aim of sterilisation is to destroy all microorganisms on the surface of an article, in a fluid or within a polymeric product for implant, to prevent disease transmission associated with the use of that item. The concept of what constitutes 'sterile' is typically measured as a probability of sterility for each item to be sterilised. This probability is commonly referred to as the sterility assurance level (SAL) of the product and is defined as the probability of a single viable microorganism occurring on a product after sterilisation. SAL is normally expressed a 10^{-n}. For example, if the probability of a spore surviving were one in one million, the SAL would be 10^{-6}. In short, SAL is an estimate of lethality of the entire sterilisation process and is a conservative calculation. SALs for implantables are 10^{-6} and the choice of a 10^{-6} SAL was originally strictly arbitrary and is not been linked with any adverse outcomes, except possibly when measured incorrectly (e.g. from a surface of a product and not within a product for implantable).

The possibilities for polymers to be implanted in the human body are vast. Polymers used for implantation must be sterile, safe and non-toxic after sterilisation. Manufacturers or healthcare facilities must ensure that products to be implanted are entirely sterile after sterilisation. The types of polymers that can be implanted without harmful effects reflect the efficiency of different sterilisation techniques.

There are always trade-offs when selecting a method of sterilisation. Depending on the inherent properties required for medical devices and

products, a sterilisation technique must be selected that is compatible with the polymer materials to be used. As the use of polymers in medical devices and implants increases, it is important to understand the purpose of sterilisation, as well as the effects of different techniques. This includes not only sterility but also biocompatibility and physical/chemical compatibility.

7.8.1 Sterility entirety

Sterilised polymer implants must be entirely sterile, both within the polymer and on its surface. It is vital that polymers and biomaterials to be used as implants are sterilised in their entirety. Microbes (spores) trapped within polymers will typically be more resistant to sterilisation than those on the surface, and over time may activate, germinate and grow out from their trap site, thus infecting the human host.

The sterility of a product must be totally evaluated, not just on surfaces but also in areas within polymers. Thus, only sterilants that are capable of penetration should be used. Hydrogen peroxide, steam and ozone are not penetrable sterilants unless materials are highly porous. In contrast, dry heat, EO and irradiation are permeable to many materials. Electron beams are less penetrable than gamma or X-ray irradiation, and steam is less penetrable than dry heat for many polymers. PE is not permeable to steam or humidity, but EO will drive humidity and moisture through LDPE films. Nylon is permeable to moisture, but not to EO; however, pre-humidification will enable EO to penetrate nylon films.

Sterility throughout the implantable material is essential when polymers are hydrophilic, biodegradable or degraded with time, such as wear degradation, for example, of ultra-high molecular weight polyethylene for load-bearing devices. Encapsulated or hidden bacteria in the material may be released after a period of time. All implantable products and polymers must be sterilised within packaging, which must be appropriate for the sterilisation used.

Handling packages that are still warm and/or wet may compromise the barrier properties of the sterile wrapper, and the potential for contamination is increased. Sterile packages should be thoroughly cooled and dried before handling. At the end of a drying cycle, packages may still be warm and moisture may be trapped inside. If warm packages are handled with unsterile hands or placed on cold surfaces where condensation may form, the sterility of the package may be compromised. If the sterility of a wrapped item is in doubt, it should not be used.

A sterilised implant must be quarantined until the biological test or dosimeter reading. If the implant is placed in the patient before the results of the biological test are received, and if the test subsequently indicates the sterilisation failed, the only treatment for the patient is antibiotics and/or possible removal of the contaminated implant. In a situation in which the

patient is anaesthetised, it may not be reasonable or safe to wait for the results of the biological test.

7.9 Comparative efficiencies of sterilisation techniques for different polymers

In the design and development of implantable devices requiring sterilisation, consideration should be given to the choice of polymers and the needs of the patient, including the performance requirements of the finished device. The final product must meet safety and efficacy requirements while providing benefit to the patient. Product requirements can limit the choice of polymers available for construction and ultimately determine the acceptable mode of sterilisation based on compatibility. Product design characteristics also influence the sterilisation technique selected.

Selection of polymers for biomaterials requires consideration of design, processing and performance, including biocompatibility, functionality and sterilisation. The effect of sterilisation on polymers is a key factor in device design. Polymers must be selected so that the final products are compatible with the sterilising technique. Optimal selection of polymers for implantation depends on the effect of sterilisation and any biological effects, which may be similar to hydrolysis or oxidation. Selecting polymers with an 'excellent' response to sterilisation and passing preclinical biocompatibility tests are both critical for implantation.

The following list indicates the response to different sterilisation techniques[1–3] of polymers and their applications.

Polymers

PE: radiation (good to excellent, but may give off gas; low and moderate density more resistant and can be resterilised; HDPE can undergo oxidation); EO (excellent); steam (poor to good, high density more resistant); dry heat (poor to fair, but lower temperature improves for high density); H_2O_2 (excellent); ozone (excellent).

Applications: orthopaedics, joint replacements, tubing, medical packaging.

PP: radiation (poor to good, stabilised, but single use only); EO (good to excellent); steam (good and excellent with heat-stabilised grades; can be resterilised); dry heat (good excellent at low temperatures (up to 135°C) with heat-stabilised grades); H_2O_2 (excellent): ozone (excellent).

Applications: catheters, sutures, syringes, surgical filaments, surgical meshes used to reinforce soft tissue where weakness exists (for example, in the repair of hernias and chest wall defects), medical packaging.

Polymethylpentene: radiation (fair to good); EO (excellent); steam (good/excellent): dry heat (good/excellent up to 170°C); H_2O_2 (unknown); ozone (unknown).

Applications: containers, covers for medical instruments, TPX film.

Copolymers (e.g. PE/PP, polyallomer): radiation (poor to good, stabilised, but single use only); EO (excellent); steam (good, excellent with heat-stabilised grades which can be resterilised); dry heat (good, excellent at low temperatures (up to 135°C) with heat-stabilised grades); H_2O_2 (excellent); ozone (excellent).

Applications: parenteral solution containers, packaging, instruments, pneumatic and lubricant lines, tubes.

Polystyrene: radiation (excellent); EO (poor to good, but millions of parts have been acceptably sterilised and some formulations can be resterilised 2–5 times); steam (poor to excellent, with syndiotactic styrene); dry heat (poor to excellent, with syndiotactic styrene); H_2O_2 (excellent); ozone (fair).

Applications: containers, parts in IV sets, petri dishes, sputum cups.

Styrene–acrylonitrile copolymers: radiation (good to excellent); EO (poor to good, but many parts acceptable); steam (poor to fair); dry heat (poor to fair); H_2O_2 (excellent); ozone (unknown).

Applications: dialysis devices, IV connectors.

PMMA: radiation (fair to good); EO (good); steam (poor to fair at low temperatures, but not re-sterilised); dry heat (poor to fair at low temperatures); H_2O_2 (fair); ozone (good).

Applications: bone cement, contact lenses, corneal prosthesis, grout for artificial joints, orthopaedics, ophthalmology lenses, in membrane oxygenators.

Polyvinyl acetate: radiation (good); EO (poor); steam (poor to fair); dry heat (poor to fair); H_2O_2 (excellent); ozone (unknown).

Applications: film.

PVC: radiation (good); EO (excellent); steam (poor to fair up to 120°C if no load); dry heat (poor to fair up to 120°C if no load); H_2O_2 (excellent); ozone (good).

Applications: blood bags, catheters, containers, endotracheal tubes, films, hearing aid components, IV tubing, drip chambers and packaging, shrink tubing, storage bags, in ventilation systems.

Vinyl chloride copolymers: radiation (good); EO (excellent); steam (poor to good (without load) up to 120°C); dry heat (poor to good up to 120°C); H_2O_2 (unknown); ozone (unknown).

Applications: films, packaging.

Polyvinylidene chloride: radiation (good); EO (excellent); steam (poor to fair up to 120°C); dry heat (poor to fair up to 120°C); H_2O_2 (unknown); ozone (Application: medical packaging. unknown).

Fluorinated polymers (polytetrafluoroethylene (PTFE), PFA, PCTFE, PVDF, ETFE, FEP): radiation (mixed, some poor (e.g. PFE, FEP and PTFE)); EO (excellent); steam (fair to excellent); dry heat (fair to excellent, up to 170°C); H_2O_2 (excellent); ozone (excellent).

Applications: artificial joints and vasculature, fibre optics, surface treatments, stopcocks, tubing.

Polyamides (nylons): radiation (poor to good, depending whether aromatic or aliphatic); EO (excellent); steam (poor to excellent); dry heat (poor to excellent); H_2O_2 (good, but one use only); ozone (good).

Applications: bags, catheters, films, kidney dialysis, laparoscopy devices, special packaging, nylon spike.

Polyesters: radiation (fair to good); EO (excellent); steam (poor to excellent); dry heat (poor to fair); H_2O_2 (excellent); ozone (excellent).

Applications: covers, films, IV infusion fluid containers.

Polysulfone (PSF)**, polyphenylsulfone**: radiation (excellent); EO (excellent); steam (excellent, can be autoclaved thousands of times); dry heat (good to excellent); H_2O_2 (excellent); ozone (good).

Applications: handles for dental instruments, ophthalmic scopes and lenses, endoscopic devices, dialysers.

Polyethylene terephthalate copolymers (PETG): radiation (good to excellent); EO (excellent); steam and dry heat (good to excellent up to 134°C); H_2O_2 (unknown); ozone (unknown).

Application: packaging.

Polyethylene terephthalate (PET): radiation (good to excellent); EO (excellent); steam and dry heat (good to excellent); H_2O_2 (unknown); ozone (unknown).

Applications: angioplasty balloons, woven vascular prostheses, vascular grafts of large diameters.

Cellulosics (cellulose esters, cellulose acetate propionate, Cellulose acetate butyrate, cellulose (paper, cardboard)): radiation (fair to good, esters degrade less than other cellulosics); EO (excellent); steam (poor to good at low temperatures, depending upon the cycle); dry heat (poor to good, but at higher temperatures, there may be charring char); H_2O_2 (poor); ozone (poor to good).

Applications: films, filters, haemodialysers, membranes, IV burette champers, packaging.

Epoxies: radiation (excellent); EO (good to excellent); steam (fair to excellent); dry heat (fair to excellent); H_2O_2 (excellent); ozone (fair to excellent).

Phenolics: radiation (excellent); EO (good); steam (fair to good); dry heat (fair to good); H_2O_2 (good); ozone (excellent).

Polyimides: radiation (excellent); EO (excellent); steam (excellent); dry heat (good to excellent); H_2O_2 (excellent); ozone (unknown).

PUs: radiation (good to excellent, better if aromatic); EO (poor to good; steam (poor to fair); dry heat (poor to fair/good, at low temperature); H_2O_2 (good); ozone (poor).

Applications: blood pumps, catheters, connectors, containers, enteral feeding tubes, lipid-resistant stopcocks, needleless syringes, vials, balloons, pacemaker leads.

Acetals: radiation (poor); EO (excellent); steam (fair to good, up to 120°C); dry heat (good to excellent, up to 120°C); H_2O_2 (excellent); ozone (good).

Applications: structural keels for prosthetic devices, stopcocks.

Polycarbonate: radiation (good to excellent); EO (excellent); steam (fair to good); dry heat (fair to excellent, up to 134°C); H_2O_2 (excellent); ozone (excellent).

Applications: blood sets, cases, covers, cardiotomy trocars, in drug delivery devices, IV connectors, reservoirs, surgical instruments, safety syringes, valve occludes.

ABS copolymers: radiation (good); EO (excellent); steam (poor to fair); dry heat (poor to fair); H_2O_2 (excellent); ozone (fair).

Applications: in IV sets: Luer syringes, roller clamps, spikes, Y connectors, in dialysis units.

Elastomers (rubber)

Butyl: radiation (poor); EO (excellent); steam (fair to excellent); dry heat (poor to good); H_2O_2 (good, but only one cycle); ozone (poor).

Applications: tubing, closures (but not implantables).

Ethylene propylene diene monomer (EPDM): radiation (good to excellent); EO (excellent); steam (good to excellent); dry heat (fair to good); H_2O_2 (fair to good); ozone (fair).

Applications: tubing, other uses (but not implantables).

Polyketones, polyether ether ketone (PEEK), polyaryletherketone: radiation (excellent); EO (excellent); steam (excellent); dry heat (excellent); (excellent); ozone (excellent).

Applications: cardiovascular, orthopaedic, dental implants and tubing.

Nitrile: radiation (good to excellent); EO (excellent); steam (fair to good); dry heat (poor to fair); H_2O_2 (fair); ozone (unknown).

Applications: surgical gloves.

Polyacrylic: radiation (fair to good); EO (fair, but only one cycle); steam (poor); dry heat (poor); H_2O_2 (fair); ozone (good).

Polychlorophrene: radiation (good); EO (good); steam (fair to good); dry heat (poor to fair); H_2O_2 (excellent); ozone (poor).

Applications: tubing.

Silicone: radiation (fair to good); EO (excellent); steam (fair to excellent); dry heat (fair to excellent, up to 200°C); H_2O_2 (excellent, but surface sterilant); ozone (excellent, but surface sterilant).

Applications: catheters, membranes, prostheses (prosthetics), tubing.

Sterilisation that is physically/chemically compatible with a polymer may not be biocompatible. Information in the above list for a specific polymer is not an indication that the polymer is biocompatible. Biodegradation and failure may occur with some polymers. It is the responsibility of the 'user' to determine the

suitability and biocompatibility of a polymer for its specific application. The presence of additives, plasticisers and stabilisers can significantly affect the stability of many polymers, including their suitability for a specific sterilisation. A material that is thought to be compatible with a technique will not be compatible if evaluated under other conditions (e.g. irradiation of HDPE under air will be different when processed under nitrogen). This incompatibility is often due to oxidation, stability, formulation and/or processing changes in the polymer.

7.10 Post-implantation effects

Post-implantation effects often result from changes to physical and chemical characteristics that manifest as slowly visible changes to polymers. Impurities may leach out to affect cells or tissue after polymers have been implanted. In a mixture of polymers, leaching from one might affect the other. The breakdown of a polymer (to monomer) can result from a variety of physical, chemical and biological forces. All polymers are sensitive to degradation, but to differing degrees.

Polymer degradation may result from one or more of the following:[6]

- heat,
- oxidation,
- mechanical energy,
- electromagnetic radiation (UV, gamma or X-rays),
- plasma,
- ultrasound,
- hydrolysis, including enzymatic-catalysed,
- bacterial contamination.

The first six conditions involve absorption of energy that breaks primary covalent bonds, forming free radicals which may continue to take part in secondary reactions. Free-radical depolymerisation may occur in carbon–carbon polymer backbones. Hydrolytic mechanisms occur with polymers with different atoms, with depolymerisation occurring via the reverse of polycondensation. Hydrolytic degradation occurs in polymers with unstable bonds, such as ester and amide bonds, both of which exist in PU.

Biological degradation may involve biological enzymes, bacteria, cellular tissue and organ effects, and either chemical or enzymatic hydrolysis. While physical and chemical polymer degradation is well known among engineers, biology has added biodegradation, the result of enzymatic, foreign body effects and hydrolytic and ionic 'rate' mechanisms on polymers.

Enzyme-catalysed hydrolysis may be highly specific. For example, primary chains of collagen or gelatin are cleaved at the N peptide bond on the lysine side, but a poly-lysine chain is not degraded. Enzymatic degradation

is typical in breakdown and restructuring of natural polymers, such as proteins in healing wounds and restructuring of tissues. It is also common in cellulosics, such as the breakdown of starches and sugars. While enzymes may influence polymer degradation, bacterial effects on an implanted polymer may be more significant. Bacterial infection will release other enzymes and acids, both involved in the hydrolysis mechanism of degradation.

Many polymeric medical devices and biomaterials, such as cardiovascular and orthopaedic devices, may appear to be initially passive in their tissue interactions. However, when heparised or with applied additives, polymers implanted for a prolonged time or permanently may not remain passive. The properties of polymers vary depending on their predisposition to physical and chemical degradation, exposure to bacterial infections and the site of contact or implantation.

Polymers will contact tissue and/or bone in devices such as orthopaedic pins and plates, pacemakers, breast implants, replacement tendons, ligation clips and drug supply devices. Implanted devices that contact blood, include pacemaker electrodes, heart valves, vascular grafts, ventricular assist devices, internal drug delivery devices and stents. The properties of implants will also vary with the length of time they are implanted in a patient. Typical times are:

- limited implantation (≤ 24 h),
- prolonged implantation (> 24 h, ≤ 30 days),
- permanent implantation (> 30 days).

One post-implantation effect of prolonged or permanent polymer implantation can be proliferation of blood vessels and connective tissue at the implant site caused by changes in chemical and physical properties and/or motion of the device.

Granulation tissue can occur as a result of healing inflammation. Its earliest appearance is three to five days post-implantation, cauterised by proliferation of fibroblasts and vascular endothelial cells. Neovascularisation, often observed as pink, soft granular structure on the surface of healing wounds, may consist of fibroblasts, proteoglycans (early), collagen (later, type I) and vascular endothelial cells. Fibroblasts resemble smooth muscle cells and are responsible for wound contraction.

A 'foreign body reaction' is considered part of the normal wound-healing response to implanted biomaterials (polymers), which may persist for the lifetime of the implant. It consists of granulation tissue components, such as macrophages, fibroblasts, capillary formation, foreign body giant cells and fused macrophages, and may be involved in biodegradation of polymeric medical devices.

Fibrosis/fibrous encapsulation may also occur post-implantation. This is an end-stage healing response, which isolates implant and foreign body reaction from surrounding tissue. There are local and systemic factors where cells may grow or differentiate following injury, such as atrophy, hypertrophy, hyperplasia and metaplasia, as well as the production of different or too many proteins.

Biomaterial selection depends on the end use. Compatibility in one application does not ensure compatibility for another. Polymer and device characteristics to consider include chemical, toxicological, physical, electrical, morphological and mechanical properties, the effect of sterilisation, the conditions of tissue exposure and the nature of any risks.

It is essential to avoid potential toxicity problems arising from the sterilisation process in the case of medical devices that come into contact with human tissue (e.g. catheters, surgical tools and containers used for transplant preparation and storage). Because it may not be possible to predict the effects of every combination of material and sterilisation process, a simple test can be performed to ensure the absence of cytotoxicity. The test involves culturing a non-adherent cell line in direct contact with the test material, in micro-wells attached to the surface of the test device. Using this approach, sterilisation may be compared for each material considered for implantation.

Implantable polymers must:

- have good handling characteristics,
- be compatible with infection,
- be strong enough to prevent failure,
- invoke favourable host response (biocompatible),
- not limit post-implant function,
- not restrict future access,
- not shrink or degrade over time,
- be easy to manufacture,
- be inexpensive,
- not transmit infectious diseases,
- be sterilisable.

Degradation properties of polymers depend on the type of polymer used, its specific biological application and sterilisation technique employed.

7.11 Dry heat sterilisation of silicones

Reasons why dry heat is the best technique for silicones are outlined. Silicones are used for breast implants and other prosthetics because they do not absorb surrounding liquids and remain stable over a long period

after implantation. Successful sterilisation of breast implants using EO depends on the quantity of viable bioburden and presence of non-viable materials, including oils, proteinaceous films and extraneous production debris. Accumulation of oils and hydrophobic substances can agglomerate microbes, protecting them from the EO sterilant. Breast implants filled with silicone gel and oils are particularly inappropriate substances for this method. In addition, EO is highly absorbed by silicone gels, requiring extremely long times for off-gassing of EO residuals, which may not be reduced to safe limits.

Breast implants and other silicone prosthesis often have multiple cavities and imperfections, which can harbour bioburden. Steam sterilisation is not a viable alternative to EO for these multiple impenetrable cavities with non-hydroscopic surfaces. Irradiation could be an alternative, but it causes cross-linking of the polymer that causes stiffness. Gross microbial contamination of silicone prosthesis and multi-lumen implants could result from the application of steam or EO sterilisation, with viable microbes constituting a significant risk of infection with prolonged implantation.

Most silicone implants cause no macrophage or other tissue reaction, except for the effect of capsule formation to provide a sheath. In a minority of patients, however, foreign body reactions occur, possibly due to silicone fragments from a fragmented implant. Silicones may induce tumours (e.g. sarcomas) subcutaneously, but this is not due to the sterilisation technique. Pulverised silicones create no tumours and thus are not chemically carcinogenic. However, solid silicone may induce tumours after implantation. Silicone gels used in breast implants have caused problems from bacterial infections of tissues and circulatory systems.

Most implants undergo one or more thermal treatment during their production, coincidental with extrusion, moulding, vulcanisation, etc., which should impart some sterilisation or decontamination of heated components. Applying good clean room conditions, subsequent dry-heat sterilisation is expected to impart sterility to silicone implants and prosthesis. Silicone is highly heat resistant. Dry-heat sterilisation is well established for silicone implants, provided bioburden quantities are kept low and under control. It is sufficiently developed and validated to yield reliable silicone products with an excellent level of sterility assurance. For practical purposes, sterilisation never leads to an absolute sterile product, unless performed at temperatures that carbonise the organic matter of which microbes are composed. Silicone is not an organic material, and thus can withstand extremely high temperatures.

After implantation, elevated enzymatic activity can be observed, but enzymes have little effect on silicones. Certain silicone rubber heart valves may absorb some lipoidal content from blood, which in turn may lead to cracks in the heart valve.

7.12 Ethylene oxide (EO) sterilisation of polymers

7.12.1 Polypropylene – steam or EO sterilisation

Polypropylene is less toxic and more biocompatible with tissues than PE. However, to be compatible with irradiation, natural PP must be modified by incorporating additives (or by copolymerisation) capable of scavenging free radicals and preventing further oxidation. Subsequently, it is less biocompatible than natural PP. Techniques other than irradiation are thus preferred for natural PP to ensure biocompatibility. PP is more susceptible to strong oxidising agents (e.g. ozone) than PE. Heat-stabilised PP for orthopaedic implants can be sterilised using steam.

PP has been used in small sections for various surgical needs. Hydrolytic enzyme effects on PP are minimal, but it may degrade due to oxidation. However, the amount needed to construct a breast implant turns out to cause significant problems for patients. PP is a spongy material that may absorb liquid and expand after implantation. The risk of rapid expansion poses serious problems, and consequently PP is not recommended for breast implants.

PP surgical mesh can be sterilised using steam or EO. Complications that may occur following implantation of any surgical mesh include infection, inflammation, fistula formation, extrusion and adhesion formation when placed in direct contact with the intestine. Any implanted material must not be physically modified by tissue fluids, be chemically inert, not incite an inflammatory or foreign body cell response, be non-carcinogenic, not produce allergic reactions, stand up to mechanical stress and be capable of low-cost fabrication and sterilisation without tissue reaction.

PP, which is frequently used as an implantable mesh, induces remarkable chemotactic activity in tissues adjacent to a hernia prosthesis. PP may stimulate the immuno-competent cells of patients with prosthetic implants. The extent of foreign-body reactions is also influenced by PP filament structure and surface area, both of which favour monofilament materials. Tissue response to lightweight PP is characterised by a lower chronic inflammatory response than heavyweight PP. PP has been used as non-biodegradable sutures in eye operations and also in heart-valve structures.

In many situations, steam sterilisation temperatures may be too high to allow polymers and biomaterials tolerant to only low temperatures to function properly after sterilisation. Whereas heat-stabilised PP is more compatible with steam sterilisation, unstabilised PP may be degraded by heat. Degradation of PP may occur after three autoclavings. Consequently, EO is the preferred sterilisation method if more than one resterilisation is needed. If not, then steam sterilisation of a PP mesh should be carried out only once.

7.12.2 Acrylics – EO sterilisation

Acrylics are available as rigid, heat-cured, preformed materials of high clarity, widely used in intra-ocular lenses, or as cold-curing 'dough' that can be moulded and shaped into any form. The latter form is widely used in bone cements for orthopaedic applications. Acrylics have been used as implantable ocular lenses, bone cement for fixation of joint prosthetics or dentures, and maxillofacial prostheses.

Perspex gamma radiation is an acrylic that must be sterilised with dry EO as wet (> 0.5% relative humidity (RH)) EO may cause crazing. However, typical and impact-modified acrylics are compatible with EO sterilisation cycles with %RH.

PMMA is used in orthopaedic surgery to fix prosthetic components. Two additional post-sterilisation uses, which rely on its moulding properties, are in dentistry. PMMA is also used as a bone graft template and as a femoral window plug in total hip replacement. The use of PMMA bone cements to fix artificial prosthesis to the human body has become common in orthopaedic surgery. Hip and knee joints have very complex biomechanics and support high loads. Hence, acrylic bone cements must comply with international standards to ensure the bio-functionality and durability of the implant.

Acrylics are borderline sensitive to irradiation, and would not last long if used for implantation. While no new chemical entity is produced in the plastic after irradiation, irradiated lenses have produced tissue responses in patients. EO is a gentler sterilant than irradiation and improves the possibilities of implantation. A heat-resistant form of PMMA would be useful. During the manufacture/processing of PMMA, the polymer should not exceed 140°C to avoid liberation of monomer. After implantation the latter could escape into surrounding tissue and cause prolonged irritation.

The problems of contact lens-induced chronic inflammation (e.g. contact lens-induced papillary conjunctivitis) and acute inflammation (e.g. acute red eye) are less well-understood. Protein deposits, lens ageing, occlusion, mechanical effects and bacterial contamination have all been implicated. There is a need to understand and avoid what stimulates low-grade irritation and inflammation by making contact lenses more comfortable and improving their compatibility with the ocular surfaces.

For other implantation sites, cure-in-place PMMA formulations are used successfully. However, in PMMA bone cements, fibrous tissue capsules may occur that give rise to a foreign body reaction. The use of acrylics in dentistry can also lead to irritation and inflammation, especially, if toxic monomers occur as a result of excess heat in polymer manufacture.

7.12.3 Polyethylene – hydrogen peroxide or EO sterilisation

Ultra-high molecular weight polyethylene (UHMWPE) is used in orthopaedic implants, particularly at surfaces subject to high stress, such as those in hip or knee replacements. However, PE of lower MW could not withstand such stress. Radiation sterilisation is feasible for high MWs, but EO is preferable at low MWs. Aeration is required to remove toxic EO residues to avoid irritation of tissues, carcinogenicity, haemolysis, etc. The higher the MW, the more difficult it is to produce a homogeneous melt, and greater the risk of degradation before sterilisation. Degradation of PE is uncommon, except with irradiation.

Oxidation of UHMWPE by gamma irradiation results in some degradation. The extensive oxidation of UHMWPE after gamma irradiation or thermal treatments (e.g. steam or dry heat) can continue after implantation. EO is a viable alternative to gamma irradiation that avoids oxidation and fatigue-related degradation of load-bearing PE surfaces in total joint implants. PE tibia inserts have been used in a two-stage exchange arthroplasty of infected knees. Increased intensity or dose may require re-evaluation of sterilisation effects on PE implantation. Tissue necrosis does not typically occur with implanted PE, but there is considerable fibrosis.

Despite its excellent material compatibility with joint replacement materials, EO may not be suitable for sterilisation for other reasons. The cost of setting up sterilisation chambers, process monitoring and environmental management may not be justifiable. Hazardous materials training, protective attire and risk management, as well as EO recovery and regulatory paperwork, also add to operating costs.

Hydrogen peroxide (H_2O_2) appears to have the least problems associated with sterilisation of PE for implantation. There are no EO residues and processing with H_2O_2 is much faster, with sterilisation cycles less than three hours, including aeration. Vaporised hydrogen peroxide (VHP) provides for faster turnaround times than EO sterilisation, including reduced incubation for quick product release. Consequently, the VHP process is preferable for UHMWPE liners – for example, in hip replacements.

7.12.4 Polyurethane – steam, EO sterilisation or irradiation

Medical device applications of PUs include blood pumps, catheters, connectors, containers, enteral feeding tubes, lipid-resistant stopcocks, needles syringes and vials. Because of the possible complex behaviour of implantable PUs in the body, fabricators of PU-containing devices must pay particular

attention to the choice of composition and component design. Subsequent treatment during qualification, fabrication, sterilisation, storage implantation and *in vivo* operation may determine the performance and enable assessment of the efficacy of PU as an implant material.

PUs are a combination of ester and amide groups, which are vulnerable to hydrolytic decomposition. However, PU is also fabricated with an ether bridge, resulting in a polyether urethane (PEU). Steam and EO sterilisation may both cause MW reduction. Their use will depend upon the degree of MW reduction, which varies with hydrophilicity of the polyether segment. EO and irradiation sterilisation provide better results. The ester bond is more susceptible to degradation and cleavage if the PU is exposed to excessive heat in the presence of water (e.g. steam sterilisation). Hydrogen peroxide sterilisation is not used because PU is an absorber of the peroxide, which degrades the polymer. Dry-heat sterilisation may be compatible with some PU formulations at lower sterilising temperatures.

Isocyanates used in PU manufacture can induce allergic responses. Potential carcinogenic activity will vary significantly between different PU formulations. Steam or radiation sterilisation of some PU formulations may create toxic by-products – for example, 4,4′-methylenebisphenyldiamine or MDA.

Some PU sponges have caused tumours. However, PU is used as a material for prolonged or permanent implantation, as in pacemaker leads. Cardiac pacemakers frequently become infected and have to be removed. The same can occur with reused instruments. However, reuse should only be the cause of infection if cleaning and sterilisation procedures have failed to achieve sterility.

Use of PU may result in less firm encapsulation than occurs around silicone implants. However, PU implants cause less allergic reaction than silicone under some circumstances. PEU elastomers replaced silicone rubber for pacemaker lead insulation because they provide superior mechanical properties, are biocompatible (causing less allergic reaction) and were thought to be bio-stable. Although initial results were promising, over two decades of experience with PEUs have shown that these materials are not always bio-stable. In the case of PEU pacemaker leads, H_2O_2, a known product of inflammatory cells involved in the foreign-body response, permeated the outer insulation. The actual degradation of the PEU occurs when the H_2O_2 reaches the outer conduction coil of the lead where it decomposes into hydroxyl radicals, which subsequently cause chain scission in the soft ether segment, as observed *in vitro*. Localised regions of intense physical damage and chemical degradation occur in sections of the lead that are at least exposed initially to a high concentration of H_2O_2 from local cellular activity and large, repeated strains due to inter-corporeal movement. Chemical degradation and physical damage may have a synergistic effect on failure of the insulation.

7.13 Sterilisation issues relating to biodegradable polymers and coatings

Biodegradable polymers are useful for fabricating implantable medical devices, and as coatings for medical devices. Biodegradable polymers are biocompatible and may be tuned to provide optimum bioactive agent elution rates as well as degradation rates. Both medical devices and medical device coatings can use biodegradable polymers.

7.13.1 Biodegradable polymers

Commercially available biodegradable polymers are used in orthopaedic fixation devices, dental implants, ligature clips, sutures, tissue staples and skin-covering devices. Examples of the most widely used biodegradable polymers are polyhydroxyl acids, such as polylactic acid (PLA), PGA and their copolymer polylactic-*co*-glycolic acid (PLGA). Implants using these polymers are only required to last for weeks or months. The behaviour of these implants is determined by their glass transition temperature, which can be as low as 10°C. Residual stresses may remain in moulded parts after manufacture, leading to deformation on heating above the transition temperature.

PLA, PGA and PLGA are hydrolytically unstable. Hydrolytic degradation is influenced by water, moisture, steam, humidity, heat, acid, alkali and enzymes. Consequently, these polymers are affected by moisture during sterilisation. Steam or dry heat can lead to hydrolysis of the implants as well as deformation at higher temperatures. EO may cause some hydrolysis from the humidification step, and chemically may lead to moisturisation of the polymer. Additionally, EO sterilisation at 50–60°C and 40–50% RH is above critical temperature for these polymers. At 40–50% RH the activated surface of PGA absorbs water, which enhances degradation. For EO sterilisation to be effective it must be performed under very low % RH conditions. Complete removal of residual traces from the gas is also difficult to achieve. H_2O_2 is a surface sterilant, and the bioresorbable implant may need to be sterilised in its entirety to preclude patient infection during degradation. However, H_2O_2 is compatible with PGA, PLA and other sutures.

PGA and PLA typically do not survive irradiation. Irradiation at 25 kGy may induce degradation of the polymer chain, resulting in reduced MW and influencing mechanical properties. However, radiation sterilisation at lower temperature (e.g. 10°C, dry ice) may be effective at low doses (e.g. 16 kGy or higher). While immediately after irradiation at some doses, the tensile strength of PGA is insignificant, tensile loss may become significant after only seven days of implantation under a physiological environment.

When a polymeric material reaches the final stages of its degradation process, biodegradable material may cause a local foreign-body reaction.

In most cases, the symptoms of this tissue response are subclinical and pass unnoticed, but in some patients a clinical inflammation ensues. Reactions include a painful erythematous papule or a sinus discharging polymeric debris for up to six months. In severe cases, extensive osteolytic lesions may develop. For implants made of polyglycolide, the average incidence of such reactions may be 5%. However, when slow-degrading polymers are used, the incidence is lower. Tissue responses to polyglycolide manifest themselves, on average, around 11 weeks after surgery. Foreign-body reactions to devices made of poly-L-lactide can emerge as late as four to five years after implantation. A poorly shaped bone section, the use of a quinone dye as a polymer additive and an implant with a large surface area may lead to factors with increased risk of a foreign-body reaction. Laboratory experiments indicate that it may be possible to diminish the risk of an adverse tissue response by incorporating alkaline salts or antibodies to inflammatory mediators in the implants.

A biodegradable PU and a naturally derived polymer, gelatin, are used for liver manufacture. The structural design of some PEUs may allow both radiation and EO sterilisation. However, steam, irradiation and EO sterilisation will cause MW reduction. Biodegradation of PEU may be due to hydrolytic action on polyester and amide groups, or due to instability of the ether bond to oxidative deterioration.

Polyhydroxyalkanoates (PHAs) – for example, polyhydroxybutyrate [poly(3HB)] and poly-3-hydroxybutyrate-*co*-poly-3-hydroxyvalerate [poly(3HB-*co*-3HV)] containing 4–30 mol% hydroxyvalerate – are plastic-like polymers produced naturally by many types of bacteria. They are among the most promising future plastics because they are biodegradable and may be produced using renewable resources. PHAs are moisture-resistant polyesters and films can be sterilised by conventional methods (heat treatment and gamma irradiation), with no impact on strength.

Collagen, a natural biopolymer, is used as a biomaterial in surgical sutures and also used in solution to eliminate scar crypts serving as drug delivery vehicles. Sterilisation of collagen solution without deterioration is complicated because heat denatures it, but other methods are not typically applicable to solution. Sterilisation of cross-linked collagen fibres, films, membranes and sponges has been performed by irradiation and EO. Sterilisation by irradiation at doses greater than 50 kGy may lead to loss of crystallinity, increase in solubility, as well as other changes. Residues must be evaluated in the case of EO sterilisation because natural materials may lead to EO by-products.

Glutaraldehyde may be another sterilisation method, but its residues may elicit tissue toxicity after implantation. Also, it is difficult to maintain sterility after processing because of the need to remove residues without a package barrier. Tests should be performed to determine how much of the product or compound is absorbed by the body and to determine its effects over time.

7.13.2 Coatings

Coatings play an important role in implantable devices by improving the functionality the polymers used. Possibilities include: improved surface quality to enhance lubricity; improved resistance to friction, chips and impact for device protection; improved adhesion of tissues to polymer materials; special bio-functions, such as inhibition of blood coagulation via coatings with anti-clotting properties; hydroscopic or hydrophobic surfaces that help to absorb body constituents or drugs or resist absorption (e.g. of drugs) so that therapeutic activity can be maintained. The use of medical devices can be expanded by 'surface modifiers' that add a variety of important properties. For example, via coatings, enhanced biocompatibility can be achieved at low cost without changing the polymers from which a device is fabricated.

Consequently, selecting a sterilisation technique that is biocompatible and physico-chemically compatible with such coatings is important. For example, the use of hydrophilic polymers as a coating for medical devices is of particular interest. Steam or humidity in ethylene oxide and ozone sterilisation may cause these hydrophilic coatings to swell and become non-functional or unuseable. However, non-hydrophilic coatings are more compatible with humidity or steam. When used subsequently in implantable devices, they must, of course, be biocompatible after the selected sterilisation technique.

7.14 Biocompatibility testing[5]

An essential material safety requirement for polymers used in medical applications is biocompatibility. New implant designs and polymers must receive careful, preclinical evaluation. The materials and the processes used in device manufacture must be selected to ensure that the device is biologically safe for its intended use. The manufacturer must take into account the sterilisation process and the intended shelf-life of the device. Biological hazards include minor symptoms, such as irritations, to obviously serious toxicological symptoms, such as mutations and cancers, reproductive/developmental toxicants, as well as malfunctions.

The effects of polymers after implantation must be analysed, evaluated and studied by a series of biocompatibility tests prior to implantation. Subsections 7.14.1 to 7.14.12 refer to ISO 10993 standard and references 7 to 15.

7.14.1 Genotoxicity – ISO 10993-3

Genotoxicity testing evaluates gene mutations, changes in chromosomes or DNA and gene toxicities caused by by-products or compounds over an extended period of time. The International Organization for Standardization (ISO) standard 10993-3 outlines tests for genotoxicity, carcinogenicity and

reproductive toxicity. The ISO guidelines for genotoxicity testing require examination of gene mutation (bacterial mutagenicity test), chromosomal aberrations (chromosomal aberration assay) and DNA effects (mouse lymphoma assay). The FDA also requires three genotoxicity tests. The bacterial reverse mutation and the *in vitro* mouse lymphoma tests are the same as those recommended by ISO. A third test, which some within the FDA recommend, is an *in vivo* test, such as the mouse micronucleus test.

7.14.2 Carcinogenicity – ISO 10993-3

This test is performed only if there are data from other sources suggesting possible difficulties. The test needs to be performed over most of the test subject's life. It looks for tumorigenicity as well as chronic toxicity.

7.14.3 Reproductive/developmental testing – ISO 10993-3

This test is performed when there is concern that the reproductive system could be affected. It tests the effects of the material or implant on the reproductive system, embryo development, as well as pre- and post-natal development.

7.14.4 Haemocompatibility testing – ISO 10993-4

These tests evaluate the effects of product or compounds on blood or blood components, directly or indirectly during routine use. Haemocompatibility testing evaluates the effects on blood/blood components of blood-contacting devices/polymers. Thrombosis, coagulation, platelets, haematology, immunology are examined via simulation of geometry, contact conditions, flow dynamics, and blood reactivity differences between species via short- and long-term testing. The degree of haemolysis is measured spectrophotometrically.

The activation of complement proteins due to the use of a medical device has been associated with adverse clinical reactions. An enzyme immunoassay is used to screen for complement components in human serum that has been incubated with the test article. Elevated levels of complement components C3a and SC5b-9 indicate activation of the complement system. Both C3a and SC5b-9 assays are available.

One test determines the time citrated human plasma takes to form a clot, when it is first exposed to the test material, then to calcium chloride and, finally, to partial thromboplastin. Test results may report the 'partial thromboplastin time' (PTT) – that is, the time it takes the recalcified citrated plasma to clot

once the partial thromboplastin has been added. The test material is removed and examined for the presence of thrombi, and the vein is examined for patency (occlusion). These observations are complemented by photographs.

7.14.5 Cytotoxicity testing – ISO 10993-5

A cytotoxicity test determines whether a product or compound will have any toxic effect on living cells. These tests are typically used to test raw materials or components at the design stage and as a periodic test of material quality during production. These tests involve exposure of substances extracted from test material to one of two cell culture lines. Cytotoxicity *in vitro* testing is also used to ensure material biocompatibility. The ISO test method is used to meet international regulatory requirements. The USP test method meets the FDA's US regulatory requirements.

7.14.6 Implantation – ISO 10993-6

This test studies the effects of products or compounds on living tissue. Exaggerated amounts of material should be used. It is important to calculate the maximum amount of material that would be used and then implant multiples of that amount in an experiment. These studies help determine whether device surface characteristics, polymer composition and physical geometry affect local tissue responses, such as inflammation, tissue in growth, vascularisation and fibroplasia.

Acute inflammation can be characterised by: neutrophils of short life (hours to days); measurements of monocytes and macrophages at their highest concentrations; observations of exudation of fluid and plasma proteins; phagocytosis; and recognition, attachment, engulfment and degradation of foreign materials by leukocytes. Chronic inflammation occurs from persistent inflammatory stimuli such as macrophages, key mediators in immune reaction development which release growth factors. Other possible aspects are lymphocytes and plasma cells, antibody production, delayed hypersensitivity response, and there can be blood vessels and connective tissue proliferation related to an implant, localised at the implant site, caused by chemical and physical properties and/or motion device(s) or polymers. Gross and histologic photomicrographs can also be used.

7.14.7 Biodegradation – ISO 10993-9

These tests evaluate how much of the product or compound is absorbed by the body and follows the product or compound through the body after it has been absorbed to determine the effects over time.

7.14.8 Sensitisation – ISO 10993-10

This test evaluates sensitivity (e.g. allergic reactions) by the body to an implanted material or device. The murine local lymph node assay (LLNA), for example, has become a standard test method with good sensitivity and specificity, especially for delayed-type hypersensitivity when combined with statistical data analysis and negative control groups.

7.14.9 Irritation – ISO 10993-10

This test determines how irritable a product, material or compound is to the body. Studies should be made in combination with how the product or compound will be used and affected areas should be tested to determine the effect over time. For ocular, dermal and mucosal tissue contact, the appropriate test is selected. For breached tissue and blood contact, an intracutaneous test is chosen and typically uses only extracts. The dermal irritation test usually involves direct contact with the test material. The mucosal irritation test can involve either direct contact or use of extracts. The ocular tests usually use extracts. Extracts are prepared using solvents that will extract either hydrophilic (polar) or lipophilic (non-polar) compounds present in the device materials.

7.14.10 Acute systemic toxicity – ISO 10993-11

This test identifies the effect of exposure to a product or compound within 24 h. Acute toxicity occurs after a single exposure or repeated exposures to the test subject. Sub-acute symptoms appear within 14–28 days of delivery.

Acute toxicity tests estimate the potential harmful systemic effects from a single exposure to polar or non-polar extracts of device materials. Sub-acute toxicity is assessed after single or multiple exposures to extracts of device materials. The exposure period is longer than typical acute toxicity tests, but not exceeding 10% of animal's life span. Sub-acute studies involve expanded evaluations and can include systemic changes, local irritation, body weight, blood values and tissue changes as part of the protocol. The length of time for the test and the parameters evaluated depend on the end use of the device.

7.14.11 Sub-chronic toxicity – ISO 10993-11

Studies that continue for 90 days or for up to 10% of a test subject's life span are considered sub-chronic. Studies that continue for longer than 10% of a test subjects, life span are considered chronic.

7.14.12 Chronic toxicity – ISO 10993-11

Chronic toxicity studies can require that animal subjects be exposed to varying doses of test agents over long-term studies lasting two years or longer. If the device involves new chemistry that (from material characterisation and exposure assessments) indicates a high enough risk, one or more of these studies may be necessary. Chronic toxicity tests carried out over at least 10% of an animal's life span determine carcinogenicity or tumour-generating potential with single/multiple insults.

7.14.13 Summary

For implantable polymers that require special processing, the supplier or manufacturer should be contacted. A polymer used as a biomaterial in an implanted medical device must be proven to be non-toxic, biocompatible and safe to FDA and other regulatory standards before use. Material selection must meet the stringent requirements of ISO 10993-1[7] (see Table 7.10). The materials are tested after exposure to the sterilisation technique. The biological testing of the polymer is dependent on the intended contact duration. Body-contact polymers are characterised as surface contact, external communicating and implant. Implanted polymers have the most stringent requirements (see Table 7.10).

7.15 Conclusions

Studies found in the literature form the foundation for the work going forward, and they provide some good guidelines and insights. However, experience with real-world polymers shows a need for more careful and thorough evaluations. For example, potential polymer toxicity does not have the same response in older patients than younger patients, who often have biological repair mechanisms that older patients no longer have. In general, the trends point towards a need for a little less optimism and more careful understanding of the interaction between design, material selection, sterilisation, biocompatibility, environment and final polymer product performance.

Sterilisation is an important challenge and polymers known to be heat sterilisable and biocompatible have intrinsic long-term advantage. Heat sterilisation enables devices to be completely sterilised, is inexpensive, enables resterilisation, is more readily available and accessible in healthcare facilities. Heat sterilisation at lower temperatures will allow more heat-sensitive polymers to be sterilisable because the new sterilisation techniques are for niche applications, provide less penetration and are small scale. Heat sterilisation uses no toxic chemicals, does not generate toxic waste and is thus environmentally safe. Polymers and packaging materials continue to become more

Table 7.10 Initial evaluation tests for consideration

Device categories		Initial evaluation test (biological effect)								Supplementary evaluation test (biological effect)			
Body contact	Contact duration	Cytotoxicity	Sensitisation	Irritation or intracutaneous reactivity	System toxicity (acute)	Sub-chronic toxicity (sub-acute toxicity)	Genotoxicity	Implantation	Haemocompatability	Chronic toxicity	Carcinogenicity	Reproductive/ developmental	Biodegradable
Surface devices													
Skin	A	X	X	X	–	–	–	–	–	–	–	–	–
	B	X	X	X	–	–	–	–	–	–	–	–	–
	C	X	X	X	–	–	–	–	–	–	–	–	–
Mucosal membrane	A	X	X	X	–	–	–	–	–	–	–	–	–
	B	X	X	X	O	O	–	O	–	–	–	–	–
	C	X	X	X	O	X	X	O	–	O	–	–	–
Breached or compromised surfaces	A	X	X	X	O	–	–	–	–	–	–	–	–
	B	X	X	X	O	O	–	O	–	–	–	–	–
	C	X	X	X	O	X	X	O	–	O	–	–	–
External communicating devices													
Blood path, indirect	A	X	X	X	X	–	–	–	X				
	B	X	X	X	X	O	–	–	X	–	–	–	–
	C	X	X	O	X	X	X	O	X	–	–	–	–

(*Continued*)

Table 7.10 Continued

Device categories		Initial evaluation test (biological effect)								Supplementary evaluation test (biological effect)			
Body contact	Contact duration	Cytotoxicity	Sensitisation	Irritation or intracutaneous reactivity	System toxicity (acute)	Sub-chronic toxicity (sub-acute toxicity)	Genotoxicity	Implantation	Haemocompatability	Chronic toxicity	Carcinogenicity	Reproductive/ Developmental	Biodegradable
Tissue/bone/dentine communicating*	A	X	X	X	O	–	–	–	–	X	X	–	–
	B	X	X	O	O	O	X	X	–	–	–	–	–
	C	X	X	O	O	O	X	X	–	–	–	–	–
Circulation blood	A	X	X	X	X	–	O†	–	X	O	X	–	–
	B	X	X	X	X	X	X	O	X	–	–	–	–
	C	X	X	X	X	X	X	O	X	–	–	–	–
Implant devices													
Tissue/bone	A	X	X	X	O	–	–	–	–	–	–	–	–
	B	X	X	O	O	O	X	X	–	–	–	–	–
	C	X	X	O	O	O	X	X	–	X	X	–	–
Blood	A	X	X	X	X	–	–	X	X	–	–	–	–
	B	X	X	X	X	O	X	X	X	–	–	–	–
	C	X	X	X	X	X	X	X	X	X	X	–	–

Notes: X = ISO Evaluation tests for consideration; O = additional tests which may be applicable.
* Tissue includes tissue fluids and subcutanous spaces; † for all devices used in extracorporeal circuits.
Contact duration: A, limited (24 h); B, prolonged (24 h to 30 days); C, permanent (>30 days).
Sources: ANSI/AAMI/ISO 10993-1, AAMI, 2003.[7]

heat stable, heat sterilisable and less costly because of demand not only for medical devices, but also for other applications. In particular, heat-resistant fluoropolymers should provide cost-effective solutions to the ever-growing demands of biocompatibility and modern medical technology.

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8

Sterilisation of healthcare products by ionising radiation: sterilisation of drug-device products and tissue allografts

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Abstract: Ionising radiation can be used to provide a terminal sterilisation process to sealed packages containing a wide range of biomaterials and healthcare products, either in solution or in soft matter and solid states. This technique is therefore an attractive alternative to other means of sterilisation particularly for expensive, low production volume items and for which sterility assurance levels (SALs) may be difficult to validate. The main challenge is to prevent degradation of the products while achieving typical SAL values of $1{:}10^6$. This chapter, therefore, reviews the current state of the various protocols used to sterilise biomaterials and their components and devices.

Key words: ionising radiation, sterilisation, biomaterials, healthcare products, proteins, drug-device combination products.

8.1 Introduction

The principle of the use of any sterilisation technique is to reduce the bioburden level on healthcare products to an acceptable sterility assurance level (SAL), while at the same time, minimising damage to the product, again to an acceptable level. Ionising radiation is used effectively to sterilise many healthcare products by the lethal action of radiation on the bacteria, viruses and spores. However, radiation will also damage the healthcare product. In some cases, such as metal devices, the damage is often regarded as negligible, in other cases, such as for enzyme preparations, there may be extensive and unacceptable changes which prevent its effective use. Here, it is important to understand the mechanisms of radiation-induced damage so that approaches can be taken to minimise it for more vulnerable types of healthcare product.

The main driving force for research into radiation chemistry and biology has been to understand the effect of radiation on living systems and, thus, the research literature is dominated by aqueous systems. This area of research is clearly relevant to many healthcare products which may contain, for example, antibodies, human-derived products such as plasma and

urokinase, other proteins and enzymes, drugs or indeed combinations of these, as drug-device combination products. The latter are, however, often solid products where, for example, both synthetic and bio-polymeric systems are used either as a purely physical structure for the other components or as a drug-release material. In addition, there are many other solid manufactured healthcare products, such as syringes, sutures, metal devices, etc., which can also be sterilised by ionising radiation. It is important, therefore, to understand the different effects of radiation on both aqueous and solid systems. These differences are outlined below and are illustrated by discussion of the effect of radiation on proteins in aqueous solution and drugs and drug delivery systems in the solid state.

8.1.1 Types of drug-device combination products which require sterilisation

In order to develop strategies to sterilise drug-device combination products by ionising radiation, it is essential to understand the challenges presented by the wide range of components used in these devices. The types of device fall generally into the following categories: drug-eluting stents, anti-microbial venous catheters, antimicrobial urinary catheters, orthopaedic device-based drug-delivery products, device integration and tissue regeneration products, wound dressings, cerebrospinal shunts and corticosteroid release devices (reviewed in Wu and Grainger, 2006).

Drug-eluting stents consist of a relatively rigid endovascular scaffold made of expandable woven metallic wire or of an etched tube. They are coated normally with a thin non-degradable polymer, typically poly-isobutylene or polymethacrylate, which controls the release of a drug. Following the initial success of the sirolimus-eluting stent Cordis` CYPHER™ in 2003, other drug-eluting stent products have been developed rapidly. Other polymer coatings are being developed, notably using polylactic acid or polylactide-co-glycolide coatings, which are biodegradable (Hermann *et al.*, 1999; Finkelstein *et al.*, 2003). The success of drug-eluting stents has stimulated the development of other drug-delivery systems. The drug-eluting bead, based on a poly (vinyl alcohol) hydrogel modified by a sulphonic acid-containing component, allows interaction with drugs having an opposite electric charge to the beads (Lewis *et al.*, 2007). Antimicrobial agents incorporated onto catheter surfaces may also be categorised as drug-device combination products (Wu and Grainger, 2006). Alternatively, antibiotic agents are incorporated into the polymeric material of the catheter (Zhang, 2000). The most effective are used in combination – for example, minocycline with rifampicin (Raad *et al.*, 1996; Darouiche *et al.*, 1999) and chlorhexidine with silver sulphadiazine (Veenstra *et al.*, 1999).

Orthopaedic implants, as new combination devices, are being developed to promote and accelerate bone neogenesis and bone healing. The delivery of small molecules with osteo-inductive properties as well as growth factors, anti-osteoporotic agents and osteo-synthetic genetic materials is an active area of development (Wu and Grainger, 2006). Biphosphonates, for example, are used widely in the treatment of postmenopausal osteoporosis because of their inhibitory effect on osteoclastic bone resorption. The control of bone infection (osteomylelitis) is also being achieved in orthopaedic combination devices and includes antibiotic-eluting bioceramics, drug-containing bone cements and polymers loaded with antimicrobial agents (Baro *et al.*, 2002). Several combination devices in which the bone cement based on polymethylacrylate (as PMMA beads) is loaded with antibiotics such as erythromycin, colistin tobramycin and gentamycin are now commercially available and approved for clinical use in both Europe and in the USA (Wu and Grainger, 2006). PMMA can also be loaded with other agents such as anti-osteoporotic molecules, proteins and peptides (growth factors) (Downes, 1991). Polyhydroxyalkanoates, polyesters of biological origin, are also being used in the treatment of osteomyelitis (Gursal *et al.*, 2001).

Combination devices involving the controlled release of mitogenic and morhpogenic agents to promote locally controlled tissue responses, tissue engineering and regenerative medicine are being developed rapidly. These involve the use of cytokines and chemokines and also proteins and plasmid DNA (Richardson *et al.*, 2001).

In other categories of drug-device combination products, wound dressings comprise typically hydrogels containing antibiotics such as neomycin sulphate, bacimycin zinc and polymyxin B sulphate. Cerebrospinal shunts include silicone ventricular catheters loaded with rifampin. Corticosteroid release products for the control of fibrosis are usually based on dexamethasone and use, for example, poly (vinyl alcohol) or poly (lactic-*co*-glycolic acid) microspheres as the release agents (Wu and Grainger, 2006).

It is clear from the above brief description of the components of drug-device combination products that there is a wide range of chemical and biochemical materials and compounds in use. Any method of sterilisation must take into account the possibility of degradation of all of the components and hence potential loss of functionality. The aim is to avoid degradation within some defined limits. Radiation chemistry and radiation biochemistry are supported by an extensive experimentally based research literature with some obvious applications – for example, to radiotherapy and to sterilisation of foodstuffs, healthcare products and tissue allografts. In the rest of this section, some recent studies focusing on the use of radiation for sterilisation will be used to illustrate the types of chemical changes induced by radiation on biomaterials and drugs in aqueous solution as well as on drugs and drug-delivery systems in the solid state.

8.2 The effect of radiation on aqueous systems

Ionising radiation interacts with water to create both radical ions (H_2O^+ and the electron, e^-) and excited states of water (H_2O^*). Subsequent reactions of these fundamental primary species take place within the spurs, localised 'pockets' of ionisation, leading eventually to yields of free radicals and molecular products distributed homogeneously throughout the solution. The effect of ^{60}Co gamma radiation on de-oxygenated water can thus be summarised (Appleby and Schwarz, 1969):

$$H_2O \rightarrow {\cdot}OH(2.87), H^{\cdot}(0.61), e^-_{aq}(2.70), H_2(0.43), H_2O_2(0.61),$$
$$H_3O^+(3.3), OH^-(0.6)$$

where the values in parentheses refer to the radiation yields, expressed as G-values. The G-value is the number of molecules, atoms or free radicals formed (or lost) per 100 eV of energy deposited in the water. The values given above are for gamma rays from ^{60}Co and would be similar for other types of radiation having equal linear energy transfer (LET) values. Thus, 3 MeV electrons would produce similar yields to those above. The yields would, however, be slightly different for 10 MeV electrons. It is now more usual to express G-values in units of μmol/J. A useful conversion between the two types of unit is given by the following relationship: a G-value of 1 is equivalent to 0.1036 μmol/J. In aqueous solutions where it is often convenient to express changes in concentration, it can be calculated that a 10 Gy radiation dose will produce 3.1×10^{-6} mol dm^{-3} of a species whose G-value is 3.0.

In the presence of proteins at low concentrations – for example, at 10^{-5} mol dm^{-3} – all the energy of ^{60}Co gamma radiation is absorbed effectively by water molecules (approximately 55 mol dm^{-3}). Thus, the radiation chemistry of such systems is determined by the yields and reactivity of the free radicals and molecular species produced from the irradiation of water alone. Free radicals are defined as species having an unpaired electron in their bonding structure and, as such, are naturally very reactive and become involved in reactions which produce more stable entities. The three free radicals produced by the radiation of water, the hydroxyl radical (·OH), the hydrated electron (e^-_{aq}) and the hydrogen atom (H·) have substantially different modes of reaction. A knowledge of the chemical properties and reactivities of these species is important when trying to understand the effect of ionising radiation on components of drug/device combination products dissolved in an aqueous environment.

The hydroxyl radical is a strong oxidising agent and the majority of its reactions are very rapid and close to diffusion-controlled limits. It can, for example, oxidise in simple one-electron transfer processes with either metal

ions or complexes or with organically derived substrates. It also participates in hydrogen abstraction reactions with, for example, alcohols and carbohydrates. Its third mode of reaction is via addition to double bonds – for example, by addition to ethylene or benzene. With the exception of some electron transfer reactions involving metal ions or complexes, hydroxyl reactions usually produce substrate free radicals which then participate in further reactions leading to the eventual degradation or stable modification of the substrate. Normally, the reaction sequence ends when two free radicals react with each other to produce a reaction product mixture containing only stable products.

In contrast to the hydroxyl radical, the hydrated electron and hydrogen atom are strong reducing agents. Both will reduce metal ions or their complexes. Hydrated electrons may also react with conjugated olefins or aromatic compounds to form anion radicals. Hydrogen atoms, like hydroxyl radicals, can also abstract hydrogen atoms from alcohols and carbohydrates.

With three free radicals being produced by the action of ionising radiation on water or on dilute aqueous solutions, it is sometimes difficult to determine which of them is producing the observed radiation products. It is thus desirable, in some cases, to convert hydrated electrons into an extra yield of hydroxyl radicals by saturating the solution to be irradiated with nitrous oxide, the reaction being:

$$e^{-}_{aq} + N_2O \rightarrow {}^{\bullet}OH + OH^{-} + N_2$$

Here, the effective yield of the hydroxyl radical is given by a *G*-value of 5.6 and thus nitrous oxide-saturated solutions produce effectively only hydroxyl radicals as the reactive intermediate. The yield of the hydrogen atom, $G = 0.61$, is not, however, negligible and should not be discounted. In the presence of air or oxygen, the hydrated electrons also react rapidly to form the superoxide anion radical, $O_2^{\bullet -}$:

$$e^{-}_{aq} + O_2 \rightarrow O_2^{\bullet -}$$

Hydrogen atoms also react rapidly with oxygen at neutral pH values to form superoxide anion radicals (via its protonated form, $HO_2^{\bullet}$):

$$H^{\bullet} + O_2 \rightarrow HO_2^{\bullet} \leftrightarrow O_2^{\bullet -} + H^{+}$$

This is also a simple method of restricting the number of water-derived free radicals available to react with substrates. Superoxide anion radicals are neither strong oxidising nor reducing agents and are much less reactive than either hydroxyl radicals or hydrated electrons (reviewed in von Sonntag, 1987). For many organic compounds, there is no detectable reaction.

However, for easily oxidisible substrates such as ascorbic acid and polyphenols, reactions do take place. Superoxide anion radicals can also reduce some quinones, such as p-benzoquinone. In the absence of a reaction with a substrate, superoxide radicals dismute to produce hydrogen peroxide and water:

$$O_2^{\bullet -} + O_2^{\bullet -}(+2H^+) \rightarrow H_2O_2 + O_2$$

The catalysed dismutation of superoxide radicals is the basis of the action of the enzyme superoxide dismutase, one form of which contains copper at its active site. In the enzymatic reaction, superoxide radicals reduce copper (II) to copper (I) at diffusion-controlled rates. The copper (I) so formed reacts equally rapidly with another superoxide anion radical to re-form copper (II). As with the majority of fast reactions involving free radicals, these rate constants were measured using the technique of pulse radiolysis (see, for example, Fielden *et al.*, 1974). It is now well established that superoxide can react rapidly with many transition metal complexes and is often involved in similar dismutation processes. In the case of some iron complexes, for example, superoxide can produce hydrogen peroxide via a metal complex-catalysed dismutation process:

$$O_2^{\bullet -} + Fe(III) \rightarrow O_2 + Fe(II)$$

$$O_2^{\bullet -} + Fe(II)(+2H^+) \rightarrow H_2O_2 + Fe(III)$$

The production of hydrogen peroxide in the presence of Fe(II) allows the formation of the much more reactive species, the hydroxyl radical, in a Fenton-like process (Fenton and Jackson, 1899):

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + {}^{\bullet}OH + OH^-$$

8.3 Sterilisation of aqueous solutions of proteins and enzymes: reactions of free radicals with proteins

The radiation chemistry of amino acids, peptides, proteins and enzymes has been the subject of several reviews (Garrison, 1972, 1979; Klapper and Faraggi, 1979; Schaich, 1980; von Sonntag, 1987; Saha *et al.*, 1995; Houee-Levin and Sicard-Roselli, 2001). As can be expected, these demonstrate both the complexity of free radical-induced chemistry and the diversity of protein structure, content and conformation. A review of protein radiation chemistry as it applies to the sterilisation of healthcare products containing proteins and enzymes has also been made by Parsons (2010). It is not

the purpose here, therefore, to focus on the radiation chemistry. Instead, the focus is placed on those studies carried out at high doses applicable to sterilisation and, in particular, those methods used to protect these sensitive biomolecules against ionising radiation. A brief outline of the major reaction pathways for the primary free radicals of water radiolysis will, however, be useful to place such studies in context, as follows.

From pulse radiolysis studies, it is known that the hydroxyl radical reacts with aliphatic amino acid components of proteins at relatively slow rates compared with its reaction with aromatic and sulphur-containing amino acids (Butler *et al*., 1984). As a general approach, therefore, hydroxyl radicals may be seen to compete for three major categories of reaction site in the protein. It may react with the aliphatic amino acids, or at the –CH- bonds in the main peptide backbone or at aromatic or sulphur-containing amino acid residues. The high rates of reactivity with aromatic and sulphur-containing residues mean that relatively small amounts of these residues can dominate the radiation chemistry. Crosslinking of proteins induced by hydroxyl radicals is a significant mode of reaction for peptides and proteins containing tyrosine and phenylalanine (Gordon *et al*., 1977; Boguta and Dancewicz, 1981, 1982, 1983).

The rates of reaction of the hydrated electron with aromatic amino acids are approximately a factor of ten lower than those measured for histidine, cysteine and cystine (Butler *et al*., 1984). Combined with the high reactivity of the peptide bond with e^-_{aq},this fact indicates that reactions of the latter with proteins will be dominated by its reactions with the peptide bond, with the protonated histidine residue and with cysteine and cystine.

The effects of oxygen on the radiation chemistry of proteins is hard to predict. In general, oxygen may either provide some protection to protein degradation or promote degradation. Reaction of the hydrated electron with oxygen is rapid and may compete with the reaction with the protein or enzyme to produce the superoxide radical anion. This latter species is often unreactive with proteins and thus this is a mechanism for protection. The effect of oxygen is, however, more complex than this and it is difficult to make generalizations about which proteins may be protected or not.

The reactions of primary radicals produced by ionising radiation upon enzymes have been the subject of a vast number of studies. A list of some of these from about the mid-1960s to 1985 is given by von Sonntag (1987). It is, of course, an easier task to follow the inactivation of an enzyme by radiation than to conduct much more difficult experiments with non-enzymatic proteins where quantitative product detection and analysis are hard to achieve.

For aqueous solutions, sterilisation at doses up to 25 kGy, has been approached through the use of free radical scavengers, such as ascorbate ions, glutathione, mannitol, glycerol, phenols, oxidised glutathione and nitrate ions. Oxidised glutathione is a good scavenger for all the water-

derived free radicals – that is, for hydroxyl radicals, hydrated electrons and hydrogen atoms, whereas nitrate ions are good scavengers of hydrated electrons and their precursors. The others in the list are good scavengers of hydroxyl radicals – ascorbate ions being the scavenger used in most published work in this area. The effectiveness of the scavenger depends both on the concentration of the scavenger and the concentration of the protein. For example, consider the following competition between ascorbate and a protein for the hydroxyl radical:

$$\cdot OH + Asc^- \rightarrow Asc + OH^-$$
$$\cdot OH + \text{protein} \rightarrow \text{protein}^{+\bullet} + OH^-$$

where Asc and protein$^+$ represent the free radicals of ascorbate and protein, respectively. Since the steady-state concentrations of hydroxyl radicals produced by gamma radiation sources are very low compared with the concentrations of protein and free radical scavenger, the relative amounts of each of the two reactions above are given by k_1 [Asc$^-$] and k_2 [protein], respectively (where k_1 and k_2 are the second-order rate constants for the respective reactions, usually expressed as $M^{-1}\ s^{-1}$). Typically, concentrations of ascorbate of up to 0.1 M have been used. For this particular concentration, k_1 [Asc$^-$] = $7.2 \times 10^8\ s^{-1}$ where $k_1 = 7.2 \times 10^9\ M^{-1}\ s^{-1}$ (obtained from a review of rate constants by Buxton *et al.*, 1988). If we then assume that hydroxyl radicals react rapidly with the protein, say at $10^{10}\ M^{-1}\ s^{-1}$, and that the protein has a concentration of 0.1 mM, then k_2 [protein] = $1 \times 10^6\ s^{-1}$. Under these experimental conditions, therefore, only one hydroxyl radical in 7200 would react with the protein, affording, in principle, good protection by ascorbate. Similar calculations may be made for hydrated electron and hydrogen atom scavengers. The selection of appropriate concentrations of both scavenger and protein are thus important, as are the conditions of irradiation. Irradiation in the presence of air, for example, will produce the superoxide anion radical (as well as the protonated form, the perhydroxyl radical, $HO_{2\cdot}$) instead of the hydrated electron and hydrogen atoms. A further consideration is the effect of radiation dose on the degradation of free radical scavenger. A 25 kGy dose will produce about 7.5 mM loss of scavenger due to the hydroxyl radical reaction. In effect, scavenger concentrations should be considerably greater than this value if complete depletion is to be avoided. In all these free radical scavenger experiments, it is also assumed that the scavenger free radicals so formed are themselves unreactive with proteins. For some scavengers, for example, where sugars are used, this may not be the case. Elimination of $HO_{2\cdot}$, for example, from the many carbon-centred peroxyl radicals formed in the reaction of hydroxyl radicals with sugars is one such possibility, among others (see the review by von Sonntag, 1987).

8.4 The sterilisation of proteins in aqueous solution

In contrast to the considerable amount of research literature on the inactivation of enzymes and on the degradation of proteins in general, there have been relatively few studies which have focused on the sterilisation and/or the degradation of proteins and enzymes at doses close to typical sterilisation doses (25 kGy). For aqueous preparations, these studies have adopted a dual approach to protection of proteins – that is, protein solutions have been irradiated in the presence of a free radical scavenger as well as being frozen. Some of these studies have also been conducted in the solid state, as lyophilised or dry samples. When aqueous solutions are irradiated in the frozen state, the primary free radicals of water and their precursors become trapped. At low temperatures, diffusion of these precursors and their products is very restricted, reducing the possibility of interaction with substrates and thus the precursors are able to recombine, reducing the yields of hydroxyl, hydrated electrons and hydrogen when solutions are thawed. It is clear, therefore, that the yields of primary water-derived free radicals and molecular species available to react with substrates such as proteins are much reduced when frozen aqueous solutions are irradiated. Irradiation at low temperatures in frozen solution is therefore very desirable in that free radical-induced degradation is much reduced although not necessarily non-existent. At low temperatures in frozen solutions, it has been shown that the inactivation of an enzyme, such as invertase, using very large doses up to 900 kGy (Lowe and Kempner, 1982), is described by the following equation:

$$A = A_0 e^{-qmD}$$

where D is the radiation dose, m is the molecular mass of the enzyme (or target) and q is a constant related to average energy deposition in each primary ionisation and also to a factor reflecting the change in radiation sensitivity with temperature. Essentially, this equation is a quantitative description of the direct effect of radiation on a large target molecule such as a protein and is in fact usually referred to as the target theory of radiation (see, for example, Kempner, 2001). In this theory, it is assumed that each random ionisation of a protein molecule results in massive structural damage and complete loss of biological function. Thus, it was observed that there was little or no detectable loss in invertase activity on extrapolation to a typical sterilisation dose of 25 kGy (Lowe and Kempner, 1982).

Adopting this dual approach of irradiating proteins and enzymes at a low temperature in the presence of a free radical scavenger, insulin monoclonal antibody preparations were irradiated at 4°C at doses of 15 and 45 kGy in the presence and absence of 0.2 M ascorbate (Grieb *et al.*, 2002). In the absence of ascorbate, SDS PAGE and ELISA experiments showed that there was almost complete loss of activity and protein at both 15 and

45 kGy doses. Although there is insufficient detail in this chapter to calculate the concentration of the antibody, these experiments show that there is considerable damage to the protein even at 4°C. It is not clear, however, whether these solutions were actually frozen or still liquid. It seems likely that the indirect production of the primary water-derived free radicals is still significant and that diffusion of them to react with the protein was still taking place. In the presence of ascorbate at 4°C, however, there was no detectable loss of antibody activity at 15 kGy, although some loss could be measured at 45 kGy. Protein bands were observed using SDS PAGE showing that the loss of protein had been reduced considerably. Freeze-dried solutions of the antibody irradiated in the presence of ascorbate showed full retention of activity at 15 kGy with some reduction in activity at 45 kGy. No measurements of retention of protein integrity were made in this case because a 100-fold excess of a bulking agent, albumin, was added to facilitate freeze-drying. The authors also showed that the radiation-induced aggregation of antibody, clearly observed in size exclusion experiments in the absence of ascorbate, was not apparent in the presence of ascorbate. Experiments were carried out at lower temperatures, –80°C, but only to show the effect of ascorbate on the reduction of levels of virus in frozen solutions spiked with the pathogen. In a similar study, (Amareld *et al.*, 2003), urokinase was irradiated at –80°C at a dose of 50 kGy. Here, in the presence of 0.2 M ascorbate, the activity of the enzyme was maintained at about 90% of the unirradiated control frozen solution. The same solution spiked with virus and then irradiated showed reduced levels of pathogen by about five orders of magnitude. In the absence of ascorbate, the activity was reduced to about 20% of the control. No attempts were made in that study to monitor the loss of integrity of the protein after irradiation. The fact that enzyme function is reduced considerably in the absence of ascorbate confirms: (i) that ascorbate is an effective free radical scavenger, presumably in these conditions, of the hydroxyl radical and (ii) that at –80°C in the absence of ascorbate, the loss of enzyme activity is probably much greater than could be expected from the direct action of radiation alone on the enzyme (based on the approach of Kempner, 2001). It seems likely, therefore, that there is a significant contribution from trapped water-derived free radicals arising from direct action of radiation on the solvent, water.

Irradiation of human plasma at high sterilisation doses, 50 kGy, at dry ice temperatures of approximately –80°C in the presence of 12.5–50 mM ascorbate has been investigated by using the formation of protein hydroperoxides and carbonyls as markers of damage to the proteins (Zbikowska *et al.*, 2006). In the absence of ascorbate but at dry ice temperatures, there was an approximately two-fold reduction in the level of radiation-induced protein hydroperoxides relative to the same solution irradiated at ambient temperature (as estimated from the low doses used, common to both

experiments). This may be due to the difference in oxygen concentrations in the two experiments and this may make comparison difficult since oxygen is required for hydroperoxide formation. It is also not clear whether protein oxidation at –80°C is attributable to direct action on plasma proteins or to trapped water-derived free radicals. At 1 mg/mL plasma protein, the amino acid concentration is relatively high and may be sufficient to scavenge significant amounts of trapped water-derived free radicals. In the presence of ascorbate at –80°C, there was an approximately 30% reduction in carbonyl formation. This reduction is very small and may be accounted for by the high concentration of protein, effectively in competition with ascorbate, or, as the authors indicate, the situation may be more complex since ascorbate itself may reduce the levels of carbonyl and hydroperoxide subsequent to their formation on irradiation. Subsequent studies, undertaken by the same team, using a 50 kGy dose and dry ice and focusing on protein degradation, in particular, on fibrinogen, and plasminogen, as well as on the coagulation and fibrinolytic activities of plasma, have confirmed the relatively small protective effect of 50 mM ascorbate. Surprisingly, a much greater protective effect was observed for 50 mM histidine (Zbikowska *et al.*, 2007a, 2007b).

Immunoglobulins, in the form of recombinant monoclonal or polyclonal antibodies derived from blood plasma, have also been irradiated at sterilising doses at dry ice temperatures. Upon irradiation of a paste, it was shown that the Fab and Fc domains of intravenous immunoglobulins (IVIG) remain essentially intact. Integrity of these domains was seen as a critical requirement for a successful application of a radiation sterilisation process, primarily designed to eliminate harmful viral agents (Tran *et al.*, 2004).

In a study of the sterilisation of β-galactosidase, tris buffer and nitrite ions were used as radiation protection agents – the irradiations being carried out under inert conditions (nitrogen de-gassing of solutions) and at both ambient and dry ice temperatures (Audette-Stuart *et al.*, 2005). Nitrite ions are good scavengers of electrons, of both 'dry' and hydrated forms, as well as being scavengers of hydrogen atoms. These reactions produce nitrogen dioxide as the major product. Here, tris buffer was used as a scavenger of hydroxyl radicals. Analyses of protein degradation and enzyme activity were made. In the presence of nitrite at ambient temperatures, where the indirect effect of the water-derived free radicals dominate the radiation chemistry, the *G*-values for the loss of enzyme activity increased nearly four-fold on addition of excess nitrite from 8.8×10^{-9} mol J^{-1} to a value of 3.18×10^{-8} mol J^{-1} – the increase being attributable to reactions of nitrogen dioxide, which is known to nitrate tyrosine residues. It is notable that both these yields are less than the total yield of hydrated electrons, hydrogen atoms and hydroxyl radicals produced by the direct action of radiation on water (equal to about 6.3×10^{-7} mol J^{-1}). In addition, the authors also noted that the loss of protein was always less than the loss of enzyme activity. This is an unusual finding and

may be attributable to modification of the enzyme with change in molecular mass. Here, nitrogen dioxide may simply nitrate the enzyme without producing fragmentation. At dry ice temperatures and in the absence of nitrite ions, the loss of protein integrity is much reduced relative to ambient temperatures – a reduction of at least 250-fold was observed. In the presence of nitrate at these temperatures, the loss of protein integrity was reduced by a factor of 45. In the presence of tris buffer, used as a scavenger of hydroxyl radicals, protection of the protein integrity was also apparent although the effect was not dramatic – for example, at ambient temperatures, addition of tris buffer reduced the loss to 40% of the solution irradiated in the absence of tris buffer. It may be here that the analysis of protein fragmentation is complicated by protein aggregation – hydroxyl radicals are known, for example, to react with tyrosine residues, to yield protein dimers. In the same study, the effect of lyophilisation on protein integrity was also considered. The authors found that, at –78°C, there was little if any difference in the loss of protein integrity upon lyophilisation. At ambient temperatures, the radiation-induced loss of protein was reduced by a factor of five relative to dilute solutions of the enzyme. It is clear from this study that again reduction of temperature to that of dry ice brings significant reductions in protein damage. It is not clear, however, how much of the damage is attributable to trapped water-derived free radicals relative to damage caused by direct action on the enzyme. It can be noted, however, that the molecular mass of β-galactosidase is high at approximate 500 kDa and this would enhance the potential for significant direct action on the protein at the high sterilisation doses used here. The study probably also confirms that the particular scavengers used are not particularly effective in protecting the enzyme.

In a recent paper on the sterilisation of human insulin, protection of the protein was attempted largely in aqueous solution without resort to either lyophilisation or reduction in temperature (Terryn *et al*., 2007) and can be compared with a previous study by the same authors in which solid-state insulin was irradiated at radiosterilisation doses (Terryn *et al*., 2006). At 10 kGy, the recovery of insulin was 96.8%. In these experiments in aqueous solution, carried out under nitrogen and at pH2 (to avoid precipitation of insulin, the most effective protection agents were found to be ascorbate and oxidised glutathione (GSSG). Although both insulin degradation and dimer/polymer yields were determined, it was not made clear how the two sets of measurements related to one another. With this proviso, it was shown that at 10 kGy and using 0.34 M ascorbic acid, there was 73.6% retention of insulin. Additional experiments at dry ice temperatures again confirmed the reduction in radiation-induced degradation of proteins. At 25 kGy, the amount of insulin was reduced to about 40% of the unirradiated value – at this dose at ambient temperature, there would be no insulin left. Nevertheless, the reduction in protein integrity at dry ice temperatures is

considerable. However, addition of 0.34 M ascorbic acid led to at least 90% recovery at this dose.

In these latter, relatively few, focused studies on the sterilisation of proteins, there are several points worthy of note for future sterilisation strategies. It is clear that sterilisation at dry ice temperatures does reduce the protein degradation yields considerably relative to experiments in dilute solution. For the single study where radiation yields were given, the reduction is at least a factor of 100 – the data may in fact underestimate the reduction since protein modification without fragmentation may not have been measurable (Audette-Stuart *et al.*, 2005). Whether this reduction is entirely due to the direct effect of radiation on the enzyme or whether it is attributable to a low yield of water-derived free radicals was not established. It may be that both mechanisms play a part. The addition of free radical scavengers, in particular ascorbate, which is known to react effectively with hydroxyl radicals, also brings about a large reduction in degradation of proteins and enzymes, an effect which is also apparent at dry ice temperatures. In aqueous solutions at ambient temperature, it is the most effective protective agent. That it protects better than other hydroxyl radical scavengers probably indicates that ascorbate not only scavenges free radicals but also repairs damaged amino acid residues. The highly protective effect of ascorbate seen at dry ice temperatures (Terryn *et al.*, 2007) may either indicate that high concentrations of ascorbate can scavenge any free radicals arising from traps in ice or it may also suggest that whatever the mechanism by which damage to amino acid residues arises in the frozen state, ascorbate can repair such damage. Most sterilisation studies have so far focused on scavengers which react with oxidising species such as the hydroxyl radical, neglecting the significant damage that hydrated electrons can do to proteins. Here, it can be noted that oxidised glutathione at low concentrations (10^{-4} M) was shown to be an effective protective agent, probably reflecting its ability to scavenge hydrated electrons as well as the oxidising radicals. Finally, lyophilisation of protein solutions was found to confer significant protection relative to dilute solution at ambient temperatures – the loss of protein being reduced by a factor of five (Audette-Stuart *et al.*, 2005).

8.5 Sterilisation of drugs using radiation

Many pharmaceutical drugs have been, and are being, developed for use in combination products. Most of these are used in stents as anti-neoplastics, immunosuppressives and anti-inflammatory agents (Hupcey and Ekins, 2007). Studies of the effects of ionising radiation on pharmaceutical drugs have been a popular area of research for radiation chemists, particularly in the last 30 years. A survey of such studies on 67 different drugs showed that most of the work focused on morphine hydrochloride, atropine sulphate,

chloramphenicol, streptomycin and tetracycline. Most of the irradiations were carried out at large doses, typically in the range 10–60 kGy (Boess and Bogl, 1996). Similar reviews have been made by others (Jacobs, 1995; Dahlhelm and Boess, 2002). More recently, such studies have been carried with the aim of reducing damage to the drug when large sterilisation doses are given. These fall into two categories: those carried out in aqueous solution in the presence of free radical scavengers and those irradiated in the solid state.

An example of the former category involved measuring the degradation of the cortisone acetate in aqueous solutions at doses up to 25 kGy and separating out the degradative abilities of the three water-derived free radicals (El-Bagory, 2007). It was found that hydroxyl radicals and hydrogen atoms were the main degradative agents, with little degradation being attributable to the hydrated electron. Methanol, 2-propanol and some surfactants were used as free radical scavengers. In another study in aqueous solutions, metoclopramide, an anti-emetic drug, was used as a model for sterilisation in the presence of radioprotective agents (Maquille *et al*., 2008a). This involved a detailed analysis of many drug products formed upon irradiation at doses up to 25 kGy. Not surprisingly, addition of hydroxyl radical reactive substrates such as mannitol, nicotinamide and pyridoxine led to significant reductions in the loss of the drug – the maximum drug recoveries were greater than 90% at 15 kGy. At dry ice temperatures, frozen solutions of metoclopramide and metoprolol were irradiated at doses up to 60 kGy in the presence of nitrogen. Only very low levels of degradation products were detected, at about 0.3% of the unirradiated drugs (Maquille *et al*., 2008b). This level of protection found for these drugs is much greater than that found for proteins and enzymes when irradiated at dry ice temperatures. This difference may suggest that damage to proteins is caused by both the direct action of radiation on the protein and also by the diffusion-limited reactions of trapped water-derived free radicals with the protein. In the case of the frozen drug solutions, presumably only reactions due to the trapped water-derived free radicals contributed to degradation of the drugs.

Irradiation of pharmaceutical drugs in the solid state provides an alternative approach to sterilisation. In such studies, degradation yields are usually reported as percentages and are usually low, at about 2% or, indeed, much less. In the solid state, free radicals will be more stable in general than in the liquid state; stable free radicals can be detected in electron paramagnetic resonance (epr) experiments on irradiated solids. Thus, at typical sterilisation doses of 25 kGy, the direct action of radiation on solids will only produce small amounts of degradation when expressed as a percentage loss of the bulk irradiated medium. This, in itself, does not imply that the damage to the drug is insignificant. The amount of radiation products, even at low degradation yields, may be sufficient to be of concern in terms of potential toxicity, colour changes, stable free radical concentrations, etc. Table 8.1

Table 8.1 Summary of data from selected sterilisation studies on pharmaceutical drugs in the solid state

Drug	Dose range (kGy)	Yield of epr radicals (nmol J^{-1})	% Degradation (dose)	Product analysis methods	Reference
Naproxen	0–25	Not determined	n.m.	n.m.	Polat and Korkmaz, 2002
Apranax	0–25	13	n.m.	n.m.	Polat and Kormkaz, 2002
Sulphanilamide	0–25	56	n.m.	n.m.	Colak and Korkmaz, 2003
Sulphafurazole	0–50	18	n.m.	n.m.	Colak and Korkmaz, 2004a
Sulphathiazole	0–50	11	n.m.	n.m.	Colak and Korkmaz, 2004b
Sulphacetamide	0–50	<11	n.m.	n.m.	Colak and Korkmaz, 2004c
Cyclophosphamide	0–30	2.2	1.7 (30 kGy)	Hplc; lc/ms; FTIR, thermal colour	Varshney and Dodke, 2004
Doxorubicin	0–30	Not determined	0.8 (30 kGy)	Hplc; lc/ms; FTIR, thermal colour	Varshney and Dodke, 2004
Metoclopramide	0–60	n.m.	0.046 (25 kGy)	hplc; lc/ms; colour	Maquille *et al.*, 2006
Paracetemol	2.5–25	Not determined	n.m.	n.m	Polat and Korkmaz, 2006
Ketoprofen	0–50	4.0	No change detectable	Hplc; lc/ms; gc/ms	Colak *et al.*, 2006
Meropenem trihydrate	0–15	Not determined	n.m.	n.m.	Tepe *et al.*, 2006
Imidazoline derivatives	25–200	0.96–41.2	Not determined	DSC; FTIR; X-ray diffraction; tlc; colour	Marciniec *et al.*, 2007
Piperacillin hydrate	0–15	Not determined	n.m.	n.m.	Tepe *et al.*, 2007
Cefadroxil	0–25	18	n.m.	n.m.	Aydas *et al.*, 2008

summarises the results from a number of recent studies on the irradiation of pharmaceutical drugs in the solid state carried out at high doses appropriate to sterilisation. Here, it can be seen that the degradation at typical sterilisation doses ranges from 0.046% to 1.7%. A similar range, 0.96–41.2 nmol J^{-1}, is also seen for stable free radicals yields (as measured by epr). The latter value of 41.2 nmol J^{-1} would be approximately equivalent to a *G*-value of 0.4 if the drug density was similar to that of water. Epr yields are minimum values for degradation of the drug – fast-decaying radicals formed in the solid state would not be detected in epr experiments. Measurements of degradation yields of drugs are subject to large errors when monitoring the loss of the drug – arising from measuring the difference between two large numbers. It is better to carry out product analysis if a more accurate degradation value is required. Thus, in the study by Varshney and Dodke (2004), product analysis, showed that cyclophosphamide degraded by 1.7% at 30 kGy. Other extensive studies involving product analysis, such as hplc, nmr, hplc-ms, epr, tlc, etc., have been carried out by Marciniec and co-workers, largely using electron beam irradiation up to doses as high as 800 kGy. Drugs such as thiamphenicol, clotrimazole and florfenicol have been investigated this way, where the yields of both drug loss and radiation product formation have been measured. Typical losses, expressed as percentages, are 0.1% for thiamphenicol and 0.95% for florphenicol at the sterilisation dose of 25 kGy (Marciniec *et al*., 2008, 2009a, 2009b, 2010). Similar studies have also been carried out on fluoroquinolones and cephalosporins (Singh *et al*., 2009, 2010). In all these studies, despite significant differences in loss of drug, it was concluded that sterilisation doses of up to 25 kGy could be safely used.

In conclusion, radiation in the solid state produces low drug degradation yields in percentage terms. However, the yields of products may still be sufficiently high to present difficulties with toxicity, colour and other key parameters when sterilising by radiation. Radiation of frozen solutions at dry ice temperatures, particularly in the presence of some free radicals scavengers, may provide a better method of reducing degradation to even lower and more acceptable values.

8.6 Sterilisation of drug-delivery systems using radiation

There is now much research effort focused on the development of both natural and synthetic polymeric carriers of drugs to enhance both drug delivery to a diseased organ or tissue and to control the release of the drug at those sites. Such carrier systems are described as controlled drug delivery (CDD) or controlled drug release (CDR). Three types of carrier system are seen to be suitable for sterilisation. They are all categorized as solid-state

systems – that is, solids, solids swollen with solvent (hydrogels) and solids dispersed in liquids (liposomes and nanoparticles). Irradiation of the polymers used in CDD/CDR systems can produce both chain scission and crosslinking, the proportions of these processes depending on factors such as macromolecular structure, presence of oxygen, temperature, dose rate and additives. Reaction of polymer free radicals with oxygen, for example, may favour oxidative damage and chain scission relative to crosslinking. Here, the ability of oxygen to diffuse to react with such free radicals is important. High dose rates, on the other hand, such as those delivered by electron beams, will favour crosslinking since higher local concentrations of polymer free radicals will be formed and so promote inter-reactions.

There have been several recent reviews concerned with the effect of sterilisation doses of radiation on polymeric carriers for drugs (Sintzel *et al*., 1997; Bhattacharya, 2000; Clough, 2001; Edlund and Albertsson, 2002; Jain *et al*., 2005; Razem and Katusin-Razem, 2008). The review by Razem and Katusin-Razem is a particularly detailed review of drug-loaded carrier systems studied since 1990. Readers are therefore referred to this work for the amount of information that it contains on drug release in irradiated carriers. It is the purpose of this section, instead, to highlight the diverse nature of the effect of radiation on drug-carrier systems by using examples of synthetic and natural polymers most commonly used in CDD/CDR.

The most frequently used synthetic carriers are based on polylactic acid (PLA) and its copolymers with polyglycolic acid (PGA). The copolymers (PLGA) are particularly common drug carriers. These are usually 50:50 copolymers and are frequently used in the form of microspheres from several μm to several hundreds of μm in diameter – many are in the range, 5–60 μm. Molecular masses range from 8 to 130 kDa. These PLGA carriers are used to deliver a wide variety of molecules within the three leading therapeutic classes of drug, viz. antineoplastic, antibacterial and anti-inflammatory agents.

Although there are studies in which the polymer systems are irradiated without drugs – for example, an analytical investigation of irradiated PEGd, 1PLA and PEG-PLGA multiblock (Dorati *et al*., 2008) – in general, the majority of radiation studies involve drug-loaded systems. In addition to monitoring the drug-release profiles before and after irradiation, other techniques such as differential scanning calorimetry, electron paramagnetic resonance, FTIR, gc/ms, hplc, lc/ms, light scattering, size exclusion chromatography, and so on have all been used to detect changes in the drug and the carrier polymers.

In some (50:50) PLGA systems, little or no effect of radiation on drug release has been detected. These include, for example: ibuprofen in 13.14 kDa microspheres of 39.3 μm diameter irradiated at dry ice temperatures where the drug, the carrier and the drug-release rate were shown to be stable to irradiation (Fernandez-Carballido *et al*., 2004) and ganciclovir

in 34 kDa microspheres of 300–500 μm diameter at dry ice temperatures (Herrero-Vanrell *et al.*, 2000). Such stability might result from the use of low temperature; however, other studies at dry ice temperatures showed that (i) the weight average molecular weight of the PLGA copolymers, as well as (ii) the release rate of the encapsulated drug, changes. Thus, for captopril in 50:50 PLGA microspheres of molecular weights ranging from 17–67 kDa and 11–16 μm, the average molecular weight of the copolymer decreased by up to 18% for the smaller microspheres. This decrease was not affected by temperature or presence of oxygen (Volland *et al.*, 1994). When melanotan I was encapsulated in 50:50 PLGA rods (2 mm diameter) and irradiated at dry ice temperatures, there were significant increases in the rate of release of the drug (Bhardwaj and Blanchard, 1997). Similarly, the release rate of clonazepam from 50:50 PLGA microspheres (34 kDa, 2–10 μm) was also found to increase with irradiation dose (Montanari *et al.*, 2001). In this study, epr was used to measure the radiation yields of both drug and polymer free radicals, the total yield of all radicals being calculated to be 0.22 μmol J^{-1}. This is a very high yield and can be compared, for example, with the yield of hydroxyl radicals formed upon irradiation of water, 0.29 μmol J^{-1}. 50:50 PLGA microspheres (1.5 μm) have also been used to release larger molecules such as recombinant human insulin-like growth factor I. Here, it was shown that (i) the average particle size increased to 1.88 μm after irradiation, (ii) the initial release rate increased and (iii) dimerisation of the drug took place. In another study of a large molecule, ovalbumin, in 50:50 PLGA microspheres (13 kDa, 25–69 μm), irradiation caused a significant increase in drug-release rate although the size distribution was unaffected. Addition of 10% NaCl, however, conferred stability in terms of drug rate release and size changes, although PLGA radicals could be detected by epr (Dorati *et al.*, 2005).

A similar diverse response to sterilising doses of radiation has been observed in the case of drug-loaded swellable polymers. For example, for hydroxypropylmethyl cellulose (HPMC) containing diltiazem hydrochloride, irradiation induced chemical modifications in the drug as well as a progressive decrease in the average molecular weight, the latter being quantified as G (chain scissions) = 1.2–1.4 μmol J^{-1} (Maggi *et al.*, 2003). These yields are very high indeed. There were also large changes in drug-release rate – under the experimental conditions employed, nearly 100% release was observed at 720 min, whereas after 25 kGy, this decreased to about 300 min. In contrast, another cellulose derivative, hydroxyethyl cellulose, was used, together with trehalose, to produce microspheres which were then loaded with vancomycin and irradiated (Bartlotta *et al.*, 2005). No effect of irradiation on the drug itself, the size of the microspheres or on the rate of vancomycin release could be seen.

Irradiation studies of sterilisation doses of drug-delivery systems continue to attract much interest and are now being extended to include the effect

on 'spiked' amounts of bacteria and toxin as well as vaccines. Doxorubicin-loaded poly(butyl cyanoacrylate) nanoparticles spiked with *Bacillus pumilus* irradiated at doses from 10 to 35 kGy showed no significant changes in mean particle size, polydispersity and aggregation ability. The drug was also stable. The addition of 100 colony-forming units per gram of bacteria demonstrated that sufficient levels of sterility could be achieved with 15 kGy (Maksimenko *et al*., 2008). The irradiation of PLGA microspheres, containing the SPf66 malarial antigen, at 25 kGy had no apparent effect on SPf66 integrity or on the formulation properties such as morphology, size and peptide loading, although slightly faster vaccine release rates were observed. Sub-cutaneous administration of irradiated and non-irradiated spheres into mice induced similar immune responses (Igartua *et al*., 2007). In a similar study on the Brucella ovis antigenic extract (HS) entrapped in mannosylated poly(anhydride) nanoparticles, doses up to 25 kGy did not modify the size, morphology and antigen content of the nanoparticles nor did they affect the integrity and antigenicity of the entrapped antigen although there was a negative effect on the rate of release of antigen from the nanoparticles (Da Costa Martins *et al*., 2009).

In summary, there are yet no clear explanations of why irradiation should affect a drug carrier such as PLGA loaded with a wide range of drugs or vaccines in such a diverse way. In some cases, little or no effect on the drug or carrier is observed, whereas in others, significant changes in the size distribution of PLGA microspheres are seen together with evidence of decreasing molecular weight of the polymer and increases in the rates of drug release. Electron paramagnetic resonance experiments show the production of free radicals, even for those systems where there is otherwise no apparent change in drug or polymer. Similarly, there are no clear distinguishing effects of conditions such as presence or absence of oxygen and temperature. It should, however, be of no surprise that radiation causes some damage to the main component, the polymeric carrier, and although this may be a small percentage in line with the known direct effects of radiation on solids, it appears to be sufficiently extensive in most cases to cause measurable changes in the drug device. Certainly, in some cases the damage is too small to be measured. The effect of other components in the drug-carrier systems may have a significant effect, perhaps in enhancing or reducing transfer of damage from localised sites of ionisation. The effect of 10% NaCl in stabilising PLGA microspheres loaded with ovalbumin (Dorati *et al*., 2005) may be attributed to such an effect.

8.7 Sterilisation of tissue allografts using radiation

Tissue allografts include bone, tendon, skin, tendons and other soft tissues and are extensively used in surgery throughout the world. Tissue banks are

the major source of these natural materials and many, particularly throughout South and Central America, Asia and India, use radiation sterilisation as a routine process to provide sterile items in a sealed package for medical use. There has been considerable research into the effects of ionising radiation on such tissues and an extensive bibliography on this was provided in the International Atomic Energy Agency Code of Practice on the radiation sterilisation of tissue allografts (IAEA, 2008). Research in this area continues, as shown by the following examples.

In a study of demineralised bone in both the dry state and in the presence of aqueous and non-aqueous carriers, it was found that the biological activity of the dry bone remained relatively stable. However, in the presence of aqueous solutions, there was considerable loss of activity. Radiation did not affect the cell attachment to the matrix but did influence both stem cell and osteoprecursor cell proliferation rates (Han *et al*., 2008). In another study of bone and soft tissue allografts irradiated at dry ice temperatures at doses up to 28.5 kGy, there were no statistical differences in the mechanical strength or modulus of elasticity for any graft compared with the control groups (Balsy *et al*., 2008). The effect of donor age on the initial biomechanical properties of human tibialis tendon allografts following irradiation at 14.6–18.0 kGy was found not to significantly affect the initial failure load, stiffness or displacement at failure (Greaves *et al*., 2008). In another biomechanical study, low dose irradiation at 15 kGy did not affect the mechanical properties of ovine bone patellar tendon-bone allografts whereas at high dose (25 kGy) there was a significant deterioration of the biomechanical integrity of the soft tissue constituent (McGilvray *et al*., 2010).

Studies designed to protect tissue allografts from the effects of radiation usually involve either the addition of free radical scavengers or the use of low temperature radiation to reduce free radical mobility. In a study using free radical scavengers such as mannitol, ascorbate and riboflavin, tendon tissues were found to be protected significantly against tensile damage, particularly for ascorbate and riboflavin (Seto *et al*., 2008).

Radioprotectants were also found to protect human bone-patellar tendon-bone allografts against biomechanical damage (Reid *et al*., 2010). At a relatively high dose of 50 kGy using radioprotectants it was found that the remodelling and osteoinduction of bone allografts from a rabbit calvarial model was equivalent to non-treated allografts, thus providing the potential for highly sterile allografts without damage to structural or biological integrity (Burgess *et al*., 2010). The use of similar radiation protection conditions at similarly high doses of 50 kGy did not affect the fusion rates in a rat spinal model when compared with unirradiated bones, thus conferring a high degree of sterility without adversely affecting the biological activity of the rat bone (Alanay *et al*., 2008). Skin allografts were also found to retain the desired histological, cytotoxicological and physical properties when

irradiated at 25 kGy and –80°C in the presence of the free radical scavenger glycerol (20%) (Rooney *et al.*, 2008).

In order to demonstrate that irradiation conditions designed to protect tissue allografts through the use of low temperature irradiation and free radical scavengers can, under these same conditions, lead to high levels of sterility assurance, tests can be conducted in the presence of known amounts of pathogens and then the inactivation rates of these pathogen 'spikes' measured. In one such study with semi-tendinosus tissues pre-treated with a radioprotectant solution and with addition of 'spikes' of several viruses followed by irradiation at a 50 kGy dose, it was found that the pre-implantation biomechanical properties of the tendons compared favourably with a non-irradiated group. Furthermore, there was 4.5 log reduction in the added Sindbis virus and larger rates of inactivation were found for two other added viruses (Grieb *et al.*, 2006).

Application of ISO methods to assure the sterilisation of tissue allografts through the attainment of SAL of 10^{-6} are followed routinely by tissue banks, although there is little information on the application of these methods to tissues pre-treated with radioprotectants. Using ISO 13409 (now replaced by ISO 2006a, 2006b, 2006c), it was shown that a 25 kGy dose could be substantiated to achieve this SAL value for lyophilised human amnion membranes (Djefal *et al.*, 2007). In a separate study of human amniotic membranes, in either the air-dried state or preserved in 99% glycerol solutions, the IAEA Code of Practice was used to substantiate a sterilisation dose of 25 kGy for an SAL of 10^{-6} and was based on a verification dose using only ten samples to achieve an SAL of 10^{-1} (Yusof *et al.*, 2007). In the application of the more recent ISO method (ISO 2006a, 2006b, 2006c), the VD_{max} method was used for frozen processed bone allografts of extremely low bioburden to show that a dose as low as 15 kGy could be used to achieve an SAL of 10^{-6} (Nguyen *et al.*, 2008). This particular study demonstrates the importance of processing tissue allografts prior to irradiation which ensures that the lowest bioburden values can be maintained before irradiation. The use of such low doses for sterilisation minimises damage to the allografts.

8.8 Conclusions and future trends

Much of the current research and R&D activity in the field of sterilisation of healthcare products by ionising radiation concerns the irradiation of the separate components of drug-device products at doses normally used for sterilisation of healthcare products – that is, at doses between 15 and 50 kGy. Thus, drugs, proteins and drug-delivery systems comprise the bulk of such studies. The studies may be in the solid or aqueous state, as appropriate, with the main focus being to establish whether the various components retain both their function and structural integrity.

A significant proportion of studies have used free radical scavengers to reduce the reactions of free radicals with the drug-device components – much of this work being carried out in aqueous solution. This latter area of study shows considerable promise particularly when conducted at low temperatures. It is equally clear, however, that the choice of free radical scavenger is critical. Studies in the solid state, particularly on drugs and drug-delivery systems, have shown a wide range of free radical yields: some where the extent of degradation is probably acceptable and some where the yields are relatively high. Even a low percentage degradation may produce unacceptably high levels of radiation products, giving potential problems with toxicity and producing easily observable colour changes. The potential benefits of terminal sterilisation of the relatively low batch volumes of manufactured drug-device combination products contained in a sealed package will certainly drive a greater research and R&D effort in this area. To this end, there will be a focus on understanding why some otherwise equally good free radical scavengers produce widely different levels of protection. Equally there must be a greater understanding of what determines the variation of drug degradation in the solid state. This may be related to structure of the drug and the solid-state arrangement of molecules whereby some geminate ion recombination processes may be more effective than others.

Regardless of whether a sterilisation process has been shown to be effective in terms of retention of structure and function of the drug-device component, there is always a need to demonstrate that bacterial and/or viral contamination can be eliminated under those specific conditions – the effect of temperature is likely to affect the rate of killing of both types of contamination. There has been relatively little work on studies which combine both the effect of radiation at sterilisation doses on function and integrity of the healthcare product as well as measuring the reduction in bioburden to achieve the desired levels of sterility assurance. There are now signs, however, of an increasing number of studies with this latter aim in mind.

8.9 Sources of further information and advice

Among the peer-reviewed journals, the following are particularly popular: *Biologicals* (for sterilisation of biologicals); *Radiation Physics and Chemistry* (for most types of irradiated materials); *Journal of Controlled Release* (for irradiation of drug-delivery systems); *Polymer Degradation and Stability* (for polymeric components); *International Journal of Pharmaceutics* (for drug irradiation); *Radiation Effects and Defects in Solids* (for drug irradiation in the solid state); *Biomaterials* (for irradiation of biopolymers and other biomaterials); *Journal of Pharmaceutical Sciences* (for irradiation of drugs and biologicals).

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9

Antimicrobial coatings for 'self-sterilisation'

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Abstract: In this chapter we analyse the different surfaces that have inherent antimicrobial properties as well as investigating the effectiveness of current methodologies. We show how simple surfaces that have functional properties can have a significant effect on the biofilms that form and the infection control consequences that thus exist. Hand touch surfaces and especially those surfaces that are near to the patients can act as harbours for bacteria and reservoirs of infection. Specially designed functional surfaces that either utilise metal ions or light-activated agents can control the bacterial colonisation by inherent and potent antimicrobial properties. This chapter is split into sections describing the different types of surface and antimicrobial agents with specific examples given for the need and use as well as the studies that have been performed. We make special example of the use of catheters in healthcare which is a significant problem where cleanliness is concerned and accounts for the major proportion of all hospital-acquired infection cases. Functional surfaces, while not a substitute for good hygiene, will play an increasingly important role in the cleanliness of healthcare environments.

Key words: self-cleaning, functional materials, antimicrobial agents, photocatalysts, hospital-acquired infections.

9.1 Introduction

To combat hospital-acquired infections and the spread of organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* (*E. coli*) and *Clostridium difficile* (*C. diff*), surfaces and coatings have been designed with inherent antimicrobial or 'self-sterilising' effects. The ultimate goal would be to have a self-sterilising property in every surface that is commonly in contact with people within the healthcare environment. Proposed surfaces include: door handles and push plates, bed frames, corridor walls, toilet-flush handles, curtains, clothes, etc. It should be noted that while self-sterilising surfaces and coatings are a reality, they will never replace general good hygiene as the primary measure for the control of human pathogens within a healthcare environment. Functional self-sterilising surfaces are an 'extra' effective measure in reducing hospital-acquired infections and lowering the associated death rates.

One little-appreciated concept in the spread of organisms such as *E. coli* and MRSA and their effect within a healthcare environment is that a third of people carry MRSA (usually in the nasal passage), and everyone has *E. coli* and *C. diff* in their lower intestine and bowels. The human body is adept at fighting off infection and very rarely suffers from problems unless the immune system is suppressed, such as after an operation or during a serious bout of illness. It is under these circumstances that the threat of infection from such organisms is particularly acute. The problem is, therefore, how to stop visitors and staff from a single patient transferring their bacteria to that patient or indeed unrelated patients, as well as moving one patient's bacteria to another.

Although the number of organisms on near-patient surfaces can be reduced by more frequent cleaning, this is expensive and it would be more cost effective to modify the surface to reduce adherence, transfer or survival of pathogens or to improve the efficiency of cleaning (Hayden *et al*., 2006; Hota, 2004). Some pathogens can persist for months or even years on dry, uncleaned surfaces (Hirai, 1991). *Enterococci* are among the most frequently isolated in the ward environment. They can survive for prolonged periods under dry conditions (Wendt *et al*., 1997). Many investigators have studied the survival of *Enterococci* (including Vancomycin-resistant *Enterococcus*) and found that they can persist from five days to four months on a variety of different dry surfaces (Belkin *et al*., 2000). Staphylococci can survive between one day (on all fabrics and plastics) and more than 90 days on polyethylene plastic (Belkin *et al*., 2000). Some Gram-negative bacteria such as *E. coli*, *Acinetobacter* spp., *P. aeruginosa, Klebsiella* spp. and *Shigella* spp. can survive for many months on dry surfaces. Increased survival time is correlated with increased inoculum (Belkin *et al*., 2000; Neely and Orloff, 2001), humidity (Jawad *et al*., 1996) and the presence of organic material such as serum (Hirai, 1991; Table 9.1). There is some debate as to whether the type of test material influences bacterial persistence in the environment. While some authors have found no correlation between survival times and particular surfaces (Bale *et al*., 1993; Kramer *et al*., 2006), others have reported increased survival on plastic versus fabrics, on polyester when compared with cotton (Belkin *et al*., 2000; Neely and Orloff, 2001) and on wood relative to steel (Wendt *et al*., 1998).

In the UK, more than 5000 people die each year as a result of healthcare-associated infections. Many hundreds of thousands of people develop healthcare-associated infections that prolong hospital treatment, increase morbidity and mortality and add to treatment costs. The total annual cost of healthcare-associated infections to the National Health Service in the UK is in excess of £1 billion (2004). The commonest types of healthcare-associated infections in Europe are urinary tract infections and 80% of these are associated with the use of catheters (Gil-Tomas *et al*., 2007). Catheters and similar

Table 9.1 Persistence of a selection of clinically important bacteria on dry inanimate surfaces

Bacterium	Duration of persistence (range)
Acinetobacter spp.	3 days to > 4 months
Clostridium difficile (spores)	5 months
Escherichia coli	1.5 h–16 months
Enterococcus spp. including VRE and VSE	5 days–4 months
Klebsiella spp.	2 h to > 30 months
Mycobacterium tuberculosis	1 day–4 months
Pseudomonas aeruginosa	6 h–16 months
Salmonella typhimurium	10 days–4.2 years
Shigella spp.	2 days–5 months
Staphylococcus aureus including MRSA	7 days–7 months
Streptococcus pyogenes	3 days–6.5 months
Vibrio cholerae	1–7 days

Notes: VRE, vancomycin resistant enterococci; VSE, vancomycin susceptible organism; MRSA, methicillin resistant *Staphylococcus aureus.*
Source: Adapted from Kramer *et al.*, 2006.

devices also contribute to 60% of bloodstream infections (Gil-Tomas *et al.*, 2007). Advances in regenerative medicine have led to increasing reliance on a variety of medical devices, of which the catheter is one of the most widely used (> 150M pa in the USA). This use has increased with an ageing population. Unfortunately, the non-shedding surfaces of the catheters, and related devices, frequently become colonised by microbes, resulting in biofilm formation. Biofilms comprise organised microbial communities embedded in an extracellular matrix consisting of polymers originating from both the microbe and the host. When in a biofilm, the physiology of the microbe is dramatically different from that displayed in the planktonic phase, and crucially, the biofilm exhibits greatly reduced susceptibility to host defence systems and to antimicrobial agents. Accumulation of a biofilm on the catheter surface inevitably results in a catheter-related infection that is extremely difficult to treat with antibiotics. These catheter-related infections are a major cause of morbidity and death throughout the world. In the USA, peripheral venous catheters are the most common of intravascular devices used, and their use is responsible for most of the annual 200 000 bloodstream infections. The mortality rate for catheter-related bloodstream infections is 10–25%. Urinary catheters are inserted in more than 5 million patients in the USA each year. Urinary tract infections associated with the use of these catheters are the most common infections in hospitals and nursing homes. In USA, they encompass more than 40% of all institutionally acquired infections.

A large number of articles have been written concerning the occurrence and prevention of catheter-related infections (Noimark *et al.*, 2009; Page *et al.*, 2009). Their content includes guidelines on the prevention and

management of infections and strict instructions regarding catheter insertion. Furthermore, hundreds of trials have attempted to quantify success at preventing catheter-related infections, these include antibiotic and heparin flushes, lubricant gels, antiseptics and silver containing catheters (Noimark *et al*., 2009). Despite this, millions of cases of catheter-related infections arise with fatal consequences, especially for the most vulnerable in society, namely newborns, infants and the elderly.

9.2 Self-sterilising surfaces

These surfaces can be separated into three fields: hard surfaces, soft surfaces and fabric surfaces – with a different approach needed for each. In addition to bacterial transfer by touching contaminated surfaces, there is also the effect of atmospheric contamination in the case of aerosol exhausts from people breathing, coughing and sneezing.

9.2.1 Hard surfaces

Hard surfaces include things such as door handles, flush handles, walls, equipment and bed frames. These surfaces can act as a breeding ground for bacteria and a reservoir for infection. They are often described as hand-touch surfaces and by definition are often touched repeatedly by multiple users from multiple backgrounds.

Many users of toilet facilities will touch the tap in order to turn it on only to touch the same tap again to turn it off, transferring bacteria back onto their newly washed hands. They will then proceed to touch the door handle on the way out and the push plates in the other areas of the hospital. There are approaches to these surfaces including those described below, but one increasingly popular method is to remove the surfaces altogether. Many UK hospitals now have automatic or movement sensitive flush mechanisms, soap dispensers and taps. There are also an increasing numbers of automatic doors, but items such as railings, walls and some door handles cannot be removed.

The bed rail is frequently touched by patients, their visitors and staff without hand hygiene and becomes a significant bacterial reservoir. Bed rails have been recognised as a reservoir of MRSA (Boyce, 2007). Wilson *et al*. (2008) showed that bed rails were one of the most frequently contaminated areas in intensive care units, and as they were frequently handled or touched by patients, visitors and staff, they represented the most likely reservoir for cross-infection (Moore *et al*., 2009b). There were marked differences in the number of organisms detected on different bed rail surfaces. At University College London Hospital, a level of contamination significantly greater for

textured polymer bed rails was observed compared with steel ones. In subsequent laboratory tests, the textured foot rail was most likely to acquire organisms from fingers. The plastic-coated side rail had the roughest surface and was the most difficult surface from which to remove organisms by cleaning. Transfer from bed rail to fingers was most likely with the plastic foot rail even after 24 h. Either the texture or the material of the surface may have influenced the level of bacterial contamination, which in turn may have affected the number of bacteria transferred from bed rail to hands.

Earlier work on computer keyboard surfaces has also suggested that adherence and spread can be influenced by the type of surface (Wilson *et al.*, 2008).

9.2.2 Soft surfaces

Soft surfaces include catheters, plasticised dressings and equipment buttons, etc. Many of these items will have internal applications which leads to concerns over cytotoxicity and hyper-sensitivity reactions. Internal-use applications would require a lower degree of cytotoxicity as the functional surface would be in contact with the human cells, as would bandages and wound dressings. The cytotoxicity is a very important aspect, as destruction of healthy living cells around the wound will have a negative impact on the healing process. It was found in burn victims that the cytotoxicity of silver-enhanced dressings had a detrimental effect on the healing process (Atiyeh *et al.*, 2007).

Catheters are usually placed as a semi-permanent fixture for continued use and as such provide a constant gateway for infection into the body (Noimark *et al.*, 2009). Urinary tract infections are observed with 100% occurrence within several days if the urine drainage system is not maintained as closed (Ha and Cho, 2006; Turck *et al.*, 1962). If the system remains closed, which is relatively difficult, then the chance of infection falls to 50% for a five-day period (Ha and Cho, 2006; Stark and Maki, 1984).

We have published a review on catheter-related infections in *Chemical Society Reviews* (Noimark *et al.*, 2009). In this review, we show that a number of catheters have been developed to try to reduce the incidence of catheter-related infections, including the incorporation of silver and silver-releasing agents, the use of ionopheric agents and the attachment of antibiotics to a catheter surface. Independent clinical studies have shown mixed results and only the high-dose antibiotic flushes have been shown to be independently effective – albeit at a low level. The later approach suffers from the drawback that long-term antibiotic use, as often required for catheterised patients, can lead to the development of bacterial resistance, especially as 'last resort' antibiotics are used. What is required is a new approach that avoids the use of antibiotics and which is shown to prevent infection. Our

approach is inspired by photodynamic therapy – using light together with a dye (i.e. a photosensitiser or light-activated antimicrobial agent) to kill bacteria (Wilson, 2004). Such light-activated antimicrobial surfaces not only kill microbes, but they also have an important additional advantage over conventional antibiotics in that they inactivate microbial virulence factors (Kömerik *et al.*, 2000; Tubby *et al.*, 2009).

9.2.3 Fabric surfaces

Fabric surfaces include curtains, dressings and clothing; many of these items routinely now contain silver ions (Section 9.2.2) so that they have a background resistance to bacteria, or are made from easily washable or non-stick materials. Some hospitals may use PVC-type curtains to help limit bacterial transfer and aid infection control.

9.2.4 Atmosphere

The contribution of airborne transmission or bacteria to nosocomial infection is less clear. However, large numbers of droplets of saliva and respiratory tract secretions are released into the environment by talking, coughing and sneezing, and many of these contain viable microbes (Papineni and Rosenthal, 1997). Showerheads and fountains may contain *Legionella pneumophila* and other pathogens (Bollin *et al.*, 1985). The UK Department of Health has put hospital cleanliness at the centre of its initiatives aimed at reducing healthcare-associated infections. The *National Specifications for Cleanliness in the NHS* (National Patient Safety Agency, April 2007) state: 'Providing a clean and safe environment for healthcare is a key priority for the NHS and is a core standard in *Standards for better health*.' Hospitals need to know which surfaces in the ward are easy to clean and slow to recontaminate. Reduction of environmental contamination by hospital pathogens would reduce colonisation or infection of patients by reducing the number of contaminated hand or surface contacts with the patient. Staff would pick up fewer organisms on their hands before touching the patient and the patients themselves would acquire fewer pathogens from nearby surfaces, such as the bed rails. Although this would be expected to be associated with a reduction in infections, at current rates a very large cluster randomised trial would be required to demonstrate such an effect (Moore *et al.*, 2009b).

9.2.5 Antibiotic use

Before the discovery of penicillin in 1928 by Alexander Fleming, there was no prevalence of genes encoding antibiotic resistance; however, since then,

continued and increased use of various antibiotics have led to significant resistance within some bacteria. The worldwide problem of resistance to antibiotics within hospital pathogens has been recognised for more than 20 years (Lesprit and Brun-Buisson, 2008). The problems are often associated with inappropriate antibiotic use, which has been recorded in 25–50% of hospital prescriptions (Lesprit and Brun-Buisson, 2008). The major cause is the prescription of an unnecessarily broad spectrum of antibiotics for too long a duration, which is exacerbated by many clinicians who have limited perception of the problems associated with over-use (Lesprit and Brun-Buisson, 2008).

There are many surfaces currently in use in hospitals that use antibiotics as a measure to prevent harbouring bacteria. It is also widely practised, though not recommended in many cases, to give a course of wide spectrum antibiotics to patients who have catheters inserted and left for more than 12 h at a time. There are numerous problems with the use of antibiotics, not least the evolution of bacterial strains to have resistance to antibiotics. The over-use of antibiotics has largely been responsible for epidemic strains of MRSA and other 'super bugs' that are now resistant to the highest level of antibiotics. The ability to 'invent' new and stronger antibiotics is very limited. In the next few years, the problem could be exacerbated with more strains of resistant bacteria emerging and few new antibiotics hitting the market. A vicious cycle exists, whereby antibiotic use leads to bacterial resistance, which in turn leads to use of more and stronger antibiotics.

9.3 Antimicrobial metal surfaces

There are many metals that are used in a healthcare environment simply as a result of their non-reactivity and ease of cleaning, such as stainless steel. It is relatively easy to keep stainless steel or indeed PVC table tops clean and sterile as the material lends its self nicely to easy cleaning and disinfection using traditional cleaning methods. Other metal surfaces, however, have an inherent self-sterilisation capability. These include silver and copper.

9.3.1 Copper as a sterilisation method

A clinical trial at Selly Oak Hospital in Birmingham, UK, showed some interesting results back in 2007. Three areas of interest were chosen: door push plates, taps and toilet seats. These were routinely swabbed for five days before being replaced with similar items that contained copper. The surfaces were then swabbed for a further five days and the results correlated. The study showed that there was a 90–100% decrease in the bacterial counts on the copper-containing items when compared with the standard items (2010). Thus, a significant impact was reported in a real-life environment.

The copper has the ability to disrupt the life-cycle of the bacteria by: causing leakage of potassium through the outer membrane, disruption of the osmotic balance in the cells, by binding to the proteins and by causing oxidative stress by generating hydrogen peroxide (2010).

9.3.2 Silver as a sterilisation method

Silver is known to have sterilisation properties and has been used for many decades, even centuries – for example, the Romans stored water in silver-lined vessels. Despite the noble metal properties of silver, in the bulk form, the surface of the silver can react with body fluids to form ionic silver, which is toxic to bacteria. The silver ions can transport across the cell walls of the bacteria organisms and have a toxic effect therein. Silver can also bind to the bacterial proteins and effect the modifications in the cell wall and membranes, thus having a detrimental effect on the reproduction of the bacteria and inhibiting the cells (Rai *et al.*, 2009). The activity of the silver can be controlled to a small degree by the surface area of the particles, hence nanoparticulate silver is popular. Additionally, silver compounds can be used to increase activity further. Highly active antimicrobial silver does, however, suffer from longevity issues as the silver is used up in the process. Silver nanoparticles have been successfully incorporated into a number of commercial applications designed to kill bacteria including towels, socks and catheters (Noimark *et al.*, 2009).

The exact mechanism of silver action is as yet not fully understood; however, it is believed to be linked with its interaction with thiol groups found in the respiratory enzymes of the bacteria cell wall and cell membrane.

9.4 Light-activated antimicrobial surfaces

We have been exploring a separate approach for making antimicrobial polymer surfaces by the incorporation of light-activated antimicrobial agents. These kill bacteria *via* the formation of oxygen-derived cytotoxic species such as singlet oxygen, superoxide and other reactive oxygen species (ROSs), such as the hydroxyl radical ($OH^{\bullet}$). These radicals are produced by the interaction of the photosensitiser with light, which generates highly reactive singlet oxygen species from the air or oxygen-containing environment. Clinical studies have shown that this photodynamic action of the light-activated antimicrobial agents can be used to kill bacteria, fungi, protozoa and viruses in humans and research has shown that it is possible to kill microbes that are on, or up to a distance of 0.65 mm from, a surface containing a light-activated antimicrobial agent which is illuminated, due to the diffusion of the free radicals generated (Wilson, 2003). Commercially, light-activated antimicrobial agents have been widely used and have regulatory approval

for use in the photodynamic therapy (PDT) of carcinomas, as fluorescence markers to detect tumours and in the treatment of periodontal diseases. The light-activated antimicrobial agent methylene blue (MB) is non-toxic and is authorised for intravenous administration as a 1% solution for the treatment of methaemoglobinaemia. The reactive oxygen species (ROSs) generated by the light-activated antimicrobial agents are unlikely to induce resistance in the target microbes as they simultaneously attack microbes by multiple pathways rather than the specific mode of action of antibiotics, hence they are suitable for longer-term use. Furthermore, our preliminary studies show that the light-activated antimicrobial agent–light combination dramatically hinders bacteria adhesion, prevents formation of a conditioning film and can actually induce detachment of an active biofilm.

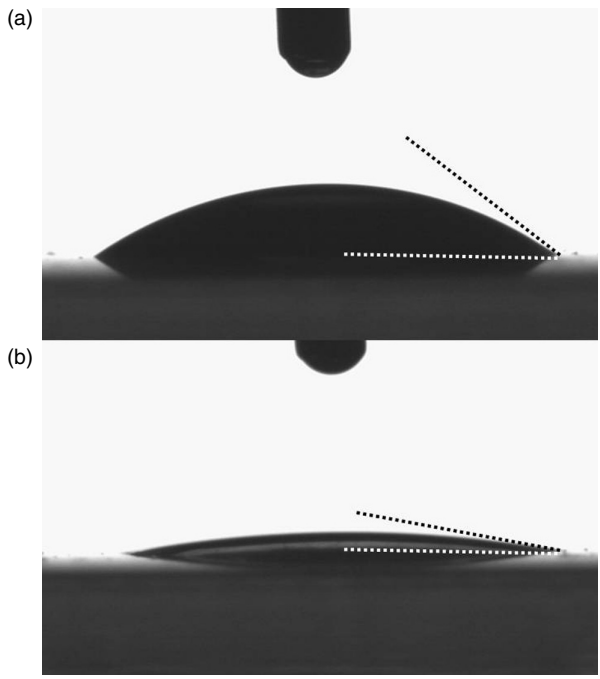
9.4.1 Titanium oxide-based systems

TiO_2 is the most commonly found and applied photocatalyst. Unlike silver additive systems, the light-activated antimicrobial coatings have the ability to regenerate their activity in the presence of light, thus retaining the maximum effectiveness against human pathogens. The self-cleaning coatings are non-specific and therefore non-discriminatory, destroying all forms of bacteria and viruses as they are simply considered to be functionalised carbon chains. This is advantageous as the organisms cannot readily evolve immunity to the sterilisation as they do with antibiotics and silver ion diffusion, which are more targeted. Some bacteria, however, are more susceptible to destruction by light-activated surfaces than other bacteria – for example, *E. coli*, a Gram-negative bacteria, is harder to kill than MRSA, which is Gram-positive (Decraene *et al.*, 2006). This is down to the make-up of the cell wall and the mechanism in which the light-activated surface kills the bacteria. Light is absorbed into the semiconductor, which then promotes an electron from the conduction band into the valance band. This creates two separate entities: an electron and a positive hole. The electron and hole pair can then migrate in different directions to the surface where a reaction with water or oxygen produces what is called 'singlet oxygen', a very toxic and reactive species that can interfere with the cell wall structure. In essence, the singlet oxygen performs a form of 'cold combustion', where carbon atoms are converted into carbon dioxide and thus the complex bacteria is simply reduced to carbon dioxide, mineral acids and water. In actuality, few bacteria are fully photomineralised; the majority are damaged by the photocatalyst – especially the outer membranes – which then leak cellular content and destroy the bacteria.

There is, however, another effect that is occurring additional to the cold combustion or photo-induced oxidation of the bacterial cell wall and that is the surface effect of superhydrophilicity. This effect concerns the relationship

between the surface and water. If the surface loves water (hydrophilic), then a droplet will spread into a pool and tend to run off the surface as long as it is not horizontal. It will also take away dirt as it does so in a uniform fashion, rarely leaving streaks. If the contact angle measured between the surface and the side of a water droplet reaches an angle of less than 10 degrees then the surface is considered to be superhydrophilic (Fig. 9.1). In a hospital setting, this will make the light-activated surfaces much easier to clean as dirt will simply not stick to them. This is the process by which commercial self-cleaning windows such as Pilkington Activ™ and BIOCLEAN™ work (http://www.selfcleaningglass.com).

The big problem with TiO_2 as a functional self-sterilising coating indoors, however, is that the band onset of the semiconductor is ~3.2 eV. It therefore requires UV light to activate it, which is relatively abundant on the outside of buildings due to sunlight but is not abundant inside. Window glass absorbs much of the UV light in sunlight and interior lighting contains very little UV, so pure TiO_2 coatings are inefficient and unsuitable for indoor application, even on the inside surfaces of windows. TiO_2 self-cleaning surfaces



9.1 Image of two 8.5 μL water droplets on glass substrates showing (a) the effect of radiation on the photocatalyst and (b) the effect of the interaction with the water.

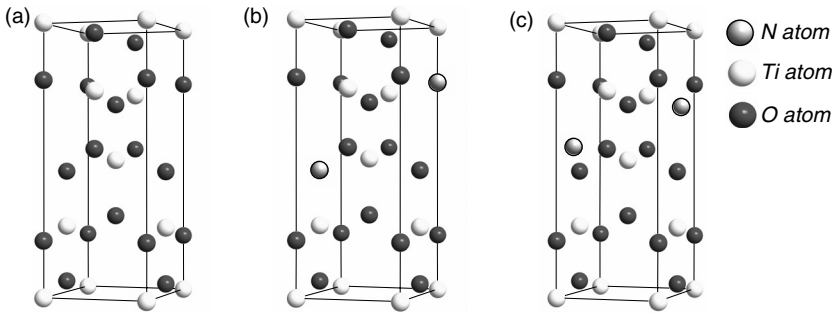
would only be suitable for inside use alongside a rigorous cleaning protocol involving portable high-powered UV lights. Much research interest has been directed at modifying TiO_2 and changing the band gap properties to develop indoor, white light photocatalysts.

Modified TiO_2 systems

TiO_2 can be modified to absorb visible light so that it acts as a photocatalyst under indoor lighting conditions (Dunnill *et al.,* 2012) Modifications can take the form of either dopants or island enhancements. In the case of dopants, many elements have been doped into the structure of the TiO_2 to alter the band structure properties to allow the material to absorb and use visible light rather than UV light. Typical dopants include nitrogen (Dunnill and Parkin, 2009; Dunnill *et al.*, 2009b, 2010), sulphur (Dunnill *et al.*, 2009a), carbon (Li *et al.*, 2007a, 2007b) and many different metals (Anpo *et al.*, 2002; Gracia *et al.*, 2004; Jung, 2001). Island enhancement is concerned with producing islands of metal nanoparticles on the surface, such as silver (Dunnill *et al.*, 2011a, 2011b). The dopants tend to have an effect on the electrical properties, by shifting the band onset of the band structure within the TiO_2. This allows the surface to absorb photons of wavelength $\lambda > 385$ nm and thus operate as a photocatalyst using visible light. A small shift in the band onset (~40–50 nm) of a photocatalyst will actually have a remarkable ten-fold enhancement on the photocatalytic properties as there is a significant increase in the number of photons available at lower energy.

Light-activated systems in general have been seen to have different effects on different types of bacteria. *E. coli*, for instance, is a Gram-negative bacteria and is much less susceptible to destruction by light-activated antimicrobial surfaces than MRSA, which is a Gram-positive organism. Some strains of MRSA have been shown to become inactive just in the presence of bright white light.

The doping of nitrogen into the TiO_2 structure can occur in two forms, substitutional and interstitial. Their names refer to the location of the nitrogen, which is critical to photocatalytic efficiency. Substitutional doping involves oxygen replacement – that is, the formation of $TiO_{2-x}N_x$ – while interstitial doping involves the addition of nitrogen to the TiO_2 lattice and the formation of TiO_2N_x, as shown in Fig. 9.2. In reality, however, isolating the individual forms is very difficult, with a $TiO_{2-x}N_y$ ($x < y$) species often formed. It is apparent that the location of the nitrogen is critical to the behaviour of the material as a photocatalyst, though there is much debate as to which position is most beneficial. In many reports, a combination of both substitution and interstitial doping could have occurred, leading to issues with reported results for one type of doping over another. The presence of nitrogen can alter the band structure or suppress the recombination efficiency of the photo-generated electron–hole pairs (Beranek and Kisch, 2007).



9.2 Schematic showing the structure of the three different forms of anatase with possible nitrogen positions: (a) anatase TiO_2; (b) substitutional doping; and (c) interstitial doping. The percentage of nitrogen would in fact be significantly smaller than two atoms per unit cell (Dunnill *et al.*, 2011b).

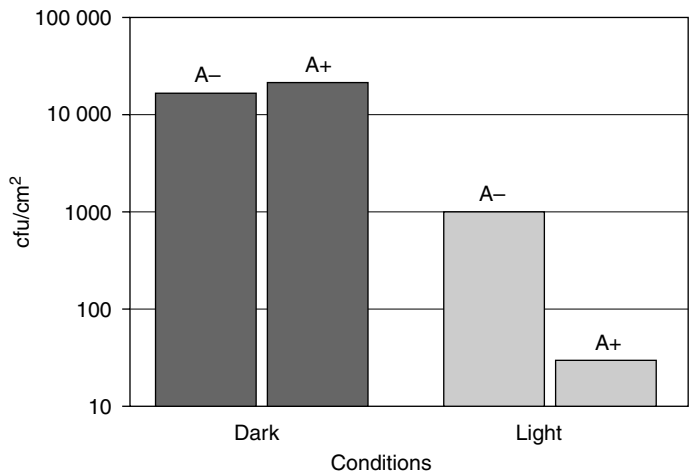
The presence of low concentrations of nitrogen within the anatase form of titania is known to promote a large decrease in the formation energy of oxygen vacancies from 4.2 to 0.6 eV (Di Valentin *et al.*, 2005; Zhao *et al.*, 2008). This occurs as a result of excess electrons created in the oxygen vacancy being trapped on the nitrogen site. These oxygen vacancies have been attributed to colour centres and indeed to the enhanced photocatalytic activity. Some authors believe, however, that the presence of oxygen vacancies instead acts to give recombination sites for electrons and holes, leading to poor performance (Prabakar *et al.*, 2007). It is entirely possible, with this disagreement, that the correlation between photocatalytic enhancement and nitrogen content is actually more complex and indirect due to the presence of oxygen vacancies created low concentration of nitrogen within the titania lattice. High nitrogen concentrations encourage substitutional doping, where it is distinctly favoured over interstitial doping (Di Valentin *et al.*, 2005), which has a profound effect on the material properties as the nitrogen fills the oxygen vacancies, removing the oxygen vacancies that may enhance the photocatalysis. In general, the literature is divided between three positions: those believing that substitutional N-doping into the titanium dioxide creates visible light photocatalysis, those believing that it is interstitial doping that is required and those who believe that the enhanced photocatalysis is, in fact, down purely to the formation of oxygen vacancies within the structure, and therefore more subtly due to the nitrogen dopant.

N-doped films by CVD have shown a significant kill in bacteria such as *E. coli* when only the interstitial site has been occupied. Samples were either exposed to two 24-h light doses (A+L+), an activating 24 h white light dose before the addition of *E. coli* (A+L–), 24 h white light irradiation after *E. coli* addition

(A–L+) or incubated in the absence of light throughout (A–L–). Bars indicate median values from the data sets. A+L+ shows a 2.8 log kill (99.9%), while A–L+ shows a 1.2 log kill (99.4%) compared with the A–L– control (Fig. 9.3).

9.4.2 Dye-based systems

Wilson *et al.* (2008) have shown that the incorporation of the dye toluidine blue O (TBO) into cellulose acetate, results in an antimicrobial material that can kill bacteria when irradiated with white light and that this can reduce the microbial load in a clinical environment (Decraene *et al.*, 2006). MB can also be incorporated into polysiloxane and polyurethane polymers and catheters along with 2 nm nanogold particles via a swell-encapsulation-shrink method (Perni *et al.*, 2009a, 2009b). The catheter-polymers with just MB or TBO achieved up to a 2 $\log_{10}$ reduction in the viable counts of MRSA and *E. coli* when exposed for 1 min to light from a low power 660 nm laser. Interestingly, the incorporation of traces of 2 nm nanogold particles significantly enhanced the ability of MB and TBO to kill bacteria to greater than 6 $\log_{10}$. That is a 10^4 increase in effectiveness with a 1 min exposure. We were the first to show that gold nanoparticles, while having no antibacterial effect alone, are effective enhancers of bacterial lethal photosensitisation (Narband *et al.*, 2008). Furthermore, a covalently linked gold–tiopronin–TBO molecule made at University College London has been shown to be the most potent light-activated antimicrobial agent



9.3 Colony-forming unit (cfu) counts for the survival of *E. coli* on an N-doped TiO_2 sample after exposure to the white light source. A± indicates an activation step consisting of 24 h white light, prior to the application of *E. coli* on the surface (Dunnill *et al.*, 2009b).

ever reported for killing MRSA, based on concentration (Gil-Tomas *et al.*, 2007). This agent at 1 μm concentration gives a 7 $\log_{10}$ reduction compared with 2 $\log_{10}$ reduction for TBO of equivalent concentration and light exposure (Perni *et al.*, 2009b). Recent work at University College London has shown the efficacy of this approach using both laser and white light (hospital bulbs) sources. In all cases studied, including a small-scale (year-long) trial in a dental clinic where settle plates were used, gold nanoparticles enhance the light-activated antimicrobial agents' ability to kill bacteria with no kill seen for the nanoparticles on their own. This study showed the long-term photo-stability of the light-activated antimicrobial agents–gold–polymers and conjugates under hospital lighting conditions. The concentrations of light-activated antimicrobial agent within the polymers is low – less than 1 ppm for covalently attached light-activated antimicrobial agent and up to 100–500 ppm for swell-encapsulated material. The amounts of gold used are significantly lower – yet still have a prominent enhancement effect (< 1–10 ppm). To be able to build effective devices there is a need to understand the underlying science – it is not yet known how the gold nanoparticles enhance the bacterial kill or what the best method to optimise the polymers and make devices is. We see the self-sterilising polymers having a myriad of applications within healthcare, including catheters, dressings, drapes, curtains, keyboards, telephones, etc., and have shown that they are very effective under indoor room and hospital lighting conditions.

There are many dyes that are available and known to have a light-activated antimicrobial effect. MB and TBO are examples of such dyes. Soft plastics such as those used in many catheters, buttons and keyboards can be encapsulated with MB molecules to give them an inherent antimicrobial nature. This can be achieved easily by the swell-encapsulation method. Here the plastic surface is soaked in a specially formulated mixture of swelling agent and dye solution. The swelling agent opens up the polymer chains allowing access for the dye into the polymer matrix. Once removed from the bath, the swelling agent simply evaporates back out of the polymer, leaving the dye fixed in place with the polymer chains.

Obviously, in the case of light-activated antimicrobial surfaces that have application inside the body such as catheters, it is important to deliver the activating light. This can be done using specialist optical fibres that fit inside the lumens of the catheters and leak light out sideways so that they irradiate both the internal and external surface of the catheter during use.

9.4.3 Combined surfaces

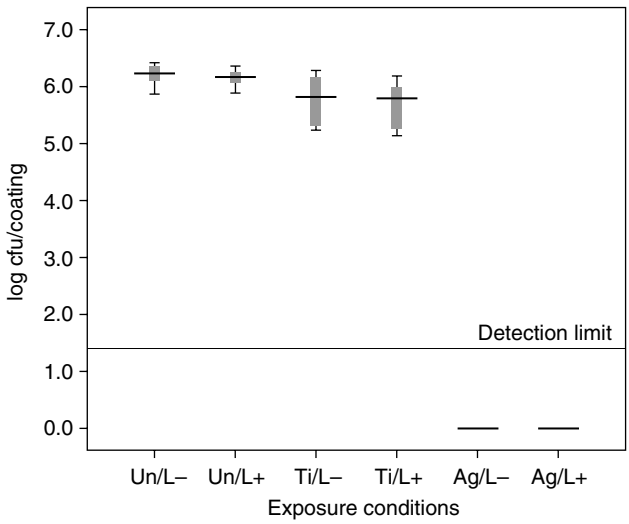
It is possible to further enhance the properties of the above antimicrobial surfaces by combining different techniques together. The soft polymers that can be given a light-activated antimicrobial effect by the swell-encapsulation

of MB or TBO can also be enhanced by the addition of gold or silver nanoparticles to the soaking mixture. These enhanced materials have a much more potent sterilisation effect than the dye alone. The additional cost is small as the concentration of the noble metal is minutely small.

Combined surfaces can be created using a dual-step approach. An example of this is the use of a TiO_2 surface that has been post-treated with silver nanoparticles in the form of nano-islands. The silver nanoparticles are easily applied using silver nitrate solution which decomposes in the presence of UV light and the TiO_2 surface (Dunnill *et al.*, 2011b). Surfaces can have controlled island size and population by varying the concentration of the silver nitrate solution and the speed at which the dip-coating occurs. The main advantage of these surfaces is two-fold. First, the silver islands leach silver ions into the TiO_2 substrate shifting the band onset towards the visible and improving the photocatalysis. Second, the combined substrates now have a dual action for antimicrobial activity. The synergistic nature of the combined surfaces means that there is a photo-activated release of silver as well as a silver-activated enhancement of the photocatalytic cleaning properties. These surfaces are, therefore, more potent than both the plain TiO_2 and silver-coated surface, as well as having considerably better lifetimes. Similar island formations can be applied to N-doped TiO_2 for even more potency (Dunnill *et al.*, 2011a). It is well known that different bacteria have different susceptibilities to control by silver and/or light-activated surfaces. *E. coli*, for instance, is one of the hardest organisms to deactivate using a light-activated antimicrobial as it is a very sturdy and stable Gram-negative organism. It is, however, very susceptible to the toxicity of silver. On the other hand, epidemic strains of MRSA, such as EMRSA 15 and EMRSA 16, are fairly resistant to silver toxicity but relatively easily killed by light-activated surfaces. These combined antimicrobial surfaces would therefore have the advantage of the dual-pronged approach whereby the different bacteria are neutralised more or less by the different types of antimicrobial agent. This gives a surface that is resistant to colonisation by all types of bacteria and viruses, not just one specific group.

In the case of combined surfaces, a significant 6 log kill was observed using *E. coli* both in the dark and in the light on a titanium oxide film enhanced by silver islands (Fig. 9.4). There is much significance to the fact that the bacteria was completely neutralised both in the light and in the dark, indicating that the most probable cause of the destruction was the silver rather than the light-activated surface.

In the case of the MRSA 16, a light-tolerant epidemic strain of MRSA currently infecting UK hospitals, a clear distinction is observed showing the increased destruction of the bacteria in the light when compared with the dark (Fig. 9.5). Here the increase is directly related to the presence of light activation using the same surfaces as those described above. A dual



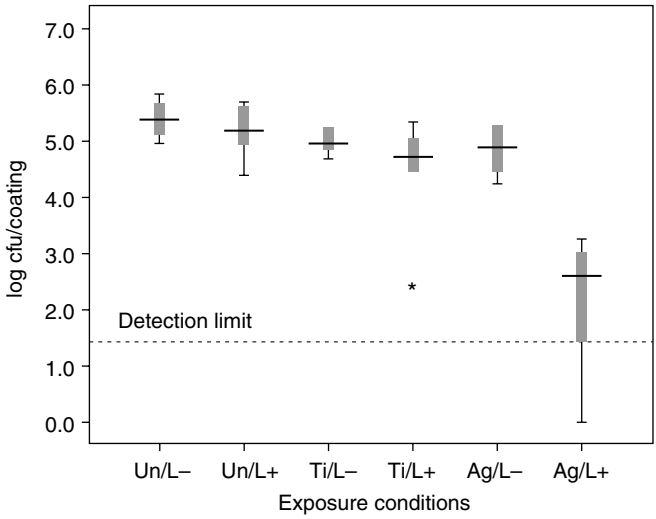
9.4 Microbiology data showing three different samples under two different conditions of testing, light on (L+, hospital lighting conditions) and light off (L–). The first two data sets are from blank microscope slides, Un, the next two data sets are sample TiO_2 and the final two data sets are from sample Ag–TiO_2. Median values are displayed in colony-forming units present on the test slides after 6 h of incubation at room temperature and are represented by the thick horizontal line. The base and top of each box represents the 25% and 75% quartiles respectively, and the error bars, the 10% and 90% percentiles. The detection limit of the assay is also shown (Dunnill *et al.*, 2011b).

approach and synergistic mechanism is therefore postulated whereby the light-activated surface promotes the silver ion release as well as the silver enhancing the light activation. These films are thus highly effective against both *E. coli* and MRSA covering Gram-negative and Gram-positive organisms and producing a universally toxic surface to bacteria.

9.5 Conclusions

Self-sterilising material surfaces could and should have significance in the field of antimicrobial control within a healthcare environment. The ability of a surface to retain some inherent antibacterial properties long term and without the destruction of healthy human cells with which the surface comes in contact is paramount.

One of the major problems with the testing of such surfaces in the real world is the ability to control the variables. Even if two similar wards were set up in nearby wings of the same hospital, it would be difficult to say with certainty that an increase or decrease in infection rates was due to the antimicrobial surfaces and not due to some other initiative or increase in



9.5 Microbiology data for MRSA 16, showing three different samples under two different conditions of testing, light on (L+, hospital lighting conditions) and light off (L–). The first two data sets are from blank microscope slides, Un, the next two data sets are the pure titania, TiO_2 and the final two data sets are from sample Ag–TiO_2. (Dunnill *et al.*, 2011b).

effectiveness of an already standard cleaning regime. Common sense, however, would dictate that functionalising the surfaces to kill bacteria would help as an added preventative measure. It should never be used to replace good hygiene standards and regular cleaning.

Nosocomial pathogens are acquired by patients through contact with the hands of carers or their visitors or directly from their local environment. Some studies suggest that cleaning of the near-patient surfaces can reduce methicillin-resistant *S. aureus* infection (Dancer *et al.*, 2009). However, a much larger study in a critical care environment was not able to demonstrate an effect of high-level cleaning on acquisition of MRSA (Moore *et al.*, 2009a). Microbes present on surfaces can be transferred to susceptible individuals (Hota, 2004). However, the association between environmental contamination and nosocomial infection is unclear and probably multifactorial.

Previous work (Moore *et al.*, 2009b) showed that when MRSA numbers were reduced in the environment, detection of MRSA on staff hands was similarly reduced. Hands acquire pathogens from environmental reservoirs and hospital pathogens are transmitted between staff and patients via environmental surfaces. Multi-resistant strains, such as MRSA, *Acinetobacter baumannii*, vancomycin-resistant *Enterococci* (VRE) and *Clostridium difficile* are particularly important because of their ability to survive on dry surfaces for

many months, even in the absence of biological material (skin scales, secretions, etc.). Methicillin-sensitive *S. aureus* (MSSA) can survive for up to ten days on formica surfaces, with strains of MRSA surviving up to fourteen days (Duckworth and Jordens, 1990). Potential reservoirs include hands, gloves and gowns of hospital staff and patients, bed rails, arterial pressure monitors, ventilators, tubing, resuscitation bags, mattresses and pillows (Dancer, 2004).

9.6 Future trends

In the future, there will likely be less reliance on antibiotic treatments and more reliance on functional surfaces in the control of human pathogens. Functional surfaces containing such technologies as light-activated antimicrobial agents could be deployed in many forms, including catheters. Developments in this field will include the need to deploy light to the active sites that could be inside the body. The cleanliness of hospitals over the next few years will continue to rise in significance as the effectiveness of front-line antibiotic drugs lowers and the bacterial resistance increases. This will prove to be an increasingly interesting field of study with real-life applications and positive consequences to all.

9.7 Sources of further information

The following reviews are suggested as a basis from which to begin researching the discussion in this chapter: Beranek and Kisch, 2007; Dunnill and Parkin, 2011; Mills and Le Hunte, 1997; Noimark *et al*., 2009; Page *et al*., 2009, Parkin and Palgrave, 2005 and Dunnill *et al.,* 2012.

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10

Prions and endotoxins: reprocessing strategies for reusable medical devices

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Abstract: The apparent high level of resistance of Creutzfeldt-Jakob disease (CJD) and variant Creutzfeldt-Jakob disease (vCJD) prions to inactivation by conventional means of device reprocessing poses an infection prevention challenge to a hospital's central sterilization department. This chapter will focus on prion inactivation methods for instrument reprocessing that are currently recommended by public health agencies for the prevention of iatrogenic transmissible spongiform encephalopathy (TSE) transmission. Important factors such as cleaning/decontamination efficacy and steel quality are discussed. Research continues to evaluate the potential use of alternative sterilizing agents and methods for prion inactivation. Additionally, the reprocessing strategies for the removal or inactivation of bacterial endotoxin are discussed briefly.

Key words: prions, Creutzfeldt-Jakob disease (CJD), variant Creutzfeldt-Jakob disease (vCJD), prion decontamination, cleaning agents, steam sterilization, endotoxin, water treatment, dry heat.

10.1 Introduction: prion disease epidemiology

Transmissible spongiform encephalopathies (TSEs) are progressive neurodegenerative diseases of humans and animals. These rare, fatal diseases of the central nervous system (CNS) are associated with the presence of pathological prions that arise via a process (the mechanism of which is a matter of debate), by which a normal cellular prion protein (PrP^{C}) characterized by alpha-helix conformation is converted to an abnormal form (PrP^{Sc}) with

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beta-sheet characteristics (Prusiner, 1998; Moore *et al.*, 2009). The major TSE diseases of humans include Creutzfeldt-Jakob disease (CJD) and since 1996, variant CJD (vCJD); three other TSEs of humans are fatal familial insomnia, Gerstmann–Sträussler–Scheinker syndrome, and kuru (the incidence of which has been all, but eliminated since the Fore people of New Guinea ceased the practice of an end of life ritual, in which surviving family members would eat the brain of the deceased) (Kretzschmar, 1993; Will *et al.*, 1996; Belay, 1999; Belay and Schonberger, 2005). The incidence of CJD in the USA occurs primarily in older adults, with increasing numbers of cases appearing in persons aged 55–9 and peaking between ages 65 and 75. Current surveillance continues to show an annual sporadic CJD incidence rate of approximately one case per million population in the USA (Holman *et al.*, 1996, 2010), while the annual worldwide incidence is estimated between 0.5 and 2.0 cases per million population (CDC, 2008).

The TSEs of humans can be assigned to three groups based on the perceived means of acquisition – idiopathic (sporadic without a known external source), genetic or familial, and acquired (from a known external source) (UK Department of Health, 2003). The idiopathic TSEs are sporadic CJD (sCJD) and sporadic fatal insomnia. Genetic TSEs include familial CJD, Gertsmann–Sträussler–Scheinker syndrome and familial fatal insomnia. Acquired TSEs are kuru, iatrogenic CJD and vCJD. Of the different manifestations of CJD, sCJD is the most common, accounting for 85–90% of cases (Belay, 1999; Belay and Schonberger, 2005). Familial CJD occurs much less frequently, being identified in approximately 10–15% of cases. Iatrogenic CJD (i.e. those cases associated with the delivery of medical care) are very rare today (< 1%). Direct contact of CNS tissues with prion-contaminated surgical instruments and devices has been associated with PrP^{Sc} transmission; this will be discussed in more detail in the following subsection (Bernoulli *et al.*, 1977; Will and Matthews, 1982). Much of the iatrogenic transmission in the past has been linked to receipt of prion-contaminated tissues during transplant procedures (i.e. cornea [two confirmed cases, one possible case] or dura mater [228 cases]), and receipt of hormone therapy using hormones derived from prion-contaminated cadaveric sources. The hormones used were pituitary growth hormone (226 cases) or gonadotropin (4 cases) (Brown *et al.*, 1992; Lang *et al.*, 1998; Will, 2003; CDC, 2008). Currently, the risk of PrP^{Sc} transmission from donated hormones and tissues has largely been eliminated through the use of several important infection prevention developments including: (1) use of recombinant DNA technology for hormone production since 1985; (2) implementation of and adherence to donor medical assessment activities and (3) diligence in using effective prion inactivation procedures when processing dura mater (Janssen and Schonberger, 1991).

The sporadic form of CJD (sCJD) is thought to arise from a spontaneous event that converts PrP^{C} to the abnormal PrP^{Sc} which, in turn, serves as the template to continue the conversion of the normal cellular protein to more

copies of the abnormal isoform (Prusiner, 1998; Weissmann *et al*., 2002). Among sCJD cases, there appear to be at least six phenotypes determined by the properties of PrP^{Sc} and several subtypes determined by the polymorphism at codon 129 (methionine and valine alleles) of the prion protein gene (PRNP) (Parchi *et al*., 1999). Clinical profiles appear to differ slightly among the phenotypes, and diagnostic test results can vary depending on the subtype as well. Some of the major symptoms of sCJD include cognitive impairment and dementia, ataxia, myoclonus, sensory disturbances and akinetic mutism. The course of illness for sCJD is fairly rapid, with death typically occurring within one year of onset (Belay *et al*., 2005). Pneumonia in the bedridden sCJD patient is often noted as aco-morbidity at the time of death.

The epidemiology of vCJD cases differs from that of sCJD in several aspects. This TSE was first recognized in the UK in 1996 and eventually was linked to the consumption of beef that was contaminated with a zoonotic prion of cattle – the bovine spongiform encephalopathy (BSE) agent (Will *et al*., 1996, 2000). Symptoms at onset include sensory disturbances and psychiatric manifestations (e.g. anxiety, irritability, insomnia), with neurologic symptoms appearing later (e.g. gait disturbances, myoclonus, other cerebellar signs) (Spencer *et al*., 2002). Cases of vCJD in the UK occur in a much younger population, with a median age of death being 28 years, and the course of illness extended beyond one year by several months (e.g. 13–14 months from onset) (Belay, 1999; Sejvar *et al*., 2008). When the ages of British vCJD patients are factored into a mathematical model along with the assumption that all of these infections are the result of exposure during the 1980s, it is estimated that the time interval between exposure to BSE contamination in beef and the onset of vCJD symptoms is 16.7 years (Valleron *et al*., 2001).

CJD was first described as an idiopathic disease condition of the CNS in 1921, and for nearly 30 years it remained under this designation with individual cases having no obvious association to any other CJD case. However, two reports from the UK in the 1950s raised the question about potential patient-to-patient transmission of CJD when, during a nosocomial disease investigation, neurosurgical instruments were found to be in common among three patients diagnosed with CJD (Nevin *et al*., 1960). At that time, the investigation did not unequivocally prove PrP^{Sc} transmission via contaminated neurosurgical instruments, but it did draw attention to: (1) the possibility that CJD could be a transmissible disease and (2) instruments and devices used in surgery (primarily neurosurgeries) and perhaps other medical care procedures could serve as fomites in transmission.

10.2 Prion resistance to inactivation: early research

The report by Nevin *et al*. in 1960 describing a possible association of presumably contaminated neurosurgical instruments with transmission of three

cases of iatrogenic CJD is interesting in the fact that there was little to no detail provided about the methods of neurosurgical instrument cleaning and sterilization. We can only assume, therefore, that the hospital used the conventional instrument reprocessing procedures of the day. This most likely would have included cleaning the instruments with a surfactant cleaner and subjecting the cleaned, dried instruments to steam sterilization in a gravity displacement autoclave, the parameters for the sterilization cycle being unknown. A single case of iatrogenic CJD attributed to exposure to PrP^{Sc}-contaminated neurosurgical instruments was described in France several years later (Foncin *et al*., 1980). Once again, the exact details of the instrument reprocessing procedures so central to the issue of interrupting prion transmission are limited in this report. The instruments were cleaned with soap and water, dried and subjected to what was considered a standard dry-heat method of sterilization (i.e. 180°C for 2 h). In the mid-1970s, two cases of iatrogenic CJD reportedly associated with implantable stereotactic depth electrodes were identified in Switzerland (Bernoulli *et al*., 1977). The definitive evidence of PrP^{Sc} transmissibility associated with contaminated fomites (e.g. devices) was generated when the implicated depth electrodes had been implanted into the brain of a chimpanzee; the animal developed TSE symptoms within 18 months of implantation (Brown *et al*., 1992; Gibbs *et al*., 1994). The reports noted that the depth electrodes were 'sterilized' by cleaning them with benzene and subjecting the devices to 70% alcohol and formaldehyde vapor. When compared with modern conventional instrument reprocessing procedures, it is clear that the methods mentioned in these reports would be considered inadequate and, in the case of the depth electrodes, unorthodox by today's standards. Additionally, the design of the depth electrode device, that of a blind, closed-end lumen, posed significant challenges to effective cleaning. In total, six cases of iatrogenic CJD were identified from the 1950s to 1976 and, interestingly, all of these occurred in Europe. To date, there have been no reports of additional cases of iatrogenic CJD definitively linked to surgical instruments or devices. Furthermore, there have been no cases of iatrogenic vCJD attributed to surgical instrument use over the past 11 years (Lumley, 2008; Health Protection Agency, 2011).

The earliest reports noting PrP^{Sc} resistance to chemicals and physical methods of inactivation came from veterinary medical research. Veterinary researchers in the 1950s perhaps made the first discovery of PrP^{Sc} transmissibility when they attempted to prepare a vaccine to prevent louping-ill in sheep herds (Greig, 1950). Neural tissue obtained from infected animals was used to prepare the vaccine, and this was treated with 0.35% v/v formalin to inactivate the louping-ill virus. Approximately 10% of the vaccinated animals came down with scrapie, a TSE common to sheep (Taylor, 1999). This experience in veterinary vaccine production heightened an awareness that a disease agent (the exact nature of which was unknown at the time) was

present in the source material of the vaccine, and this agent was apparently unaffected by a chemical treatment known to inactivate viruses without adversely affecting the antigenic properties of the source material. There followed an active research program to study the properties of this disease agent and to develop effective inactivation methods that could be used during preparation of tissue homogenates and macerates for veterinary medicine. Kimberlin and colleagues were among the first to demonstrate that two strains of mouse-passaged scrapie PrP^{Sc} in 10% brain homogenates could be inactivated when exposed for 30 min to a sodium hypochlorite (NaOCl) solution containing 13 750 parts per million (ppm) available chlorine (Kimberlin *et al*., 1983). Kimberlin hypothesized that an inactivation treatment for PrP^{Sc} should effect some reduction in titer of at least 10^4 $\log_{10}$ units. This experiment with NaOCl achieved a 10^4–10^5 $\log_{10}$ unit reduction in titer. Other researchers determined that NaOCl solutions containing 20 000 ppm were effective against the BSE agent during a 1 h exposure period, then its producing a 10^5 $\log_{10}$ reduction in titer (Taylor *et al*., 1994). Experiments to evaluate the potential use of sodium hydroxide (NaOH) for prion inactivation had produced mixed results that most likely were attributed to prion strain differences. Exposures of scrapie PrP^{Sc} and BSE agent to 1M–2M NaOH for up to 2 h also did not result in complete inactivation, but reductions in prion activity of ≥ 5 $\log_{10}$ were observed (Taylor *et al*., 1994). One group reported what appeared to be successful prion inactivation of CJD prions and scrapie agent by using 1M NaOH for a 1 h exposure period (reductions of ≥ 5 $\log_{10}$ lethal doses [LD_{50}]), but problems were noted for the animal assay due to toxicity of the samples (Brown *et al*., 1986). Other researchers, however, had noted some residual prion infectivity in animal assays when CJD, scrapie, or BSE prions were treated with 1M–2M NaOH for at least 1 h (Diringer and Braig, 1989; Ernst and Race, 1993; Taylor *et al*., 1994). One important observation about these early experiments with chemical treatments is that most researchers used an experimental design that, from a total protein perspective, posed an enormous challenge to the inactivation chemical. Furthermore, these relatively large amounts of protein (i.e. milligram quantities of 10% brain macerate from infected animals) were characterized as being of very high prion titer (~10^5 to $\geq 10^8$ LD_{50}/mg). The observation by Taylor and colleagues regarding the inactivation of the 263 K strain of scrapie agent via use of 2M NaOH was that while the titer was reduced by > 5 $\log_{10}$ LD_{50}, titrations using animal assay indicated that ~4 $\log_{10}$ LD_{50} prion activity remained (Taylor *et al*., 1994). More importantly, however, such an experimental design revealed the point of failure for this method of inactivation. Nevertheless, use of either chemical (~20 000 ppm NaOCl or 1M NaOH) for 1 h at room temperature for initial prion decontamination purposes was viewed as providing significant reductions in titer (4 to ≥ 5 $\log_{10}$ LD_{50}). Either of these chemicals would eventually be included

as part of several multi-step strategies for prion inactivation (Kimberlin *et al*., 1983; Brown *et al*., 1986; Taylor *et al*., 1994; Taylor, 2000).

During this same period (late 1980s and throughout the 1990s), prion inactivation research expanded to examine the effects of physical methods of inactivation and, in particular, research focused on the use of steam sterilization. Inactivation treatments using steam sterilization at temperatures 134–8°C in a porous load autoclave for up to 1 h did not completely inactivate high titers of either the BSE agent or two strains of scrapie (263K and ME7), but prion activity was reduced to ≥ 3 $\log_{10}$ using these sterilization cycle parameters (Taylor, 1999). The amount of brain macerate used in this experiment was 340 mg. Interestingly, in a separate experiment, prion activity in 340 mg of infected brain tissue was inactivated under those conditions. Earlier studies of steam sterilization in porous load autoclaves examined the effect on prion inactivation when the amount of brain macerate was increased (i.e. 50 mg vs 375 mg) and different parameters of the sterilization cycle were used (i.e. 134°C, 136°C and 138°C, and various cycle times) (Dickinson and Taylor, 1978; Kimberlin *et al*., 1983). Varying levels of thermoresistance were noted among the different strains of scrapie agent tested. Interestingly, increased scrapie agent thermoresistance was observed when small amounts of brain tissue macerate (50 mg) were spread thin on a lab coupon and autoclaved for 9 min at 138°C. The interpretation of this result was that prions in thin films would become rapidly 'heat-fixed', thereby maintaining the infectious beta-sheet configuration. This observation was confirmed in follow-up studies and would later underscore the recommendation to prevent infectious tissue from drying out on surgical instrument surfaces (Taylor *et al*., 1998).

While early experiments had shown that separate exposure to concentrated solutions of NaOCl or NaOH, or autoclaving at 134°C for at least 18 min often resulted in incomplete inactivation of prions (reductions of ≥ 3–5 $\log_{10}$ LD_{50}), these results were useful in demonstrating the limitations of each method in the face of high protein, high prion titer challenges, given that prion titers in brain tissue can range from 10^8 to 10^{11} LD_{50} per gram. Several researchers evaluated the effect of combining these two methods (i.e. chemical exposure and physical inactivation via autoclaving) either sequentially or performed together. Studies were conducted to examine the combined effect of 1M–2M NaOH as an immersion chemical and autoclaving the immersed items at 121°C in a gravity displacement autoclave for up to 90 min; complete inactivation was achieved for CJD prions and several strains of scrapie agent (Taguchi *et al*., 1991; Ernst and Race, 1993; Taylor *et al*., 1994). An alternative approach is to immerse the contaminated surface in a container of NaOH or NaOCl for an exposure time period, transfer the contaminated item to a container of water and then place this container in the gravity displacement autoclave to continue the decontamination process. If chemical exposure and autoclaving are performed in sequence, surfaces

with tissue contamination would be immersed in either NaOH or NaOCl for an exposure period first, removed and rinsed, dried and then autoclaved in an open pan. Each of these approaches to prion inactivation has reduced prion titers to below detection levels in animal assays (≥ 5 $\log_{10}$).

10.3 Current recommendations for instrument reprocessing

For the past 40 years it has been evident that prevention of iatrogenic CJD cases is a necessary challenge for healthcare institutions, especially so for those hospitals, hospital trusts and medical centers that perform any type of neurosurgery. At present the incidence of iatrogenic CJD associated with surgical instruments is extremely rare. The possibility exists that this may be due to the fact that it is often difficult to pinpoint the antecedent medical event believed to be the source of exposure to PrP^{Sc}, and disease investigations often encounter information gaps in medical records that add to that difficulty (Stricof *et al*., 2006). Nevertheless, it is important to acknowledge that the medical community since the 1980s has expressed interest in developing and implementing important infection control strategies to prevent iatrogenic CJD transmission where surgical instruments or other invasive devices are concerned (Brown *et al*., 2000). In the late 1990s, hospitals looked to the prion academic community to assess the state of prion inactivation research so that prevention of some prion infection and instrument reprocessing recommendations could be developed.

The World Health Organization (WHO) convened a working group that evaluated the scientific evidence available and, based on that evaluation, assembled several options with which prion-contaminated surgical instruments could be decontaminated, cleaned and terminally reprocessed with a reasonable margin of safety (WHO, 2000). Much of the prion inactivation research up to this point in time indicated that no one method of prion inactivation was adequately potent to completely render surfaces of instruments free of prion activity (e.g. affect a reduction of 8–11 $\log_{10}$ LD_{50}) in one step. The consensus of the WHO working group was to acknowledge that incineration of contaminated instruments is the method for maximum patient safety. Research conducted after the publication of the WHO recommendations indicates that when using incineration, temperatures under ~1000°C either normal or starved-air conditions should be effective for prion inactivation in residual tissues on instruments (Brown *et al*., 2004). The WHO working group, acknowledging that incineration may not be the first choice of a healthcare institution for managing prion-contaminated instruments, recommended several decontamination strategies that make use of alkaline pH chemicals for immersion followed by use of steam sterilization. Table 10.1 lists these prion inactivation options for decontamination as put

Table 10.1 Accepted methods of reusable instrument management/decontamination for prion infection prevention[a]

Public health agency	Source document and date of most recent revision	Accepted methods[b]
World Health Organization (WHO)	Annex III(2000)[c]	Incineration is preferred for maximum safety If incineration is not selected, use one of the following methods for heat-resistant instruments:[d] 1. Immerse in 1N NaOH and heat in a gravity displacement autoclave at 121°C for 30 min; clean; rinse in water; subject to routine sterilization 2. Immerse in 1N NaOH or sodium hypochlorite (20 000 ppm available chlorine) for 1 h; transfer instruments to water; heat in a gravity displacement autoclave at 121°C for 1 h; clean; subject to routine sterilization 3. Immerse in 1N NaOH or sodium hypochlorite (20 000 ppm available chlorine) for 1 h; remove and rinse in water; transfer to open pan and heat in a gravity displacement autoclave (121°C) or porous load autoclave (134°C) for 1 h; clean; subject to routine sterilization 4. Immerse in 1N NaOH and boil for 10 min at atmospheric pressure; clean; rinse in water; subject to routine sterilization 5. Immerse in sodium hypochlorite (20 000 ppm free available chlorine) (preferred) or 1N NaOH (alternative) at ambient temperature for 1 h; clean; rinse in water; subject to routine sterilization 6. Autoclave at 134°C for 18 min.[e]
	Annex III and Section 5.1, Table 8	Use for heat-sensitive instruments:[f] Incineration is preferred for maximum safety If incineration is not selected, use the following method: Keep instruments moist between time of use and reprocessing; thoroughly clean; subject to disinfection

US Centers for Disease Control and Prevention (CDC)	CDC website: CJD Infection Control (August, 2010)[g]	Keep instruments moist between time of use and reprocessing Use for heat-resistant instruments: 1. Incineration is the safest option, but may not be cost effective or practical 2. If incineration is not selected, choose a method among WHO methods 1–3.[h] Use for heat-sensitive instruments:[f] 1. Incineration is the safest option, but may not be cost effective or practical 2. If incineration is not selected, keep instruments moist between time of use and reprocessing; thoroughly clean; subject to disinfection.[i]
UK Department of Health (DOH)	ACDPTSE Annex I (May 2007)[j]	Surgical instruments: incinerate for maximum safety
	ACDPTSE Annex F (January 2011)[k]	Single-use endoscope or incinerate
	ACDPTSE Annex L (January 2011)[l]	Single-use ophthalmic instruments, sufficient reusable ophthalmic instrument inventory to keep instrument sets intact For contact lenses and devices that contact the outer surface of the eye: rinse in water; clean and rinse; immerse in sodium hypochlorite with 10 000 ppm available chlorine for 10 min; rinse and dry
Health Canada	CCDR Volume 28S5 Part C (November 2002)[m, n]	Incineration is preferred for maximum safety If incineration is not selected, use one of the following methods after cleaning to remove gross tissue: 1. Immerse instruments in 1N NaOH or sodium hypochlorite solution (20 000 ppm available chlorine) for 1 h; remove from solution; rinse with water; immerse in water and place in a sterilizer selecting the liquid cycle and heat at 121°C for 1 h (WHO decontamination option #2)

(*Continued*)

Table 10.1 Continued

Public health agency	Source document and date of most recent revision	Accepted methods[b]
		2. Immerse instruments in 1N NaOH or sodium hypochlorite solution (20 000 ppm available chlorine) for 1 h; remove from solution; rinse with water; transfer to an open pan; place in prevacuum sterilizer and heat at 134°C for 1 h, or at 121°C in a gravity displacement sterilizer for 1 h (WHO decontamination option #3) Endoscopes and other complex instruments: incineration for maximum safety
Australian Department of Health and Aging	Infection Control in the Health Care Setting, Chapter 31 (2007)[o]	Incineration is preferred for maximum safety; or reprocess reusable instruments (according to AS/NZS 4187) separately and keep for that patient's exclusive use; incinerate when the instrument is no longer needed

Notes: [a] These methods are used primarily for decontamination of instruments that have come into contact with high infectivity tissues of known or probable patients with CJD or vCJD, or for instruments that are released from quarantine based on an affirmative diagnosis of CJD or vCJD. Consult the individual guidance documents for specific information on the application of these methods to lower infectivity tissues.

[b] The methods (other than incineration), especially those for heat-resistant instruments, are intended as decontamination processes. Note that routine cleaning and sterilization follows decontamination when NaOH or sodium hypochlorite is used.

[c] WHO Infection Control Guidelines for Transmissible Spongiform Encephalopathies (WHO, 2000). Used with permission from the WHO.

[d] WHO notes in the text that these instruments should be kept moist between time of use and reprocessing to facilitate tissue removal (as part of the quarantine strategy).

[e] WHO notes that prion infectivity may not be completely inactivated if large amounts of tissue are dried onto the instrument surface.

[f] WHO indicates that heat-sensitive instruments can be flooded with 2N NaOH. However, many complex semi-critical, heat-sensitive instruments (e.g. endoscopes) are damaged in the process. CDC also lists use of sodium hypochlorite as an option here, but instrument damage would result from using this chemical as well.

[g] CDC Questions and Answers: Creutzfeldt-Jakob Disease Infection-Control Practices (CDC, 2010).

[h] Before using sodium hypochlorite on instruments, contact the manufacturer for information on the instrument's tolerance to this chemical.

[i] Select a disinfecting chemical and process appropriate for the use of the instrument (i.e. heat-sensitive semi-critical instruments should be subjected to high-level disinfection after cleaning. Avoid use of protein-fixing disinfectants (alcohols, aldehydes) if possible.

[j] UK Department of Health. Guidance from the Advisory Committee on Dangerous Pathogens (ACDP) TSE Risk Management Subgroup. Annex I: Outline Protocol for Management of Instruments and Tissues from Brain Biopsy Procedures on Patients with Progressive Neurological Disorders (UK Department of Health, 2007). Used with permission from the UK Advisory Committee on Dangerous Pathogens, Department of Health.

[k] UK Department of Health. Guidance from the Advisory Committee on Dangerous Pathogens (ACDP) TSE Risk Management Subgroup. Annex F: Endoscopy (UK Department of Health, 2011a). Used with permission from the UK Advisory Committee on Dangerous Pathogens, Department of Health.

[l] UK Department of Health. Guidance from the Advisory Committee on Dangerous Pathogens (ACDP) TSE Risk Management Subgroup. Annex L: Managing CJD/vCJD Risk in Ophthalmology (UK Department of Health, 2011b). Used with permission from the UK Advisory Committee on Dangerous Pathogens, Department of Health.

[m] Health Canada. Classic Creutzfeldt-Jakob Disease in Canada: An Infection Control Guideline. Canadian Communicable Disease Report (CCDR) 2002; 28S5: 1–84. Reproduced with the permission of the Minister of Health, 2012.

[n] Canada's recommendations for instrument decontamination (when incineration is not selected) mention instrument cleaning first, followed by either of the two decontamination options. Although their decontamination options appear to use the sequential exposure of the instruments to chemical and then steam sterilization, there is no mention of follow-up cleaning and routine sterilization as the final step.

[o] Australian Department of Health and Ageing. Infection Control in the Health Care Setting, Chapter 31 – Classical Creutzfeldt-Jakob Disease (ADHE, 2007). Used by permission of the Australian Government.

forth by the WHO along with the guidance from several national public health agencies that have used the WHO recommendations as the foundation for their guidelines. The methods are presented in Table 10.1 with all the recommended use details for chemical concentration, exposure times, autoclave cycle parameters and type of autoclave equipment. The WHO-preferred options of alkaline chemical exposure (i.e. 1M NaOH or NaOCl solution containing 20 000 ppm available chlorine) and steam autoclaving, either in sequence or combined into one step (limited to methods using NaOH) are considered to be most effective. The least effective option is to autoclave the contaminated instruments at 134°C for 18 min. At present, the UK recommends the most conservative approach of using incineration as its principal method to manage prion-contaminated instruments, while other countries do allow use of the chemical immersion/autoclaving methods. For example, CDC in USA acknowledges that while the destruction of surgical instruments is the safest and most unambiguous method, this may not be practical or cost-effective. In the early 2000s, the UK also mandated the use of single-use disposable surgical instruments for procedures involving tissues with noted high concentrations of vCJD prions (e.g. tonsils). While this seemed reasonable from a contaminated instrument management perspective at the time, this decision had unintended consequences in that compared with the tonsillectomy complication rate with reusable surgical instruments, the complication rate linked to disposable instrument use was four times higher (Maheshwar *et al*., 2003). This policy has since been rescinded.

One important point to make about these WHO recommended methods is that, with the exception of incineration, the first objective in methods 1–5 is to 'decontaminate' the instrument surfaces. Given the fact that infected brain tissue can contain large numbers of prions (~10^5–10^8 LD_{50}/mg), cleaning and autoclaving instruments without a preliminary decontamination step may not be effective at completely inactivating high prion loads (Taylor, 2003; Brown *et al*., 2004; Yan *et al*., 2004; Jackson *et al*., 2005; Peretz *et al*., 2006; Belay *et al*., 2010). From risk analysis and mathematical modeling, the risk of prion infectivity from surgical instruments that have not been fully decontaminated is greatest for the first to the sixth subsequent surgical cases. Modeling suggests that numerous conventional autoclave cycles are needed to reduce residual prion infectivity to negligible levels (i.e. at least ten cycles for medium-risk tissue contamination, at least 20 cycles for high-risk tissue contamination) (Lumley, 2008). After performing the decontamination step of a WHO recommended method, it is important that the instruments be subjected to conventional cleaning and sterilization. Cleaning the decontaminated instruments will remove the alkaline chemical residues and strip away residual organic matter, thereby enhancing the effectiveness of the terminal conventional sterilization step.

Concerns have been raised over the safety of autoclaving containers filled with NaOH solutions, due to the fact that NaOH dripate from the container can damage the autoclave chamber surfaces and create caustic fumes that can be hazardous to technicians when opening the chamber at the conclusion of the cycle. One approach to minimize the potential for equipment damage and operator injury is to use a durable container with a lid and a rim specially designed to prevent dripate release (Brown and Merritt, 2003). Another precaution to take is to allow the autoclave chamber temperature to cool down as much as possible before opening its door to minimize release of noxious fumes. Additional concerns regarding autoclaving instruments immersed in NaOH are the effects of both the chemical and the process on the instruments. Brown and colleagues evaluated the effects of this combination method on instruments made of various types and qualities of steel and those made of other metals (Brown *et al*., 2005). Briefly, their findings were as follows: (1) autoclaving instruments in NaOH will darken some instruments; (2) soaking in 1M NaOH at room temperature will damage carbon steel instruments, but not those made of stainless steel or titanium and (3) these effects are noticeable with the first exposure to NaOH. Additionally, they evaluated the effects of immersing instruments into solutions of sodium hypochlorite (NaOCl). Gold-plated instruments became badly corroded. Some corrosion was also noted for some stainless steel instruments, especially if the instrument had welded and soldered joints, articulated joints, mated surfaces and otherwise complex surfaces. With respect to using concentrated NaOCl solutions, one important safety note is the fact that NaOCl solutions should never be subjected to autoclaving, as this will cause the release of toxic gases. Furthermore, there is the potential for explosive damage to the autoclave.

10.4 Factors that impact the decision to use prion-inactivating methods

10.4.1 Surgical instrument policy issues

In 2005, an estimated 125 000 neurosurgeries were performed in the USA (FDA, 2005). Given that the current recommended instrument reprocessing methods for prion inactivation are capable of inflicting some degree of damage to the instruments and thereby shortening their use life, it is important to target these reprocessing methods to those instances for which prion inactivation is prudent or necessary. Equally important, however, are those efforts to identify those surgeries during which there is potential for instruments to become contaminated with prions. This concern also extends to endoscopic procedures when a diagnosis of vCJD is considered. In order to minimize the risk of prion transmission via instruments and devices, it is important to have

policies and procedures in place to identify at-risk patients as early as possible during their care and to have effective instrument reprocessing methods in place when needed. Furthermore, healthcare facilities should also develop policies and procedures that detail medical, epidemiological, ethical and legal strategies for post-exposure management of patients and instruments, when prion-contaminated instruments are inadvertently returned to inventory for use on subsequent surgical cases (Keeler *et al.*, 2006). Therefore, all hospitals and hospital trusts should have infection prevention strategies in place to prevent transmission of iatrogenic CJD and iatrogenic vCJD. Some countries have issued directives to this effect either from an accreditation standard approach or as part of national health policy. The Joint Commission in the USA, issued a Sentinel Event Alert in 2001 in response to reports of possible patient exposure to CJD prion-contaminated surgical instruments in two major hospitals (The Joint Commission, 2001). This Sentinel Event Alert identified the minimum basic strategies healthcare professionals would need to develop in order to prevent similar exposure events from occurring in their hospital. The UK Advisory Committee on Dangerous Pathogens issued a directive in 2006 indicating that all hospital trusts must have an infection prevention policy in place to prevent transmission of iatrogenic CJD (Health Protection Agency, 2005; Lumley, 2008).

Although the definitive diagnosis of CJD or vCJD is accomplished with histopathological examination of brain tissues obtained at autopsy or sometimes from biopsy, it may not be always necessary to perform biopsy neurosurgery if the patient's symptoms and other clinical information are suggestive of sCJD or vCJD (Belay *et al.*, 2005). However, brain biopsy procedures are important in the effort to rule out other neurologic diseases (Belay and Schonberger, 2005). Ultimately, this will help to limit the use of prion-specific decontamination and instrument reprocessing methods to only those instruments having prion contact, thereby avoiding the logistical problems associated with surgical instrument inventory replacement. Should it be necessary to perform a brain biopsy for TSE diagnosis, those neurosurgical instruments (for sCJD and vCJD) and instruments contacting potentially infectious lymphoid tissue (for vCJD specifically) should be kept moist in quarantine (i.e. in containment in a holding state) until the diagnosis is confirmed (WHO, 2000; CDC, 2010). (Additional discussion of instrument quarantine follows in the next subsection.) Alternatively, the instruments could be considered as prion-contaminated and put immediately through a recommended prion decontamination and reprocessing method. This is a practical approach, especially if the time interval between the surgery and the return of the histopathologic examination of the brain tissues is anticipated to be lengthy, and it permits the decontaminated/reprocessed instruments to be put back into available instrument inventory quickly.

10.4.2 Patient assessment

When surgery is ordered, there are three major points to consider when making the decision as to whether a prion inactivation reprocessing method is indicated for instrument decontamination: (1) patient risk factor history; (2) diagnostic testing and assessment of symptoms; and (3) instrument contact with tissues and the attendant risk level of those tissues (i.e. high, medium, low, no risk).

Patient risk factor history

The patient's risk factor history provides important context with which to interpret the results from diagnostic testing and clinical symptom assessment. The patient's interview should ascertain the status of the following factors at the minimum: (1) familial history for any TSE disease; (2) prior surgery involving the CNS (e.g. brain and/or spinal cord); and (3) receipt of human source-derived hormone therapy. The UK Department of Health approach to the patient interview provides a good example for how the patient's responses will help with instrument management decisions (UK Department of Health, 2011c).

If the patient is at increased risk for developing CJD or vCJD, more information is needed to determine the specific risk factor(s). If the patient is scheduled for surgery but is unable to provide additional information, prion inactivation methods for instrument reprocessing are utilized if the surgery involves a high-risk tissue. If the patient is scheduled for surgery involving a high-risk tissue, interview questions typically include ascertaining family history of TSE disease and/or human-derived hormone therapy prior to 1985. At present, therapy involving hormones produced from recombinant technology is not considered a risk factor. Affirmative answers to these questions indicate use of prion inactivation methods for instrument reprocessing. If the patient has a history of surgery involving the CNS or is a recipient of a dura mater transplant prior to August 1992, prion inactivation methods for instrument reprocessing are used. In the USA, the details of prior neurosurgeries in the patient's history are evaluated before the instrument reprocessing decision is made. In the UK, additional guidance indicates the use of new neuroendoscopes and new reusable surgical devices as appropriate for high-risk procedures performed on children born after 1996 (i.e. from 1 January 1997 forward), who have no prior high-risk procedures (UK Department of Health, 2011c). The UK patient interview also includes questions regarding previous blood transfusions. As to date, there have been four cases of transfusion-transmitted vCJD reported among UK recipients of blood components and plasma products (Llewelyn *et al.*, 2004; Peden *et al.*, 2004; Wroe *et al.*, 2006; Hewitt *et al.*, 2006; Turner and Ludlam, 2008). Patients in the UK who have a history of receiving blood or blood components from more than 80 donors are considered to be at increased risk for vCJD (UK Department of Health, 2011c; UK Department of Health, 2011d), and it is prudent to employ prion inactivation

reprocessing methods for instruments used in high-risk procedures for these patients. If the patient's transfusion history involves blood and/or blood products prepared from less than 80 donors, the risk to the patient for developing vCJD is reduced and prion inactivation methods for the instruments are not indicated. Public health surveillance from the late 1990s forward has not identified transfusion-transmitted cases of classic CJD in the USA (Evatt *et al*., 1998). A 2009 look-back study to assess the status of 436 US recipients of blood from 36 CJD-infected donors found that none of these recipients developed CJD (Dorsey *et al*., 2009). Therefore, the need to ask patients about prior receipt of blood or blood products is not necessary in the USA.

Non-surgical diagnostic evaluation of the patient

The diagnosis of TSE infection is the responsibility of neurologists and pathologists. Recent advances in TSE diagnostic mehtods have made the process easier and more accurate. Patient assessment for symptoms includes consideration of the epidemiology of CJD and vCJD, clinical presentation, laboratory diagnostic tests, family risk history, codon 129 polymorphism, evaluation of neuroimaging, electroencephalograms (EEG) and neuropathology findings (Belay *et al*., 2005). Two diagnostic procedures routinely ordered in the clinical work-up of the patient involve evaluation of the EEG patterns and the detection of brain-specific proteins in the cerebrospinal fluid (CSF). With regards to the EEG, a periodic sharp wave complex (PSWC) is detected in approximately 67% of sCJD patients, and is especially noted for those patients whose codon 129 polymorphism contains methionine (i.e. MM, MV) (Furlan *et al*., 1981; Levy *et al*., 1986; Steinhoff *et al*., 1996; Wieser *et al*., 2006). The appearance of PSWC on the EEG is not specific for CJD, as this pattern has been noted on EEG examination of other forms of dementia (e.g. Alzheimer's disease) (Steinhoff *et al*., 2004). However, the appearance of PSWC with triphasic morphology on EEG, coupled with an elevated 14-3-3 protein in CSF and the typical signs and symptoms of a rapidly evolving dementia, is suggestive of a probable case of sCJD or iatrogenic CJD (Kretzschmar *et al*., 1996; Wieser *et al*., 2006). The incorporation of the EEG as part of a diagnostic approach to CJD was adopted by WHO in 1998 (WHO, 1998). Additionally, the absence of PSWC on the EEG is considered diagnostic for vCJD when other laboratory tests and clinical symptoms are suggestive of prion infection (WHO, 2001).

Several brain-specific proteins have been observed at elevated levels in the CSF in TSE patients, including 14-3-3, tau-protein, S100b and neuron-specific enolase (NSE) (Otto *et al*., 1997a, 1997b; Zerr *et al*., 1998; Beaudry *et al*., 1999). While the presence of these proteins in CSF is not specific for TSE diseases (e.g. the 14-3-3 protein is a marker for acute neuronal damage and can be elevated for other neurological diseases including but not limited to encephalitis, stroke and epileptic seizures), their presence or absence can help to rule in or rule out a TSE disease and help to distinguish CJD from

vCJD (Belay *et al*., 2005; Collins *et al*., 2006; Chohan *et al*., 2010; Zanusso *et al*., 2011). In an effort to increase the positive predictive value (PPV) of CSF testing for the diagnosis of CJD or vCJD, it is important to evaluate more than just one of these target proteins. While the most sensitive marker for sCJD in CSF is 14-3-3 (86% compared with 81% for tau-protein and 65% for S100b), the highest PPV for sCJD is achieved when 14-3-3 is detected along with an elevated level of either tau-protein or S100b (94-95% PPV) (Bahl *et al*., 2009; Chohan *et al*., 2010). The highest levels of 14-3-3 have been associated with classic sCJD with PrP^{Sc} identified as type 1 (Castellani *et al*., 2004; Gmitterová *et al*., 2009). Interestingly, when analysis of the CSF is part of the laboratory diagnosis of vCJD, the PPV of 14-3-3 and an elevated tau-protein is 91% (Green *et al*., 2001). Therefore, testing for 14-3-3 and other brain-specific proteins may not be as useful in making a vCJD diagnosis (Green *et al*., 2001).

More recently, the use of magnetic resonance imaging (MRI) has been evaluated as a diagnostic technology for sCJD. With the use of fluid attenuated inversion recovery (FLAIR) and diffusion-weight imaging (DWI) modalities, basal ganglia hyperintensity and signal increase in other brain regions can be detected, and characteristic MRI lesion patterns have been noted for CJD subtypes (Tschampa *et al*., 2007; Galanaud *et al*., 2008; Meissner *et al*., 2008, 2009). Zerr and colleagues – following the completion of an international multi-center effort to conduct a comparative analysis of results from EEG, CSF proteins and MRI patterns – have made the recommendation that FLAIR or DWI MRI be included among the clinical criteria for diagnosing sCJD (Zerr *et al*., 2009). Clinical symptoms (e.g. dementia, ataxia, visual problems, pyramidal or extrapyramidal signs and akinetic mutism) evaluated in context with laboratory and imaging profiles for EEG, 14-3-3 detection in CSF and MRI would be useful to identify probable sCJD patients and possible sCJD patients (Zerr *et al*., 2009).

10.4.3 Tissue risk assessment

Once a potential source patient (i.e. a known, probable, or possible/at-risk patient for CJD or vCJD) is identified, the next consideration is the risk levels of the tissue that may be contacted during surgical or non-surgical invasive procedures. The WHO and the UK Department of Health have reviewed the CJD and vCJD pathology literature from the previous 30 years and compiled prion risk information for human organs and tissues (WHO, 2010; UK Department of Health, 2010). Table 10.2 is a summary of the prion risk levels and the tissues assigned to each risk level. Both the WHO and the UK Department of Health summaries in this regard are comprehensive in scope. For certain patient and surgery situations, the UK Department of Health recommendations are more conservative (i.e. neurosurgical

Table 10.2 PrP^{Sc} infectivity levels of human tissues and body substances from infected patients[a]

Tissues and body substances	CJD	vCJD
High infectivity levels		
Brain, spinal cord	+	+
Pituitary gland, retina, dura mater[b]	+	NT
Posterior eye (specifically the posterior hyaloid face, retinal pigment epithelium, choroid, subretinal fluid, optic nerve[c])	+	NT
Spinal ganglia[d], trigeminal ganglia	NT	+
Lower infectivity levels		
Spleen, lymph nodes	+	+
CSF[e]	+	–
Blood	–	+
Cornea, lung, liver, kidney	+	NT
Placenta	(+)	NT
Peripheral nerves, skeletal muscle	(–)	+
Tonsil	NT	+
Adrenal	–	NT
Bone marrow	(–)	–
Appendix	NT	(–)
No detectable infectivity		
Heart, saliva, urine, feces, sweat, tears, nasal mucus, gingival tissues	–	NT
Milk, testes, semen, prostate, thyroid	(–)	NT
Bone, dental pulp	NT	NT

Notes: [a] Adapted from Tables IA-IC from the most recent WHO update on tissue infectivity risk levels (WHO, 2010), with modifications from UK Department of Health guidance Annex A1 (UK Department of Health, 2010). Used with permission from the WHO.

[b] Additional entry from UK Department of Health Annex A1 (UK Department of Health, 2010). Used with permission from the UK Advisory Committee on Dangerous Pathogens, Department of Health.

[c] Subsequent studies have found no detectable infectivity associated with dura mater from CJD patients, but because this tissue has been associated with iatrogenic transmission possibly due to other factors (e.g. the graft may have been contaminated with brain tissue), dura mater remains categorized as a high infectivity tissue.

[d] UK Department of Health cites unpublished results on infectivity that place spinal ganglia into the medium risk category.

[e] Although PrP^{Sc} has not been detected in CSF, experimental transmission of TSE has been demonstrated in intracranial experiments in primates. The level of infectivity for CSF is thought to be less than that for central nervous system tissues.

Infectivity levels were determined by experimental exposure of either primates or bioassay (i.e. mouse assay) to tissues or body substances from infected patients.

+ Presence of infectivity
– Absence of detectable infectivity
NT Not tested

( ) Limited or preliminary data

Prp^{Sc} has been detected, however, by immuno histological assays or Western blot in some tissues that have demonstrated little or no infectivity in either primate or mouse assays:
CJD: skeletal muscle, blood vessels, nasal mucosa.
vCJD: jejunum, ileum, appendix, colon, rectum, adrenal, skeletal muscle, blood vessels.

Additional tissues listed in Table A1 from the UK Department of Health guidance document (Annex A1) that are mentioned in instrument reprocessing decisions based on tissue category include:
Cranial nerves (high infectivity level).
Olfactory epithelium (medium infectivity level).
Gut-associated lymphoid tissue (low (CJD) infectivity level, medium (vCJD) infectivity level).

instruments contaminated with high-risk tissues are incinerated, whereas the WHO allows the use of instrument decontamination followed by cleaning and sterilization for these same circumstances). Table 10.3 presents a comparison of WHO and UK recommendations for instrument reprocessing method selection, taking into account the patient's status (i.e. definite, probable, or suspect case of TSE) and the risk level for tissues. For CJD, the surgeries for which a determination of appropriate instrument reprocessing method is indicated would include, but may not be limited to brain biopsy for diagnosis of non-lesionous neurologic diseases, neurosurgeries involving the CNS on known or suspected/probable CJD patients, and ophthalmic surgery on the posterior segment of the eye of known or suspected/probable CJD patients. For vCJD, the list of surgeries and invasive procedures includes those listed for CJD, surgeries involving the tonsils and other lymphoid tissue, and those endoscopy procedures in which the endoscope makes potential contact with lymphoid tissue.

10.5 Important issues associated with cleaning/decontamination

When developing a reprocessing or microbial inactivation strategy for a hard surface in the environment or for a reusable medical instrument or device, it is important to understand that reprocessing consists of several distinct steps, each of which contributes to the overall success of the process. This is evident regardless of whether the goal is to inactivate conventional pathogens, such as bacteria, viruses, fungi, or protozoa, or unconventional agents such as TSE agents. The final step (usually sterilization or high-level disinfection) is sometimes referred to as 'terminal reprocessing', as this activity is the last in a sequence of steps that makes a surface or an instrument ready and safe for

Table 10.3 Compare and contrast heat-stable, reusable instrument strategies based on tissue infectivity and patient category for CJD and vCJD[a]

Tissue infectivity level	Patient categories					
	Definite, probable, or suspect[b]		At increased risk[c]		Possible[d]	
	UK DOH[e]	WHO[f]	UK DOH	WHO	UK DOH	WHO
High (CJD and vCJD)						
Brain Spinal cord Cranial nerves (optic nerve, intracranial components of other cranial nerves) Posterior eye (posterior hyaloid face, retina, retinal pigment epithelium, choroid, subretinal fluid) Pituitary gland	Single use or destroy or quarantine for same patient reuse	Annex III[g]	Single use or destroy or quarantine for same patient reuse	Annex III[h]	Single use or quarantine for same patient reuse pending diagnosis	Annex III

Medium (CJD)						
Spinal ganglia Olfactory epithelium	Single use or destroy or quarantine for same patient reuse	(Annex III)[i]	Single use or destroy or quarantine for same patient reuse	(Annex III)	Single use or destroy or quarantine for same patient reuse pending diagnosis	(Annex III)
Medium (vCJD)						
Spinal ganglia Olfactory epithelium Tonsil Appendix Spleen Thymus Adrenal gland Lymph nodes and gut-associated lymphoid tissues	Single use or destroy or quarantine for same patient reuse	Annex III	Single use or destroy or quarantine for same patient reuse	Annex III	Single use or quarantine for same patient reuse pending diagnosis	Annex III
Low (CJD)	No special precautions	Annex III[j]	No special precautions	Routine cleaning and disinfection (or sterilization)[k]	No special precautions	Routine cleaning and disinfection (or sterilization)[k]
Low (vCJD)	No special precautions	Annex III	No special precautions	Annex III	No special precautions	Annex III
No detectable infectivity	No special precautions	Routine cleaning and disinfection (or sterilization)[i]	No special precautions	Routine cleaning and disinfection (or sterilization)[i]	No special precautions	Routine cleaning and disinfection (or sterilization)[i]

(*Continued*)

Table 10.3 Continued

Notes: [a] Adapted from information in Table 9 in the WHO (2000) document and Tables 4c and 4d in Part 4 of the UK Department of Health TSE guidance (UK Department of Health, 2011d). Used with permission from the WHO and the UK Advisory Committee on Dangerous Pathogens, Department of Health.

[b] This category includes patients previously diagnosed with a TSE, or whose symptoms and diagnostic test results fit current diagnostic criteria. Patients with active neurological disease of unknown etiology are also included in this category.

[c] The UK Department of Health guidance includes persons at risk from genetic forms of CJD and persons at risk through iatrogenic exposures. With respect for risk for vCJD, persons are included in this category who had: (1) received blood or blood components from >80 donors since January 1980; or (2) received blood from an individual who later developed vCJD; or (3) been treated with certain implicated UK plasma components between 1980 and 2001; or (4) donated blood to an individual who later developed vCJD; or (5) received blood from someone who had also given blood to another person who later developed vCJD.

[d] Part 4 of the UK Department of Health guidance does not appear to define this patient category, but lists it in their Tables 4c and 4d.

[e] United Kingdom Department of Health.

[f] World Health Organization.

[g] WHO Annex III options are incineration or any of six decontamination methods (see Table 10.1 in this chapter for these options).

[h] The WHO document indicates that no consensus was reached on whether or not a prion decontamination process was needed for instruments used on patients at risk from genetic forms of CJD.

[i] The WHO document lists these tissues in a category described as 'lower infectivity'.

[j] The WHO documents notes that CSF and peripheral organs and tissues are considered as less infectious compared with those for the central nervous system.

[k] The WHO entries for these infectivity categories list only 'routine cleaning and disinfection'. However, the majority of heat-stable, reusable instruments will be used in surgery and, therefore, the appropriate terminal step in the reprocessing of these instruments would be sterilization, preferably in a steam sterilizer.

next patient use. The state of the surface or instrument at the completion of terminal reprocessing depends on the intended use of the item. This concept is central to a rational approach of instrument reprocessing as developed by Dr E. H. Spaulding, known as the 'Spaulding Classification' (Spaulding, 1972). Dr Spaulding grouped medical instruments and devices into three categories: critical, semi-critical and non-critical. Critical instruments (i.e. those that contact the bloodstream and normally sterile tissues in the body) must be free of microbial contamination before use (i.e. subjected to sterilization, a process that inactivates all microbial pathogens including high numbers of bacterial endospores). Typically, many surgical instruments are heat-tolerant, and the logical choice for the terminal reprocessing step is steam sterilization (Favero and Bond, 1991, 2001). Semi-critical instruments (i.e. those that come into contact with mucous membranes or non-intact skin) are sterilized if the instruments can tolerate the process, but if this is not the case then use of high-level disinfection to inactivate vegetative microbial pathogens and some bacterial endospores is indicated as the terminal reprocessing step (Favero and Bond, 1991, 2001). If a semi-critical instrument is heat-sensitive and therefore cannot tolerate steam sterilization, then low-temperature methods of sterilization (e.g. hydrogen peroxide gas plasma, ethylene oxide, ozone) may be considered for sterilizing these instruments. However, even these low-temperature methods may be impractical to reprocess semi-critical complex instruments (e.g. endoscopes). Materials compatibility with the sterilant must be considered first. There may be sterilant penetration issues for lumens (a major consideration when using hydrogen peroxide gas plasma) or lengthy periods of aeration to remove toxic sterilant residues (which is the case for ethylene oxide use). Non-critical instruments that make contact with intact skin usually carry the lowest risk of infection from their use. Non-critical instruments are cleaned at the minimum, and many are subjected to low-level disinfection for next patient use.

Although different instruments and devices will have differing intended uses and therefore require differing terminal reprocessing steps, it is important to recognize that all instruments, devices and surfaces require a thorough cleaning/decontamination step. Instruments must be free of residual salts, protein and other organic matter before being subjected to a sterilizing process. This is important for two reasons: (1) the presence of these residuals on the instrument surfaces interferes with the sterilizing process for those instruments; and (2) residual foreign matter on an instrument that has undergone sterilization can potentially pose a risk of patient-to-instrument-to-patient transmission of infection and/or cause localized inflammatory reactions at the surgical site for the next patient.

One major concern regarding the overall effectiveness of reusable surgical instrument reprocessing is the potential failure of the process to remove residual bioburden or organic matter. Residual protein matter on

instruments presents a daily challenge to central sterile department staff as instrument design becomes increasingly complex, making the instruments more difficult to clean. This situation has prompted several research groups to evaluate sensitive methods to detect residual protein on instrument surfaces. Using a commercially available protein stain, Murdoch and colleagues found in one study that 17% (35/206) of instruments tested had residual protein contamination in excess of 200 μg after routine cleaning and sterilizing (Murdoch *et al.*, 2006). Extremely complex instruments (e.g. McIvor gag, Draffin rod [child]) had milligram quantities of residual protein on their surfaces after cleaning. Factors in addition to instrument design problems that can contribute to large amounts of residual bioburden on instruments include inefficient manual cleaning methods, human errors when operating automated cleaning equipment, failure to use appropriate cleaning chemicals according to manufacturer instructions, and failure to keep cleaning equipment clean and fully maintained.

Lipscomb and colleagues used epifluorescence and a fluorescent reagent (SYPRO Ruby®) to detect residual brain tissue on stainless steel coupons. He also compared visual assessment of reprocessed surgical instrument cleanliness to epifluorescent microscopy of these same instruments for unspecified residual protein (Lipscomb *et al.*, 2006a). In his study of 23 surgical instruments, 56% had severe levels of contamination especially in areas with complex design such as hinges (e.g. ≥ 21 μg protein/mm^2 over 50% of the instrument surface). Across all determinations, Lipscomb found that epifluorescence was a more sensitive method than visual inspection for detecting residual protein. This same research group also evaluated the sensitivity of Ninhydrin and biuret testing for residual protein detection, comparing their minimum level of detection to that of epifluorescence testing. Epifluorescence was ~95% more sensitive in detecting residual protein on instrument surfaces (Lipscomb *et al.*, 2006b). They estimated that with respect to prion infectious doses, approximately 1×10^6 LD_{50} could be present on instruments deemed clean by either Ninhydrin or biuret testing. This level of prion activity on the surface of the instrument is presumably sufficient to transmit infection. Other investigators examined instruments deemed ready for use (i.e. reprocessed) from five hospital trusts for residual proteins. They found that instruments used for tonsillectomy and adenoid surgeries had the highest levels of protein contamination (Baxter *et al.*, 2006). Collectively, what this information suggests is that central sterile department staff should revisit instrument cleaning to find ways to improve the efficacy of these processes for bioburden removal, thereby enhancing overall quality control. Many central sterile departments are phasing out manual cleaning of instruments wherever possible in favor of automated cleaning equipment, such as washer-disinfectors or ultrasonic equipment. Additionally, it is important that equipment surfaces, especially the inner

surfaces of ultrasonic baths, are cleaned daily and the fluid reservoir is replaced on a regular basis or when it becomes cloudy to prevent biofilm build-up.

Since the publication of the WHO recommended reprocessing methods (WHO, 2000), surgeons and infection preventionists have voiced concerns about the instrument damage caused by those methods involving instrument exposure to harsh alkaline chemicals. Much of the continuing research has been focused on trying to find replacement chemicals/cleaners and use conditions for this decontamination step. One major experimental design development that helped to advance this research was the use of stainless steel wires to represent the material of a surgical instrument. Steel wires 5 mm in length would be immersed in prion-infected brain homogenate of various amounts and titers and then be allowed to dry. These would then be subjected to an inactivation process for a designated time, after which these wires would be inserted into the brains of appropriate assay animals (e.g. hamsters, transgenic mice). Assessment of residual prion activity in these animal titration experiments was based on the number of animals for each dilution of treated homogenate that became symptomatic/died and the time interval in days for the appearance of symptoms post-wire insertion (Zobeley *et al.*, 1999; Flechsig *et al.*, 2001; Fichet *et al.*, 2004; Yan *et al.*, 2004). This assay approach was found to be well tolerated by the animals, and in many instances the time required for the completion of the experiments was less than one year.

Two categories of cleaning chemicals have been investigated in the interests of finding effective prion infectivity removal and increased compatibility with the metals and other materials present in surgical instruments. These are the alkaline-based cleaners and those cleaners based on enzyme chemistry (generally using a subtilisin type of enzyme). Experiments evaluated the efficacy of these cleaning chemicals either as is or in combination with other prion inactivation steps. Fichet and colleagues determined that the WHO method of exposure to 1M NaOH or NaOCl (20 000 ppm available chlorine) followed by immersion in water and autoclaving at 134°C in a porous load autoclave was able to reduce prion infectivity by > 5.6 $\log_{10}$ LD_{50}, whereas autoclaving the chemically exposed items in a dry pan was less effective (i.e. a reduction of 4–4.5 $\log_{10}$ LD_{50}) (Fichet *et al.*, 2004). When evaluated individually, alkaline cleaners showed greater reduction of prion infectivity compared with that for enzymatic cleaners (e.g. > 5.6 $\log_{10}$ LD_{50} for an alkaline cleaner at 1.6%, 43°C for 15 min vs ~3.5 $\log_{10}$ LD_{50} for an enzyme cleaner at 0.8%, 43°C for 5 min) (Fichet *et al.*, 2004). Yan and colleagues observed the same phenomenon for alkaline and enzyme-based cleaning agents. Their experiments evaluated prion inactivation for combinations of the cleaning agents with several terminal reprocessing technologies or high-level disinfectants (i.e. hydrogen peroxide gas plasma, steam

sterilization, *ortho*-phthalaldehyde (OPA) immersion) (Yan *et al*., 2004). Baier and colleagues evaluated the mode of action of alkaline cleaners in the inactivation of prions. Alkaline cleaners denature PrP^{Sc} causing loss of the beta-sheet conformation, rendering the protein susceptible to proteinase K (Baier *et al*., 2004). This denaturation alters PrP^{Sc}, such that the denatured protein cannot be detected by Western blot analysis. Denatured prion proteins are also unlikely to spontaneously reconvert back to their beta-sheet conformation when the denaturing agent is diluted (Lemmer *et al*., 2004). This observation is interesting when considering the question as to whether or not the sides of cleaning equipment reservoirs become contaminated with active prions after surgical instruments are immersed in the alkaline cleaner. The results observed with PrP^{Sc} denaturation would suggest that such contamination of the equipment does not occur, but it should be noted that this has not been confirmed with experimentation.

Studies on the efficacy of enzyme-based cleaners have shown that these cleaners generally require higher temperatures (i.e. 50–60°C) and longer exposure times to produce prion activity reductions of ≥ 3.5 $\log_{10}$ LD_{50} (Jackson *et al*., 2005; Lawson *et al*., 2007; Hervé *et al*., 2010). Different strains of prion agents have different levels of resistance to enzyme digestion and heat (Somerville *et al*., 2002; Hervé *et al*., 2010).When enzyme use is followed with autoclaving at 134°C for 3 min (porous load) or 121°C for 20 min (gravity displacement), the prion inactivation reduction can be boosted up to ≥ 5 $\log_{10}$ LD_{50} (Fichet *et al.*, 2004; Lawson *et al.*, 2007). Lawson and colleagues note, however, that enzyme cleaners can vary greatly in their potency and that even the sequential use of an enzyme cleaner and autoclaving may be ineffective in completely eliminating infectivity if prion titers in high-risk tissue exceed 10^8 LD_{50}/mg (Lawson *et al*., 2007). The capability to remove proteins effectively from surfaces also varies among different enzyme cleaning products tested (Hervé *et al*., 2010). Genetically engineered proteases have shown promise as effective prion decontamination agents. When challenged with a 10% brain homogenate of a strain of BSE [MC3], a proprietary genetically engineered protease is capable of digesting ~7 $\log_{10}$ of prion contamination in 30 min at alkaline pH (e.g. pH 8, 10, or 12) (Dickinson *et al*., 2009). Edgeworth and colleagues determined via the use of the standard steel binding assay (SSBA) that decontamination/cleaning products with prion inactivation claims have different efficacies in prion inactivation (Edgeworth *et al*., 2011). When challenged with 10% brain homogenates, Prionzyme® and Rely+On PI® each was able to inactive > 5 $\log_{10}$ of prion activity on 5 mm steel wires. These log reductions (LR) were equivalent to that obtained with 2M NaOH treatment of steel wires. In contrast, steam autoclaving at 134°C for 18 min results in ~3.5 $\log_{10}$ reduction, leaving 0.03 $TCIU_W$ of prion infectivity, which is sufficient to transmit prion infection in laboratory animals (Taylor *et al*., 1998; Jackson *et al*., 2005).

A more detailed summary discussion of experiments evaluating the efficacy of various other cleaning chemicals has been published (Rutala and Weber, 2010). Briefly, oxidative chemicals in low concentrations (e.g. peracetic acid, hydrochloric acid, hydrogen peroxide, chlorine dioxide) effect a ≤ 3 $\log_{10}$ ID_{50} reduction within 1 h exposure. Alcohols and aldehydes are protein-coagulating chemicals that tend to fix protein contamination to surfaces, thereby providing some protection to any PrP^{Sc}, proteins beneath the surface of the contaminating biomaterial/tissue (Taylor, 1999). These two categories of chemicals should be avoided in the decontamination step early in the prion inactivation process (Rutala and Weber, 2010). Cleaners whose formulations include sodium dodecyl sulfate (SDS) show promise with ≥ 5.5 $\log_{10}$ LD_{50} reductions in prion activity (Peretz *et al.*, 2006; Beekes *et al.*, 2010). Given the wide variety of alkaline and enzyme cleaners, it is important to validate the efficacy of the cleaner of choice as part of a comprehensive validation experiment when developing a prion inactivation strategy that uses contemporary proprietary chemicals.

As prion inactivation research shifted focus from veterinary medicine issues to that of human healthcare instrument reprocessing, studies in the late 1990s showed that scrapie agent PrP^{Sc} could bind to steel and still retain infectivity (Zobeley *et al.*, 1999; Flechsig *et al.*, 2001). This finding was important in two ways. First, this development had great impact on use of animal assay for prion inactivation research. Steel wires contaminated with prion material could be easily inserted into the brain of assay animals (i.e. mice and hamsters) and was generally well tolerated by the animals. Second, PrP^{Sc} binding to steel meant that the cleaning step of steel surgical instrument reprocessing strategy would need to be as effective a protein removal process as possible. A common practice in surgical instrument management is to immerse the used instrument into water or saline or otherwise wrap the instrument in moist towels when it is no longer needed during surgery in order to keep blood, tissue and other patient material moist, thereby preventing this patient material from drying hard onto the instrument's surface. Dried blood and tissue are harder to remove during instrument cleaning and, given that PrP^{Sc} has an affinity for steel, it is imperative that patient blood and tissue not be allowed to dry. Interestingly, steel quality influences prion binding. Luhr and colleagues noted that stainless steel containing nickel and molybdenum, binds PrP^{Sc} more efficiently (Luhr *et al.*, 2009). This finding would suggest that manufacturers of neurosurgical instruments take steps to avoid the use of steel with nickel and molybdenum when fabricating the instruments. Lipscomb and colleagues evaluated the dynamics of the binding process using the ME7 mouse-passage scrapie agent in brain homogenates and made several observations. They found that PrP^{Sc} adsorbed onto steel within 30 min and could not be rinsed off with deionized water. Furthermore, the binding process had three distinct phases: (1) an initial period during

which very little PrP^{Sc} is adsorbed; (2) a short period of rapid adsorption; and, finally, (3) a plateau phase during which little additional adsorption occurs (Lipscomb *et al.*, 2007). Temperature also had an effect on this adsorption process in that ambient room temperatures (i.e. 23–25°C) appeared to slow down and prolong the initial period, thereby helping to limit prion adsorption to surgical stainless steel. On occasion, there will be surgical instruments inadvertently allowed to dry. Secker and colleagues looked at the influence of drying on prion adsorption and the impact of steps to remove the dried matter. They found that increased PrP^{Sc} adsorption is directly proportional to the length of time the instrument remains dry; if the dry period is less than 15 min, prion adsorption is minimized. Furthermore, if a dry instrument is subjected directly to enzymatic cleaning, the dried matter diminishes the effectiveness of the enzymatic cleaners. They also measured protein and prion removal efficiencies for tokens kept moist at room temperature for 24 h and compared these to protein and prion removal from dry token controls; they found that rinsing the moistened tokens following their 24 h immersion briefly with water removed 99.8% of the protein and 99.6% of PrP^{Sc} (i.e. approximately a 2 $\log_{10}$ reduction) (Secker *et al.*, 2011).

When the WHO working group developed the recommendations for instrument reprocessing for prion infection prevention, they knew that dried smears of contaminated brain macerate exhibited increased thermoresistance of PrP^{Sc} (Taylor *et al.*, 1998). They also acknowledged that prion inactivation strategies should be targeted to those instruments for which prion contamination was highly probable (e.g. neurosurgeries on known CJD or vCJD patients, instrument contact with high- or medium-risk tissues of probable CJD or vCJD patients). The concept of 'quarantine' for the instruments was put forth to address these concerns (WHO, 2000). Instruments that were potentially contaminated with prions would be set aside in a moist state until such time as the diagnosis of a TSE was confirmed or ruled out. This approach works best if the hospital has sufficient inventory of neurosurgical sets and other instruments such that placing potentially contaminated instruments in quarantine does not adversely impact the surgical work flow by limiting available inventory. Once a diagnosis of a TSE is confirmed, the instruments undergo one of the recommended prion inactivation strategies for instrument reprocessing. The UK Department of Health's Annex E provides a more conservative approach, with instruments in this situation being sent for incineration (UK Department of Health, 2011e). Whether a hospital follows the WHO recommendations or those of the UK, if the diagnosis rules out a TSE disease, quarantined instruments can be released to undergo conventional cleaning and sterilization.

Despite the diligence with which hospitals and hospital trusts work to prevent inadvertent exposure of surgical patients to prion-contaminated instruments, occasionally there will be circumstances in which a patient's

TSE diagnosis is confirmed after the instruments have already been reprocessed with conventional methods of cleaning and sterilization. Attempts to identify potentially exposed patients are initiated, and there is usually action to identify the instruments used for the source patient's surgery and return these back to the central sterile department to be subjected to incineration or one of the WHO methods for decontamination and subsequent cleaning and sterilization. This is problematic when there is no notation in the patient's chart to identify specific instruments used in the surgery or if the instrument can be shared among sets of instruments. More recently, there is interest in central sterile departments in hospitals in several countries to embrace a unique identifier tracking system for instruments, devices and materials management. Tracking systems make use of radio frequency identifiers (RFI) or bar coding (Eastern Research Group, 2006). Such a system is useful for device management in that a hospital can keep track of the numbers of uses for any particular device. In the future, however, perhaps a tracking system such as bar coding can be used to facilitate instrument recalls and help to minimize the number of potentially exposed patients that may need to be contacted for follow-up.

10.6 Research developments towards a validated reprocessing strategy

Since the release of the WHO prion reprocessing recommendations in 2000, there has been continuing research interest in developing an efficacious prion inactivation strategy in the interests of patient safety, while minimizing cosmetic and functional damage to surgical instruments and sterilizers. This is a key part of an overall goal to establish instrument reprocessing methods that incorporate microbial inactivation technologies currently available to hospitals and hospital trusts (Sehulster, 2004). The most recent research literature for this endeavor can be put into two groups: (1) inactivation technologies existing and new; and (2) early studies with respect to defining an appropriate inactivation method validation process.

Copper, when complexed with oxidative chemicals such as hydrogen peroxide or peracetic acid, has been shown to reduce prion infectivity in brain homogenates by $> 5.25 \log_{10} LD_{50}$ (Solassol *et al.*, 2006; Lehmann *et al.*, 2009). Cu^{2+} ions bind to prion protein and generate an active hydroxyl radical from the breakdown of hydrogen peroxide. This hydroxyl radical causes direct damage to proteins including PrP^{Sc}. This approach to prion inactivation may have some potential application for the high-level disinfection of heat-sensitive instruments such as endoscopes.

The investigation into the use of hydrogen peroxide gas plasma for prion inactivation has been underway for several years now, and the topic has been thoroughly reviewed (Rogez-Kreuz *et al.*, 2009). This review updates

the previous work by this group of researchers (Yan *et al*., 2004). Briefly, a new generation of the Sterrad® plasma sterilizer was able to achieve ≥ 5–6 $\log_{10}$ LD_{50} reductions of prion infectivity on contaminated stainless steel wires and was able to match the LR of a two-step combination of exposing the contaminated wires to an alkaline cleaner followed by exposure to hydrogen peroxide gas plasma in the new and previous generations of this technology. The mode of action of this technology against prions is not known precisely, but the presumption is that highly active hydroxyl radicals are generated that will interact with PrP^{Sc} and degrade it.

Despite all the research being done today in the field of prion inactivation, the fact remains that all countries are using prion inactivation strategies that have not had the full benefit of determination by validation. At present, in the USA there is no Food and Drug Administration (FDA)-cleared sterilizer equipment with set parameters for specific cycles intended to inactivate prions. Research is needed to establish validated inactivation processes, as currently there are no validated claims from sterilizer manufacturers supporting complete elimination of prion activity.

The development of a validated instrument reprocessing strategy for the inactivation of prions starts by identifying key components of the process and conducting research to determine the most appropriate conditions for each of those components (UK SEAC, 2006). The first item to be determined is the appropriate challenge prions. Prion agents derived from human sources would be a logical choice for challenge agents to the process, and validation studies should evaluate CJD and vCJD prions in order to support prion-specific inactivation claims (FDA, 2005). However, it also would be important to identify prions that are known to pose the greatest challenge to an inactivating chemical, gas, or sterilant and include these prions as part of a challenge panel for the proposed reprocessing strategy. For example, if moist heat (e.g. steam sterilization) is being evaluated, the most appropriate challenge prion agent would be one that presently demonstrates the highest level of resistance to heat. Additionally, it is important to determine the state of the prion contamination associated with resistance to heat (e.g. macates vs thin smears of protein material). Recent research has demonstrated that vCJD prions in brain macerates posed significant challenge to a heat-based inactivation process such that conventional porous load sterilizer cycles (e.g. 3 min at 134°C or 3 min at 137°C) resulted in titer reductions of $10^{2.3}$ to $> 10^{3.6}$ LD_{50}, with residual infectivity detected in animal assay (Fernie *et al*., 2012).

The next item to consider is what residual protein load on an instrument surface would reflect a worst case scenario for anticipated prion activity levels. This would also have to take into account the range of maximum titers determined for the source tissues of the challenge prions. High levels of residual protein can remain on conventionally cleaned instruments

(Murdoch *et al.*, 2006). This can be due to any number of factors such as instrument design complexities (i.e. hinges, mated surfaces, blind lumens, articulating parts). When there is the sense of how much residual protein to expect on surgical instrument surfaces, the titer of contaminating prions on those instruments can be determined. This piece of information will indicate the minimum potency benchmark that must be reached to inactivate prions, and it will be a starting point when attempting to determine if exposure (for chemicals) or cycle parameters (for technologies) can be extrapolated by more than one $\log_{10}$ to ensure a margin of safety.

The validation of an assay strategy is critical. Key elements of this task are the selection of an appropriate carrier or coupon on which the challenge prion is placed, the selection of an appropriate assay system (e.g. animals, cell culture) and the appropriate delivery mechanism by which residual prion contamination is introduced to the assay system. Many researchers have used 5 mm steel wires as the carriers, finding these easy to handle and the wires are well tolerated by the animals. Two potential drawbacks, however, are the observations that these wires appear to have a capacity of ~5–5.5 $\log_{10}$ LD_{50} (Lemmer *et al.*, 2008; Edgeworth *et al.*, 2011), and that they are easier to clean compared with small steel discs (Lipscomb *et al.*, 2006c). With respect to the prion load limitation, using carriers with < 6 $\log_{10}$ LD_{50} of prion contamination will not allow the accurate measurement of a potency endpoint if the typical residual prion load on an instrument is ≥ 6 $\log_{10}$ LD_{50}. The fact that wires are easier to clean compared with other carriers may give the erroneous impression of artificially high potency results. Animal assays have been a mainstay in prion research, but they are expensive and require months of 'incubation' before data can be analyzed. Nevertheless, animal assays (an *in vivo* method for detecting residual prion activity) would be suitable to determine the potency of the inactivation process, and once the decision has been made on what strains of prions are used as challenge, it is important to select an animal assay that provides the greatest sensitivity to those specific prions (e.g. a transgenic mouse assay model). Alternative assay methods are becoming available for consideration, such as an *in vivo* mouse assay that does not use contaminated carriers, an *ex vivo* scrapie-cell assay developed from a mouse neuroblastoma cell line, or a method known as the standard steel binding assay (SSBA), but their use still requires validation studies to determine their sensitivity and limitations (Solassol *et al.*, 2004; Vadrot and Darbord, 2006; Edgeworth *et al.*, 2011).

If proprietary cleaning or other reprocessing equipment is used in the prion inactivation strategy, determine if this equipment needs to be validated for this purpose. As pointed out earlier in this chapter, it is the initial decontamination step (i.e. prion inactivation step) in the reprocessing strategy that was determined by consensus of prion laboratory scientists. This important first step needs to be validated, but this is especially important for those parts

of the process that take place in proprietary equipment such as an autoclave. For example, some researchers have been evaluating prion inactivation in proprietary cleaning equipment (i.e. washer-disinfectors) or evaluated prion decontamination in procedures designed to simulate the cycle events in washer-disinfectors (Howlin *et al.*, 2010; Schmitt *et al.*, 2010). Schmitt and colleagues compared the prion inactivation efficacy of a standard cycle of a washer-disinfector with that for a modified cycle that included an extra oxidation step. The modified cycle for the washer-disinfector produced a prion infectivity reduction of > 7 $\log_{10}$ LD_{50}, but because the decontamination process under these circumstances constitutes an off-label use of the equipment, the manufacturer would need to consider performing the validation study. The experiments conducted by Howlin and colleagues to determine instrument handling factors (i.e. immediate reprocessing of instruments by immersing in an enzyme cleaner or pre-soak in order to prevent residual tissue drying onto the instrument) are similar to what is done typically in a validation study – each step in a process is examined and the factors that impact the outcome of that step are evaluated. For example, Howlin and colleagues determined that exposing surgical instruments immediately to either an enzyme cleaner or a pre-soak wetting agent facilitated tissue removal. The effects of various drying time periods were evaluated to determine the maximum hold time for instruments in quarantine without adversely impacting tissue removal processes and decontamination (Howlin *et al.*, 2010).

Each component of the prion inactivation process, plus the inactivation process as a whole, must be validated. This is most critical in that the potency of each component is determined and compared with the potency of the whole process. In the past, the assumption was that the efficacy of a prion inactivation process was the sum of the $\log_{10}$ LD_{50} reductions noted for each step. However, this may not be a valid assumption, as in some experiments the overall $\log_{10}$ LD_{50} reduction was less than the sum of the reductions for each step in the process (Lemmer *et al.*, 2008). Caution should be used when evaluating assertions of effectiveness for contemporary prion inactivation strategies in the absence of bona fide validation studies. As mentioned previously, many of the evaluation studies for these inactivation strategies made use of steel wires that, in hindsight, were unable to support extremely high prion titers that would have enabled researchers to determine end points of infectivity (i.e. inactivation results could not be accurate beyond 5.5 $\log_{10}$ LD_{50}). This further complicates the assessments of the overall efficacy of contemporary inactivation methods.

Finally, it is important for national regulatory agencies to become engaged in the endeavor to validate prion inactivation methods that would be effective and make use of technologies and chemicals in healthcare institutions today. Such agencies (e.g. US FDA) would be positioned to help with developing criteria for simulated use testing for instruments and validation

research for cycle parameters or chemical use conditions for other materials commonly used in hospitals (e.g. rubber, plastics, soft metals) in addition to stainless steel (FDA, 2005). As much as possible, the experimental conditions should be chosen to approximate the clinical situations expected for surgical instrument reprocessing. The goal is to advance prion inactivation science to prevent inadvertent transmission of iatrogenic CJD or vCJD while maintaining the highest level of patient safety possible.

10.7 Bacterial endotoxins

The outer surface of the outer membrane of most gram-negative bacteria is covered with a monolayer of a molecule identified as lipid A (Raetz, 1990). This unique phospholipid is composed of a glucosamine backbone moiety as opposed to a diacylglycerol moiety that is more typical of phospholipids in general. This main biochemical difference is central to lipid A's resistance to the activity of phospholipase enzymes. In addition to its role in outer membrane structure and integrity, lipid A serves as the membrane anchor for acyl chains known as lipopolysaccharide (LPS). LPS has two distinct components – an inner core and an outer core – and may have a third component, O antigen, attached to the distal end of the chain. The inner core consists of KDO (a 3-deoxy-D-*manno*-octulosonic acid) and heptose. The outer core consists of a variety of sugars including, but not limited to, glucose, galactose and *N*-acetylglucosamine. O antigen, if present, exhibits high chemical variability that helps to distinguish strains within a bacterial species (e.g. strains of *Escherichia coli*) and leads to great antigenic diversity.

LPS is commonly known as 'endotoxin'. However, both lipid A and LPS share those properties that trigger physiological responses associated with the gram-negative bacteria, namely activation of macrophages and induction of synthesis of a variety of protein factors (e.g. tumor necrosis factor (TNF), interleukin 1 and platelet activating factor) (Beutler and Cerami, 1985, 1988; Kiener *et al*., 1988; Old, 1988; Suffredini *et al*., 1989; Raetz, 1990). Release of LPS occurs after bacterial cell lysis and death. Clinically important sources of endotoxin are associated with the following gram-negative genera: *Escherichia, Proteus, Pseudomonas, Enterobacter* and *Klebsiella.*

In the past we have typically associated exposure to endotoxin with the production of fever (i.e. a pyrogenic reaction), but we know now that the clinical response to endotoxin depends on several factors: (1) source of the endotoxin (i.e. important genus/species differences in the LPS or lipid A chemistries); (2) weight of the patient; and (3) the portal of entry into the patient's body (e.g. the eye vs the gastrointestinal tract) (AAMI, 2007). Septic shock can occur when LPS or LPS-induced TNF enters the bloodstream (Morrison and Ryan, 1987; Natanson *et al*., 1989). High mortality rates have been associated with this clinical event (Kreger *et al*., 1980).

Endotoxin exposure through other portals of entry may result in an adverse clinical outcome but may not end in death. A recent example of this type of clinical event is described in an investigation of a multistate outbreak of toxic anterior segment syndrome in the USA (Kutty *et al.*, 2008). In this outbreak, the patients' exposure to endotoxin occurred during cataract surgery via the use of endotoxin-contaminated balanced salt solution; patients experienced blurred vision, anterior segment inflammation, or cell deposition, but no patient died during this outbreak.

Endotoxin poses the greatest concern when it is introduced into normally sterile tissues of the body and the bloodstream. The potential for introducing foreign matter such as endotoxin into these areas of the body is greatest when using a contaminated surgical device or instrument to make contact with these sterile tissues. The presence of residual endotoxin on surgical instruments and devices presents a constant challenge in hospital central sterile departments (Williams, 2003). The epidemiology of post-surgical adverse events identifies three body fluids and tissues at high risk for adverse outcomes if contact with endotoxin occurs: (1) the patient's bloodstream; (2) CSF; and (3) the anterior chamber of the eye (Mamalis *et al.*, 2006). Furthermore, the amount of endotoxin that gets transferred from an endotoxin-contaminated device or instrument during patient contact will also influence the clinical outcome (AAMI, 2007). Small amounts of endotoxin may be insufficient to elicit a pyrogenic reaction, but the weight of the patient will affect this response. Suffredini and coworkers in 1999 demonstrated that ≥ 50 ng of endotoxin injected into the bloodstream of a patient weighing 50 kg (i.e. ≥ 1 ng endotoxin/kg body weight) will result in fever and migration of leukocytes to the injection site. Endotoxin-contaminated surgical instruments and percutaneous devices (i.e. needles) can be effective tools in this regard as they first create the critical portal of entry and then deliver the contamination to sterile sites where severe inflammatory responses will be realized.

Device reprocessing depends on validated microbial inactivation methods and established standards. Endotoxin is an important bioburden concern, and its elimination from the surfaces of sterile reusable surgical instruments is crucial to the prevention of adverse surgical outcomes. The ability to measure endotoxin concentrations and amounts has been important to the development of successful instrument reprocessing strategies. Currently, the endotoxin unit (EU) is the standard unit of measurement for endotoxin activity. This activity was initially established relative to the activity contained in 0.2 ng of the US Reference Standard Endotoxin Lot EC-2 (AAMI, 2007). With regards to international standardization, the US Food and Drug Administration's (FDA) reference endotoxin Lot EC-6, the US Pharmacopeia's (USP) Lot G and the World Health Organization's (WHO) primary international

endotoxin standard (IS) are sublots of the same endotoxin preparation, thereby establishing the EU and the international unit (IU) as equal (Poole *et al.*, 1997; AAMI, 2007).

10.8 Device reprocessing strategies for endotoxin

10.8.1 Endotoxin removal strategies and sterilization processes

Endotoxin is not reliably destroyed by disinfection, steam sterilization processes, or ethylene oxide sterilization. And although recent research on the use of plasma exposure to inactivate endotoxin shows promise for the future (Shintani *et al.*, 2007; Hasiwa *et al.*, 2008), currently available low-temperature plasma technology sterilizers may not have the same source gas for plasma generation as that used in research (i.e. nitrogen vs hydrogen peroxide) and may not be validated for endotoxin inactivation. Therefore, when protocols for preparing water for injection or for instrument reprocessing call for sterilization, the general and most practical strategy is to prevent endotoxin contamination of items to be sterilized rather than try to remove it. Endotoxin contamination sources include water used as a solvent, water used in instrument cleaning and terminal reprocessing, packaging components and raw materials or equipment used in production (FDA, 1985). As an example, water that contains high numbers of gram-negative bacteria will be expected to have a high concentration of endotoxin, and if such water is used during instrument reprocessing it follows that this endotoxin will be deposited onto the surfaces of the instruments. Steam sterilization is not an effective depyrogenating process, so endotoxin as a clinically important biocontaminant remains active on the surgical instruments. Therefore, the logical endotoxin control strategy for heat-stable instrument sterilization is to control the bacterial contamination levels in the water used to rinse the cleaned instruments. Water containing ≤ 100 EU/mL has been determined to leave very little endotoxin residue on instrument surfaces, thereby minimizing the potential for a pyrogenic reaction in the patient after surgery (AAMI, 2007). Therefore, central sterile departments in hospitals and other healthcare venues will have a water treatment system in place to provide water that meets the quality requirements for sterile instrument reprocessing.

Water treatment systems typically consist of three components: (1) a pretreatment stage; (2) a water treatment process; and (3) a distribution system. Pretreatment is used to remove hard contaminants such as sand and other insoluble objects (e.g. bits of rock). Incoming water is treated to remove organic and soluble inorganic impurities, including the antimicrobial chemicals used by the municipal water authority to treat the water for community use (e.g. chlorine,

monochloramine). There are three water treatment options available for use: (1) deionization; (2) reverse osmosis (RO); and (3) distillation. Depending on local conditions of the municipal water, it may be necessary to validate the performance of the distillation unit, as high levels of organic contaminants such as endotoxin can diminish the unit's effectiveness. Similarly, it may be necessary to have several RO filtering units connected in series in order to provide effective microorganism and endotoxin removal, when municipal water has a high heterotropic plate count (HPC) reading (FDA, 1985). RO filtering units should be disinfected regularly to prevent bacterial build-up. The treated water is then distributed to the various points of use within the central sterile department via a dedicated distribution system. One important quality control task is to prevent any amplification of bacteria and establishment of biofilm in the distribution system. The presence of large populations of planktonic gram-negative bacteria can eventually lead to increase in endotoxin concentration downstream from the main water treatment. Two methods used to keep the distribution system clean are the disinfection of the pipes on a periodic basis and the continual recirculation of the water. Disinfection of the interior pipe surfaces can be accomplished through the use of ultraviolet light (UV), ozone, hot water temperatures, or disinfectant chemicals such as hydrogen peroxide (H_2O_2) or peracetic acid (AAMI, 2007). Periodic microbiological monitoring is an important part of the effort to maintain water quality in the distribution system, as several factors can enable any residual bacteria to increase in number (e.g. increase in water temperature, distribution system bacterial build-up, use of holding tanks). Should an increase in bacterial counts occur (detected via the use of (HPC) obtained by conventional water sampling methods), the problem can be identified quickly and remedial action to lower the bacterial count can be initiated (APHA *et al.*, 1998).

Endotoxin is removed during the water treatment process. Of the available treatment methods, RO and distillation are each more effective for endotoxin removal compared with deionization, as deionization does not remove microorganisms or organic matter (AAMI, 2007). The finished water from a central sterile department water treatment process is described as a high-purity water (AAMI, 2007), and it is typically indicated for the final rinsing of cleaned critical and semi-critical medical and surgical instruments. According to the Association for the Advancement of Medical Instrumentation (AAMI), instruments rinsed with high-purity water are expected to have < 20 EU residual on their surfaces (AAMI, 2007). Additionally, in order to keep residual bacteria counts and endotoxin concentrations to a minimum, high-purity water is generated on-demand. In some specific instances (i.e. the rinsing of delicate ophthalmic surgical instruments), sterile distilled water is recommended for the final rinse (ASCRS and ASORN, 2007).

High-purity water is occasionally checked for endotoxin levels, with acceptable concentrations being those < 10 EU/mL (AAMI, 2007). The bacterial

endotoxins test (BET) is an assay method for active endotoxin in which a liquid sample is mixed with *Limulus* amebocyte lysate (LAL) reagent; the resulting proportional reaction is measured via visual, turbidimetric, chromogenic, or other validated means of detection (AAMI, 2010a). The gel-clot technique (a visual method) is simple to perform, requires minimal equipment and data analyses are easy. Details for this and other test methods are beyond the scope of this chapter, but these are summarized in the ANSI/AAMI standard (AAMI, 2010a) and in Chapter 85 in the US Pharmacopeia standard (US Pharmacopeia, 2011a). Water samples are collected from the following locations within the water treatment system: (1) the reprocessing (cleaning and rinsing) area; (2) storage tank (if this equipment is present); and (3) immediately downstream from the treatment equipment (e.g. the RO filtering unit). Endotoxin levels are typically checked when the water treatment system is installed and whenever any modifications or repairs are made. If elevated endotoxin levels are detected, remediation is initiated and the system is tested repeatedly until the levels fall below the action level of 10 EU/mL.

Many of the instrument reprocessing procedures are either automated or involve use of equipment with some manual activity. Although high-purity water is not indicated for the initial instrument cleaning processes, it is nevertheless important to keep the cleaning equipment fully maintained so that all surfaces are kept clean and any fluid reservoirs (e.g. ultrasonic baths) are drained and replaced regularly or when it is evident that the solution has a high organic matter load. These steps will help to keep residual waterborne bacteria levels to a minimum.

The quality of the water used to generate steam is important for the success of the steam sterilization process. Water for steam must be treated to remove minerals, suspended solids and other contaminants to ensure production of as close to 100% saturated steam as is possible (AAMI, 2010b). However, it is not necessary to use high-purity water for steam generation. Studies have shown that despite the presence of low numbers of microorganisms in water intended for steam production, instruments exposed to steam from such water do not appear to have significant levels of residual endotoxin (Martin and Daley, 2001; Steeves and Steeves, 2006). Consequently, monitoring the water intended for steam production for bacterial counts and endotoxin levels is generally not recommended (Whitley and Hitchins, 2002; Flocard *et al.*, 2005).

10.8.2 Depyrogenation with dry heat

Whereas moist heat (i.e. steam) and ethylene oxide are ineffective sterilants for endotoxin removal or inactivation, dry heat and incineration have been well established as effective means of destroying endotoxin (depyrogenation). However, studies to determine the kinetics of endotoxin inactivation/

destruction were not undertaken until the late 1970s, when the endotoxin detection methods using the LAL reagent became available. In general, destruction kinetics for endotoxin appear to be independent of concentration, are second order, and D values (i.e. the time required to effect a 1 $\log_{10}$ reduction at a specified temperature) can be easily determined (Tsuji and Harrison, 1978). In general, the higher the temperature, the shorter the time needed to reduce endotoxin concentration to 1 $\log_{10}$. For example, the destruction kinetics for LPS from *E. coli* were determined at 170°C and 250°C. The total time needed to reduce the LPS concentration by 1 $\log_{10}$ at 170°C was 190.5 min (D^1 = 20.5 min, D^2 = 170 min), whereas at 250°C the time needed was 6.13 min (D^1 = 0.53 min, D^2 = 5.6 min) (Tsuji and Harrison, 1978). D values for LPS inactivation using other gram-negative bacteria, such as *Serratia marcescens* or *Salmonella typhi* were similar to those for *E. coli*. A later study involving the inactivation of endotoxin on glass surfaces showed that at temperatures < 250°C the destruction curves did not fit second order kinetics, whereas second order kinetics were suitable to describe curves for temperatures between 250°C and 325°C (Ludwig and Avis, 1990). Differences in experimental conditions (e.g. aluminum vs glass surfaces) are a possible explanation for such observations, and the existence of heat-resistant sub-populations in residual endotoxin may also be possible. If heat-resistant sub-populations were present, the D-value transition point would be expected to shift when increasing amounts of endotoxin are subjected to heat. However, Tsuji and Harrison (1978) demonstrated in their destruction curve kinetics studies that the time point of the transition from D_1 to D_2 remained unchanged regardless of the amount of endotoxin used, which argues against the presence of sub-populations.

Studies in the mid-1990s demonstrated that residual endotoxin on surfaces could be reduced in concentration by 3 $\log_{10}$ when exposed to dry-heat cycles of 250°C for 30 min (Nakata, 1993, 1994). The Pharmacopeia standards of the USA, Japan and Europe established this 3 $\log_{10}$ reduction as the standard for endotoxin inactivation during this same period (Nakata, 1993; Hecker *et al.*, 1994; USA, Pharmacopeia, 2011b). The mode of action for dry heat sterilization is heat transfer from chamber air to items on contact (AAMI, 2010c). There are three phases to a dry-heat cycle – the initial heat-up phase, the exposure period, followed by a cool-down phase. Various time/temperature combinations have been validated for dry heat sterilization/depyrogenation of laboratory glassware and other heat-stable items and devices. Examples of some of these cycle parameters are: (1) 180°C for 4 h; (2) 250°C for 45 min; and (3) 650°C for 1 min (FDA, 1985). Dry heat sterilization, in addition to accomplishing endotoxin inactivation, is a sterilizing method of choice for heat-stable powders and oils, reusable needles, glassware, glass syringes, dental instruments and burrs and metal instruments.

10.9 References

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11

Future trends for the sterilisation of biomaterials and medical devices

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Abstract: Sterilisation technologies have remained essentially unchanged over the past 30 years. This chapter looks to the future and reviews how changes in materials, the incorporation of new technologies into current methods and the modification of existing methods could expand the horizons of medical device sterilisation in the future.

Key words: materials for sterilisation, modification of sterilisation techniques, new sterilisation techniques.

11.1 Introduction

The medical device industry has changed significantly in the last 30 years. Many new devices have been introduced to the market and devices on the market have become more complex. Novel combination and biological devices have been developed in recent years. Today, device manufacture is performed in a wide range of countries and the international regulatory environment continues to evolve. Despite these major changes, the sterilisation processes routinely used in the manufacture of medical devices have remained essentially unchanged in this time period.

Sterilisation is an essential process in the manufacture of many medical devices. The ways in which most medical devices are used requires them to be sterile and pyrogen-free in order to be safe for patient use. However, it is often assumed that the selection of a sterilisation process for a particular device will be simple and straightforward, and the characteristics and adverse effects of sterilisation processes are often considered late in the product development process. Sterilisation processes are often not well understood and techniques are usually selected based on successful use on similar products in the past.

Medical devices are sterilised to eliminate living organisms including bacteria, yeasts, mould and viruses.[1] Many sterilisation techniques are available today and these include the traditional methods of autoclaving, ethylene oxide (EO) and gamma irradiation, the more recently introduced systems involving low-temperature gas plasma and vapour phase sterilants and a

range of new sterilisation technologies not in widespread use. Despite the availability of a range of techniques, it is generally agreed that no single sterilisation process is capable of sterilising all medical devices without adverse effects. All processes have their own inherent advantages and disadvantages and many adverse effects relate to incompatibilities between the materials used in medical devices and the parameters of the sterilisation processes. Additionally, some processes also involve environmental issues for manufacturing staff and adverse reactions in patients.

Sterilisation processes act on micro-organisms in a chemical or physical way. Generally, each process results in a change in the structure or function of the organic macromolecules in the microorganism, leading to death or the inability to reproduce. When selecting a sterilisation method, an analysis of the compatibility of each device, particularly the chemical composition of the materials, with the process parameters of the sterilisation method and the chemicals used, is necessary. Metals and metallic alloys are generally not adversely affected by sterilisation processes. However, the macromolecules of biomedical polymers can be affected by the same mechanisms that affect micro-organisms and different forms of sterilisation may result in hydrolysis, oxidation, softening, melting, chain scission and depolymerisation. Research has shown that sterilisation can modify the bulk and surface properties and alter the physiochemical stability of biomedical polymers.[2–5] Sterilisation may also result in the formation of degradation products, which may present a toxicological risk.[6]

The chapters in this book have described in significant detail the process parameters and effects of most sterilisation techniques. Details of steam and dry-heat sterilisation, ionising radiation, ethylene oxide, plasma discharge and new technologies have been covered. The issues relating to drug-device products and the eradication of prions and endotoxins have also been discussed. The use of antimicrobial coatings has also been outlined.

Although these techniques successfully sterilise the vast majority of medical devices produced today, all of them have their inherent disadvantages and issues.

11.2 Common sterilisation techniques

There are four techniques routinely used today to sterilise medical devices and biomaterials: steam, ethylene oxide, radiation and gas plasma.

11.2.1 Steam sterilisation

As described in Chapter 2, steam sterilisation or autoclaving is a relatively simple process that generally exposes the device to saturated steam at 121°C

for a minimum of 20 min at a pressure of 121 kPa.[7] The process kills microorganisms by destroying metabolic and structural components essential to their replication. It is the method of choice for sterilisation of heat-resistant surgical equipment and intravenous fluid as it is an efficient, reliable, rapid, relatively simple process that does not result in toxic residues.

Medical devices manufactured from metallic alloys are well suited to sterilisation using steam. However, the high temperature, humidity and pressure used in the process can lead to hydrolysis, softening or degradation of many biomedical polymers. Several workers[8,9] have reported that autoclaving is unsuitable for the sterilisation of many biomedical polymers due to unacceptable changes in mechanical properties, and a further issue is the potential formation of degradation products during autoclave sterilisation of these materials.

11.2.2 Ethylene oxide sterilisation

Ethylene oxide (EO) sterilisation is used routinely to sterilise materials that cannot withstand the high temperatures of autoclaving. It has long been used as the sterilisation method of choice for a wide range of products although its usage has dropped recently due to its environmental impacts. As described in Chapter 4, the EO sterilisation process involves drawing a vacuum in the sterilisation vessel, after which EO is injected at a concentration of 600–1200 mg/L. The steriliser is maintained at the desired conditions of 30–50°C and 40–90% humidity for the duration of sterilisation, usually between 2 and 8 h. Following the sterilisation cycle, the chamber is then evacuated to remove residual EO. Further aeration is usually required after removal of the packages from the chamber, with aeration time ranging from 2 h to two weeks, depending on the device and packaging.

EO is the most widely used industrial sterilant for medical devices today. Its bactericidal, sporicidal and viricidal effects result from alkylation of sulfhydryl, amino, carboxyl, phenolic and hydroxyl groups in nucleic acids, causing cell injury or death. Its primary advantages are the low processing temperature and the wide range of compatible materials. However, EO has some significant disadvantages which relate to the toxicity and suspected carcinogenicity of the gas and residuals in the manufacturing environment and the device itself. A long aeration process is also required to remove EO and its by-products from sterilised materials and this can affect costs and inventory levels adversely.

Although alkylating reactions have been reported in some polymers when EO interacts with some chemical groups,[10] EO exposure has little effect on most biomedical polymers.[11–13] However, potential formation of degradation products is an issue with EO sterilisation.[14]

11.2.3 Radiation sterilisation

Radiation sterilisation utilises ionising radiation to sterilise medical devices. Its usage has grown in recent decades as more facilities have been built, radiation resistant materials developed and dosage levels more tightly defined. The introduction of electron beam sterilisation has also expanded the use of radiation for sterilisation.

Either gamma rays from a cobalt-60 (^{60}Co) isotope source or machine-generated accelerated electrons are used. Gamma irradiation is the most popular form of radiation sterilisation and is used when materials are sensitive to the high temperature of autoclaving but compatible with ionising radiation. Exposure is achieved when the packages are transported around an exposed ^{60}Co source for a defined period of time. The most commonly validated dose used to sterilise medical devices is 25 kGy.[7]

The bactericidal effect of gamma irradiation is dependent on oxidation of biological tissue. It is a simple, rapid and efficacious method of sterilisation. However, high capital costs are a major disadvantage. Most metal-based medical devices can be sterilised using radiation. However, sterilisation of biomedical polymers using gamma irradiation is known to result in physical changes, including embrittlement, discolouration,[15–17] odour generation, stiffening,[18,19] softening, an increase or decrease in melt temperature[20] and decrease in molecular weight.[11,13,18]

The two mechanisms involved in these changes are chain scission and cross-linking and mechanical properties including tensile strength, elastic modulus, impact strength, shear strength and elongation may be affected. Decrease in fatigue strength have also been reported in some biomedical polymers following gamma irradiation.[19] Embrittlement may occur and crystallinity may also change.[21] Gamma irradiation has also been reported to magnify surface defects in some biomedical polymers[10,21] and Fourier transform infrared (FTIR) studies[12,19,22] have indicated significant oxidation of the surface of some biomedical polymers.

Gamma irradiation also has undesirable consequences due to the potential production of toxic degradation products such as 4,4'-methylenedianiline (MDA) that can be produced when a high-molecular-weight polyurethane material decomposes as a consequence of irradiation.[6,14,23] Cytotoxic effects have also been reported after contact with gamma-irradiated polyurethane samples believed to result from the effect of a low-molecular-weight by-product.[24]

11.2.4 Gas plasma sterilisation

Gas plasma sterilisation is a promising alternative for low-temperature sterilisation of medical devices. Although penetration is reduced compared with traditional EO, gas plasma offers generally good material compatibility

and shorter cycle times. Cold plasma is a partially ionised gas comprising ions, electrons, ultraviolet photons and reactive neutrals such as radicals, excited and ground-state molecules. It is created by the application of an electric or magnetic field to a sterilising agent such as hydrogen peroxide (H_2O_2) or H_2O_2/peracetic acid (PAA). One procedure comprises a 45 min cycle during which vapourised H_2O_2 is diffused through the treatment chamber, after which 300 watts of radio-frequency power are applied at a pressure of 0.5 Torr to create the plasma. The plasma is maintained for a period sufficient to ensure complete sterilisation with a standard phase lasting 15 min. The total procedure takes approximately 1 h.[25] Another process uses PAA and H_2O_2 vapour treatment, which is alternated with downstream plasma treatment by microwave excitation of the low-pressure gas mixture comprising oxygen, hydrogen and argon. The equipment operates by vapourising the chemical agents and diffusing the vapour into the chamber, alternating with the plasma. At the end of sterilisation, the reactive species combine to form water and oxygen, eliminating the need for aeration.[8]

H_2O_2 works by the production of destructive hydroxyl free radicals, which can attack membrane lipids, DNA and other essential cell components.[26] Inactivation of micro-organisms is dependent on time, temperature and concentration. PAA is an oxidising agent that denatures protein, disrupts cell wall permeability and oxidises sulphur bonds in proteins, enzymes and other metabolites.[26]

Gas plasma sterilisation is reported to be suitable for the sterilisation of metals, natural rubber, silicone and various polymers such as polyvinyl chloride, polyethylene and polyurethane.[8,10] However, the process uses strongly oxidative chemical sterilising agents and it is well known that these agents can induce surface oxidation of some biomedical elastomers.[2,19,27,28]

Gas plasma is not suitable with liquids, oils, powders, biological tissues, paper, cotton and linen. It has inferior penetrating ability compared with EO, but both PAA and H_2O_2 perform more effectively than EO in terms of biological kill and sterilant removal.[7] Other advantages of plasma sterilisation are that it is a fast, low-temperature process with no requirement for aeration.

11.3 Future trends

The characteristics of the ideal sterilisation method were outlined in some detail in Chapter 1. In summary, it was defined as efficient, highly reliable and safe for workers and patients. It did not damage the device, did not produce residues or toxic by-products and was rapid and inexpensive. When compared with these criteria, most routinely used sterilisation procedures are efficient and reliable. However, all have significant disadvantages when

compared with at least some of the other criteria. No one process meets all requirements.

The major disadvantages of the main sterilisation processes have been outlined in earlier chapters. Although emphasis is usually placed on immediately identifiable unfavourable effects on the device and later impacts on the patient, conventional sterilisation methods can also adversely impact the shelf life of many medical devices. The shelf life of metal-based devices is generally not affected by most sterilisation processes. However, many studies have reported that the surface and mechanical properties of polymer-based devices can be adversely affected by conventional sterilisation techniques[29] and the effects observed immediately after sterilisation can be magnified with aging of the device.[30] The long-term mechanical and physical properties of medical polymers are particularly susceptible to the effects of sterilisation processes.[31]

Another issue often ignored by manufacturers is the effect of the sterilisation process on the integrity of the packaging materials used for medical devices. Not only do the properties of some packaging materials fail to withstand the initial sterilisation process but some packaging materials are known to degrade with time after sterilisation.[32] This represents a significant risk to the sterility and integrity of the device with time.

The fundamental nature of technologies that are capable of sterilising medical devices also make them potentially unsafe for manufacturing staff and patients exposed to the process or device. Sterilisation processes act on micro-organisms in a chemical or physical way and each process results in a change in the structure or function of the organic macromolecules in the microorganism, leading to death or the inability to reproduce. These effects are not exclusively limited to unwanted micro-organisms. Healthy cells can also be affected by these processes and the impact on humans exposed can be significant.

Developing a process that is lethal to unwanted micro-organisms and yet safe for workers and patients is technically unrealistic and current technologies consequently fail in this regard. EO and its by-products are known to be toxic and potentially carcinogenic. Exposure to the chemicals used in chemical-based sterilisation processes such as hydrogen peroxide and peracetic acid is also not without risk. The adverse effects of exposure to radiation-based processes including gamma irradiation are also well known.

A further issue confronting the medical device industry is the development of novel and complex medical devices that incorporate, among other components, pharmaceutical agents or biological materials. Many combination products and tissue-engineered devices are currently under development and these devices represent the future of medical devices for some applications. However, the inherent nature of these products can pose specific difficulties for incorporating sterilisation as the final step in the manufacturing process.

Commonly available sterilisation methods are known to cause alterations in biomolecules such as collagen and can have effects on their physical and mechanical properties. Some of these can enhance the properties of the product and the commonly used sterilisation method for acellular dermal matrix is irradiation. However, most result in reduced performance and many researchers have tested the effects of a variety of sterilisation techniques[33] with limited success. Although sterility is achieved, the adverse effects on the biological material are often significant and these include changes in the morphological, physical and mechanical characteristics of the material. Marreco *et al*.[34] concluded that no currently available sterilisation process was suitable for chitosan membranes. In a separate study, Wiegand *et al*.[35] observed a pronounced effect on the physical properties of collagen after sterilisation.

The future of these combination and biological-based products relies to a large extent on the development of techniques that can be used to sterilise biological material with minimal compromise to tissue integrity.

Terminal sterilisation is one aspect of the ideal sterilisation technology, and the conditions required to achieve sterility using the conventional methods such as EO, gamma irradiation and autoclaving are tightly defined and can be, in most cases, directly measured. Alternative processes are technically complicated, more expensive and inherently less reliable. Most involve some form of aseptic processing, which involves the manipulation of the device components in a manner that precludes microbiological contamination of the final sealed product. Because there is no process to sterilise the product in its final container, it is critical that manufacture and assembly be conducted in a controlled, high-quality environment. The ISO 13408 suite of standards that cover aseptic processing of healthcare products outline the requirements for these processes and also cover validation and routine control of the manufacturing process for aseptically processed healthcare products. The FDA also publishes guidance on methods of aseptic processing and their validation and control including 'Sterile drug products produced by aseptic processing – current good manufacturing practice' (2004). However, ongoing issues include the development and validation of robust processes as well as release procedures that maintain high levels of reliability and traceability.

The use of an alternative sterilisation method that uses supercritical carbon dioxide has been reported.[36] The bactericidal effect of carbon dioxide is well known and various studies have demonstrated its effectiveness on a number of micro-organisms with various treatment parameters, which include temperature, pressure, treatment time and the use of additives. The promising aspect about supercritical carbon dioxide is that it has been found to be compatible with a range of biological materials including bone and formed no toxic residues in treated materials.[37] However, only limited

use of supercritical carbon dioxide for the sterilisation of biological materials has been reported.

11.4 Conclusions

Sterilisation technologies have remained essentially unchanged over the past 30 years. Some new processes have been introduced and these have offered some benefits. However, on the whole, the traditional techniques of EO, irradiation and steam have survived as the primary techniques used for the vast majority of devices. Sterilisation will continue to be an integral part of the medical device industry and, looking to the future, developments in medical device sterilisation may lie in three areas. First, the development of materials that are more compatible with current sterilisation technologies would allow wider use of irradiation and steam, both of which result in less environmental impact and safety issues for workers and patients. For example, researchers have developed radiation-stable polymers that demonstrate limited loss of mechanical properties following sterilisation with gamma irradiation.[38]

The second area relates to more customised use of established techniques. Currently, international standards and regulatory guidelines set out strict requirements for the sterilisation of medical devices. In the future, some of these requirements may be revisited to allow sterilisation protocols that involved modified cycles of existing processes or indeed, a combination of technologies to minimise both adverse effects on the devices and the production of unwanted by-products or residuals. Existing, novel, combination and biological-based products may be able to be sterilised using a reduced cycle in order to preserve functionality and integrity. Developments in the gamma sterilisation process could include validation of doses lower than 25 kGy for products susceptible to adverse effects. Manipulation of the parameters defined for steam sterilisation may also allow longer time, lower temperature processes that allow more devices to be sterilised using this technique.

Conditions to allow these reduced cycles could be partly achieved by the establishment of improved manufacturing procedures and controls that reduce bioburden levels before sterilisation. Additionally, substantial developments in clean-room technology as well as improved control over component suppliers to medical device companies would contribute to this goal.

Advancements in aseptic processing may also allow the requirement for terminal sterilisation to be eliminated for some devices. Technology improvements such as isolators, clean-room HEPA-filtered respirators and improved barrier gowns and gown materials that increase the reliability of existing processes would contribute to this aim.

Finally, the holy grail in the sterilisation of medical devices is the development of a dramatically different technology that offers all of the attributes of the ideal process outlined earlier in this chapter. The challenge is to develop a process that eliminates or deactivates unwanted micro-organisms while not harming the device or the biological structures of workers and patients. It will take a total shift in paradigm to achieve this aim and no process currently available or known to be in development is likely to achieve this goal in the near future. Although such a total shift in technology is unlikely due to the constraints previously discussed, medical device manufacturers and end users look forward to the future with a sense of expectation.

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