Inactivation of Bacterial Spore, Endotoxin, Lipid A, Normal Prion and Abnormal Prion by Exposures to Several Sorts of Gases Plasma

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This review discusses the application of several sorts of non-equilibrium gas plasma discharges for sterilization and disinfection treatments against spores or bioburden on/in the healthcare products or biological indicators. The basic properties of electrical discharges are briefly reviewed and thereafter the paper discusses the interactions of gas plasma with several sorts of biological systems such as bacteria, bacterial spores, endotoxins, lipid A and normal and abnormal prion proteins.

Key words : Sterilization / Non-equilibrium gas plasma / Spores / Endotoxins / Prion proteins.

INTRODUCTION

Some nosocomial infections can be attributed to various microorganisms such as Staphylococcus aureus, E. coli, and Bacillus cereus that may be present on the surface of healthcare products as bioburden and can come into contact with human tissues during operations. Several studies have demonstrated that some of these microorganisms are resistant to antibiotics such as MRSA and VRE and so on (http://www.betterhealth.vic.gov.au/bhc2/bhcarticles.aspx/pages/Antibiotic_resistant_bacteria). This indicates the need for improved sterilization procedures to ensure medical products are free from bioburden, airborne falling microorganisms or adhesive microorganisms as far as possible (Shintani et al., 2003; Shintani et al., 2006; Shintani, 2013; Shintani, 2014). However, some 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 health concerns because residue pathogens can be transmitted to pathogen free patients who then develop iatrogenic diseases. Some of the most serious pathogens include the abnormal prion proteins, the principal factors for the transmission of Creutzfeld Jakob Disease (CJD); this disease could potentially be transmitted via contaminated instruments during brain operations.

Another concern is related to pyrogens (fever-inducing substances) deriving from gram-negative bacteria (lipopolysaccharides (LPS)), endotoxins or Lipid A (Figures 1, 6-8). Lipid A is an active site of endotoxins. These LPS, bacterial endotoxins or lipid A can cause fever when in contact with the bloodstream and provoke fever shock, which is a major cause of death among, for example, dialysis patients. Endotoxins are extremely resistant to temperature and are not removed by conventional sterilization procedures such as autoclave sterilization or y-ray or e-beam sterilization. A few papers have been published on the effect of gas plasma exposure on endotoxins to cause more than a 5 log reduction during 30 min exposure to nitrogen gas plasma (Shintani et al., 2007, Shintani et al 2010: Shintani, 2012: Sakudo et al., 2013, Figure 2). In general a 3-log reduction is required by the authorities and GMP (good manufacturing practice) and, according published papers by Shintani et al in 2007 and Shintani in 2012, during 10 min at 28-45°C operation, a 3-log reduction was completed in Figure 2, which is a significant achievement.

Sterilization procedures must follow official rules such as ISO 14937 and 14161 and so on. This kind of subject will be discussed in a coming book (Shintani, 2015,
Moreover, the usual sterilization techniques have several drawbacks including the potential degradation of heat-sensitive healthcare products (e.g., autoclave sterilization at 121.1°C for 20 min is normal): a relatively long operation time is required due to toxic gas degassing (ethylene oxide gas or hydrogen peroxide gas sterilization). Autoclave sterilization is performed at 121.1°C for 20 min, γ-ray sterilization at 25 kGy is conducted for 2.5h (10 kGy/h is normal) and electron-beam sterilization requires a few s. Sterilization procedures do not always require long process times in every case, but depend on the procedure selected.

Alternative sterilization procedures are under investigation including the use of non-equilibrium gas plasma discharges: we will see that plasma processes are able to sufficiently kill bacterial spores with more than a 6 log reduction without leaving residues of toxic gases (Shintani, 2015) and when conducted at a relatively low temperature at 25-60°C, endotoxin inactivation is achieved at more than a 5 log reduction (Shintani et al., 2007, Figure 2). Non-equilibrium gas plasma sterilization studies have been carried out to show that many types of pathogens can be inactivated (Lerouge et al., 2001: Moisan et al., 2001: Rossi et al., 2006: Shintani et al., 2007: Shintani et al., 2010: Shintani, 2012) and the mechanism and inactivation factors of gas plasma sterilization against microorganisms or biomolecules have been extensively studied. According to our recent studies, the major factors causing sterilization by nitrogen gas plasma exposure were not well clarified and defined. This will be described later in this article (Shintani et al., 2015, Figure 3).
In this review we present the basic principles of atmospheric and low pressure gas plasma sterilization and the active species produced and discuss their interactions with organic materials, pathogens (endotoxins, abnormal prion proteins), and bacterial spores (Shintani et al., 2007; Shintani, 2012), showing how these factors could be used for sterilization and inactivation.

**BRIEF DESCRIPTION OF PLASMA GENERATION**

Plasma denotes in physics a quasi-neutral ionized gas—that is, a gas in which a certain fraction of particles is charged (Figures 3-5). The presence of charged species turns plasma into a highly conductive gas that responds readily to electromagnetic fields. As a consequence of this, plasma presents unique properties as compared with solids, liquids or gases, and is therefore often referred to as the fourth state of matter. 99% of the fourth state of matter exists in the world without our everyday awareness.

Plasma is normally generated by supplying sufficient energy to a neutral gas and inducing the formation of charged species, electrons and ions (Figures 3-5). This process proceeds by means of 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: 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 and plasmas created in this way are called thermal plasmas at a high temperature such as 3000°C. 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 and application for gas plasma sterilization.

Another way to produce 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 discharge, will be described briefly.

**1. Properties of electrical discharges**

Any neutral gas contains a certain amount of charged 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 (http://web.mit.edu/sahughes/www/8.022/lec10.pdf) up to kinetic energies sufficient for the ionization of atoms or molecules in a volume of gas, which happens principally through electron-impact ionization.

This can occur through the emission of newly charged particles from electrodes produced 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 energies compared with heavier atomic or molecular ions, whose kinetic energy remains relatively low and close to the temperature of neutral species. Since the temperatures of electrons and other neutral species (atoms, molecules, radicals, Figures 3-5) are different, such plasmas are called non-equilibrium plasmas or non-thermal plasmas.

Moreover, collisions between particles present in plasma do not lead solely to their ionization. In fact, a
significant portion of energy supplied to the plasma is used for the excitation of atoms and molecules to produce metastables or radicals (Figure 3). The presence of excited species has two important consequences. First, excited species, and particularly long-living metastables or radicals, can act as energy carriers: their internal energy can be released when they strike on the surfaces of objects placed into the volume of plasma, which can lead to the physical sputtering of their surface, or can contribute to volume ionization by a process known as Penning ionization (http://www.jstor.org/discover/10.2307/3573984?sid=21106032981603&uid=4&uid=3738328&uid=2&uid=70&uid=2129). Second, excited species can be the source of intense light emission, which is connected with their radioactive transition to lower energy levels. 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. Nitric oxide (NO) radicals in nitrogen gas plasma sterilization were detected using a chemical indicator specific to NO radicals and published in Japan Patent 5465749 (Shintani et al., 2014). The life period of NO radicals was 3-6 s, and thus long enough compared to, for example, the life period of OH radicals that is of a few μs.

Furthermore, collisions between electrons and molecules can also cause their dissociation ionization. This triggers subsequent chemical reactions leading to the presence of species initially not present in a gas. Typical examples are the production of atomic oxygen in plasma in O2 gas, or OH radicals in discharges containing water vapor (Figures 3 and 5). Species created in this way can chemically react with the surfaces of objects placed into the volume of plasma, which can cause modifications in the physical or chemical properties of their surfaces (Figure 4).

Although the processes described above by no means provide a complete description of plasma, they illustrate the key features of plasma discharges that make them highly interesting for a wide range of applications and for the sterilization of bioburden existing on/in the healthcare products. 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 energy levels sufficient to enable the physical sputtering of the treated objects (Figures 3 and 5).
2. Plasma discharges can be operated at moderate temperatures (and in some cases even at room temperature to 80°C, Figure 2), thereby allowing the treatment of heat-sensitive materials.
3. Plasma is a potent source of radiation, comprising of germicidal UV (UV254 nm) and VUV photons.
4. In plasma discharges, species not present in the initial working gas can be created (Figures 3 and 5). Such atoms, molecules, radicals, ions or metastables can interact with treated objects, resulting in modification of their properties.
5. The interactions between an immersed object and the plasma surrounding it are limited to a thin surface layer of the treated object (around 10-20 nm level, Shintani et al., 2007; Shintani, 2012) and thus do not induce significant modifications of its bulk properties.

**INTERACTION OF PLASMA WITH BACTERIA AND BACTERIAL SPORES**

1. **Sterilization with atmospheric-pressure plasma discharges**

The use of atmospheric plasma for sterilization of different types of spores has been a major subject of research. Different types of discharge have been used, and the results summarized (Montie et al., 2000: Laroussi 2002, 2005; Gaunt et al., 2006; Moreau et al., 2008: Shintani et al., 2010).

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 bacterial spores is more difficult 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 such studies have been conducted to date. Second, contamination by air backflow in the discharge occurs in the majority of cases unless particular precautions are clearly taken. In other words, the working gas mixture is not well-defined, since it generally contains a non-negligible fraction of impurities, for instance, water vapor or air, which may markedly alter the interactions between plasma and biological pathogens.

Nevertheless, the role of UV (Trompeter et al., 2002: Park et al., 2003: Heise et al., 2004: Lee et al., 2005) against spores was shown to be similar to what was observed for low-pressure plasmas. In addition, two further mechanisms that have not been observed with low-pressure plasma discharges are active in this case. However, in general, UV and VUV contributions to the sterilization of bacterial spores are considered not to be significant among plasma researchers. One example is reported by Deng et al in 2006.

1.1 **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 to lower extent, also on gram-positive bacteria (Deng et al., 2006).

1.2 Interaction with reactive oxygen and nitrogen species

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) such as the hydroxyl radical (OH), hydrogen peroxide (H₂O₂), the superoxide anion (O₂⁻), nitric oxide (NO) or peroxynitrite anion (OONO⁻) interact with various classes of biomolecules present in cells and in the interior of bacteria, leading to a complex chain of events that can cause cell death (Shintani, 2015; Shintani and Sakudo, 2016). Surface sterilization or modification (Figure 4) does not result in bacterial death. The molecular targets for ROS are DNA, RNA, lipids and proteins existing in the interior of the bacteria (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 is speculated to lead to cell death. The outer layer of gram-negative and gram-positive bacteria can be seen in Figure 6. Lipopolysaccharides exist only in the gram negative bacteria, not in gram positive bacteria. The teichoic acid contained in gram-positive bacterial cell walls is not a lipopolysaccharide (http://www.nature.com/nsmb/journal/v17/n5/fig_tab/nsmb.1819_F1.html, Figures 6-7).

The reaction of ROS with proteins has also serious implications for the function of cells, since the accumulation of injured proteins can significantly disrupt the cell metabolism. Finally, ROS and in particular, the OH radical, can cause a DNA strand to break. Nevertheless, OH radicals cannot diffuse freely through the cell to reach DNA due to their short period of life of a few µs. Therefore, it is assumed that OH radicals are created in the vicinity of DNA from hydroxide peroxide by the Fenton reaction indicated below. However, where is hydrogen peroxide from? Gas plasma sterilization does not produce hydrogen peroxide (Figures 3-5). It is impractical to consider this explanation when seriously discussed. Fenton reaction explained from the following equation: Fe²⁺ (or Cu²⁺) + H₂O₂ → Fe³⁺ (Cu³⁺) + OH⁻ + OH⁻

INTERACTION OF PLASMA WITH ENDO-TOXINS AND PRION PROTEINS

Unlike bacterial spores, endotoxins and prion proteins are not living organisms. They mostly consist of lipopolysaccharides or proteins. No DNA or RNA is involved (Shintani et al., 2007; Shintani, 2012). Because there is no DNA or RNA to target, different strategies for the inactivation of endotoxins and prion proteins have to be followed. The main results related to the application of
sterilization processes at health care facilities. The outer coating of gram-negative bacteria contain endotoxins and lipid A consisting of lipopolysaccharides (Figure 6). Their presence in the bloodstream leads to physiological events such as fever and so on, and at higher doses, to patients’ death, especially in the case of dialysis patients (Beutler et al., 2003). Endotoxins are extremely resistant to temperature and difficult to remove by conventional methods: their inactivation by low-pressure plasma for treatment of endotoxins and prion proteins are summarized in two sections emphasizing the effect of the reactive species.

1. Effect on endotoxins

Endotoxins consisting of lipopolysaccharides as shown in Figures 1 and 8 are another common types of surface contaminant that are not addressed in the

![Chemical structure of lipopolysaccharide.](image1.png)

![Chemical structure of Lipid A.](image2.png)

![MS spectrum before nitrogen gas plasma exposure (a), after nitrogen gas plasma exposure (b) and solvent injection alone (c).](image3.png)

From the MS fragmentation, it can be seen that ester or acid amide bonds are cleaved by nitrogen gas plasma exposure.
nitrogen gas plasma has been studied by Shintani et al in 2007, 2010 and 2012 (Figure 2). Nitrogen gas plasma was the most efficient, and oxygen gas plasma destroyed the outer layer of endotoxins by etching but could not inactivate them.

The mechanisms of the inactivation of endotoxins was the cleavage of lipid A at the ester bonding or acid amide bonding due to the lower bonding energy involved (Figures 10 and 11, Table 1). The chemical structures of endotoxins and Lipid A are presented in Figures 1, 8 and Figure 9, respectively. Oxygen gas plasma causes the etching phenomenon of the targeted material including bacterial spores, which is inferior to the real application to attain material/functional compatibility. The result that sterilization is accomplished, but the material is rendered useless must be avoided. Therefore, oxygen gas plasma is recommended neither in research nor in practical use. GMP requires attaining both a SAL (sterility assurance level) of $10^{-6}$ and material/functional compatibility, but oxygen gas plasma sterilization is not suitable according to GMP regulations because it causes significant etching and shrinkage which means material degradation (Shintani, 2015).

2. Effect on normal prions and abnormal prions

Protein residues remaining on the surface of instruments constitute another group of possible contaminants. These residues might contain pathogens, and in particular abnormal prions, since abnormal prions have been found mostly in the brain of CJD patients. CJD could possibly be transmitted by contact with instruments contaminated with abnormal prions. Unlike bacterial spores, prions do not contain genetic material

<table>
<thead>
<tr>
<th>C-C bonding</th>
<th>D (kJ/mol)</th>
<th>C-H bonding</th>
<th>D (kJ/mol)</th>
<th>*-H bonding</th>
<th>D (kJ/mol)</th>
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<tbody>
<tr>
<td>CH3-CH3</td>
<td>368</td>
<td>H-CH3</td>
<td>434</td>
<td>H-OH</td>
<td>499</td>
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<tr>
<td>CH3-C2H5</td>
<td>357</td>
<td>H-CH2</td>
<td>461</td>
<td>H-O</td>
<td>427</td>
</tr>
<tr>
<td>CH3-C(CH3)3</td>
<td>344</td>
<td>H-CH</td>
<td>427</td>
<td>H-O2H</td>
<td>376</td>
</tr>
<tr>
<td>CH3-C6H5</td>
<td>417</td>
<td>H-C</td>
<td>339</td>
<td>CH3O-H</td>
<td>436</td>
</tr>
<tr>
<td>CH3-CHCH2</td>
<td>466</td>
<td>H-C2H5</td>
<td>412</td>
<td>CH3COO-H</td>
<td>442</td>
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<tr>
<td>CH3-CCH</td>
<td>465</td>
<td>H-C(CH3)3</td>
<td>387</td>
<td>(N-H)</td>
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<tr>
<td>CH3-CH2OH</td>
<td>350</td>
<td>H-C6H5</td>
<td>460</td>
<td>H-NH2</td>
<td>432</td>
</tr>
<tr>
<td>CH3-COOH</td>
<td>403</td>
<td>H-CHCH2</td>
<td>455</td>
<td>H-NH</td>
<td>388</td>
</tr>
<tr>
<td>CH3-COCH3</td>
<td>355</td>
<td>H-CCH</td>
<td>500</td>
<td>H-N</td>
<td>352</td>
</tr>
<tr>
<td>CH3-CN</td>
<td>513</td>
<td>H-CHO</td>
<td>360</td>
<td>(S-H)</td>
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<tr>
<td>C2H5-C2H5</td>
<td>346</td>
<td>H-CH2OH</td>
<td>393</td>
<td>H-SH</td>
<td>383</td>
</tr>
<tr>
<td>C6H5-C6H5</td>
<td>468</td>
<td>H-COOH</td>
<td>374</td>
<td>H-S</td>
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</tr>
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such as DNA or RNA. The chemical structures of normal and abnormal prions are presented in Figure 12 (Shintani, 2012). Prions have been found to be extremely resistant to conventional sterilization procedures because of their unique and stable secondary and tertiary structures that cannot be easily altered (Figure 12). As explained in Figure 12 captions, normal prions have more \( \alpha \)-helix and less \( \beta \)-sheet compared with abnormal prions.

However, the possibility of removing prions by means of non-equilibrium plasma discharges has already been demonstrated (Shintani, 2012, Figure 13). In Figure 14 are the test results for BSA (bovine serum albumin) analysis by electrophoresis before and after nitrogen gas plasma exposure (Shintani, 2012). BSA is the most widely used protein in biological studies. We also tested myoglobin, which contains an abundance of \( \beta \)-sheet resembling abnormal prions. The results show that myoglobin was completely destroyed by plasma discharges similar to the BSA data presented in Figure 14 (data for myoglobin are not shown as they resembled Figure 14).

The mechanism of degradation of abnormal prions has not been completely clarified. However, according to Sakudo’s paper in 2013, a higher order structural change may occur. The effects of non-equilibrium plasma discharges have been studied on non-pathogenic models of proteins in order to identify general mechanisms useful for prion elimination. The results of these studies reveal the possibility of removing proteins by nitrogen gas plasma (Shintani, 2012), which may induce their deformation as speculated. Fragments could not be identified, so fragmentation did not seem to be observed, and deformation may be the likely event (Sakudo et al., 2013).

The protein removal follows two-phase kinetics, composed of an initial fast phase followed by a second slow phase. The first phase was attributed to the fast deformation 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). It was also found that the same mechanism of chemical sputtering observed for bacterial treatment was also operative for proteins, in close correlation with the measured rates between post and direct discharges (Kylian...
et al., 2009a). Here again, synergy was observed between ion bombardment and radicals such as atomic H or O, as well as O₂ or N₂. Moreover, it was also found that the initial rate did not depend on the primary structure of the polypeptides contained in the proteins (Kylian et al., 2009b), leading to the speculation that most proteins could be deformed with their higher order structure of proteins (Sakudo et al., 2013). The most important parameters found were the radical content of the discharge and the plasma density, both of which are responsible for the synergy observed during chemical sputtering.

The treatment of proteins by atmospheric plasma discharge has also been the subject of research (Deng et al., 2007a, 2007b; Bayliss et al., 2009; Shintani 2012). By using an atmospheric pressure glow discharge with pure nitrogen gas, the authors showed that proteins could be destroyed at low temperatures, and the speculated but not confirmed main agents were oxygen and metastable and excited nitric oxide (NO), with a possible synergistic effect between the two species speculated. Electrophoresis of the protein films before and after plasma treatment was used to show that the proteins were completely degraded and fragmented by the treatment as Shintani presented in Figure 14, and that the plasma action could be speculated as protein degradation by the sputtering effect on proteins (Shintani, 2012, Figure 14).

It was reported that spore inactivation kinetics was also secondary to thirdly kinetics, but it was the first order kinetics when a clump-free biological indicator was used. Therefore the above speculation must be further clarified.

THE USE OF PLASMA FOR STERILIZATION PURPOSES

Although there has been progress in the field of plasma-based sterilization, there are still some questions. The aim of this part is to mention briefly some of unresolved questions as well as to delineate further perspectives of sterilization techniques.

1. Optimization of the plasma sterilization process

The results from the literature clearly show that atmospheric-and low-pressure plasma can be used to sterilize bioburden on/in surfaces. Different mechanisms have been clearly identified, namely reaction with reactive species as shown in Figures 3 to 5 that were mostly not the major contributors except for metastables. The speculated major contributor was peroxynitrite anions (OONO⁻). Peroxynitrite has several biological activities (Goldstein and Merenyi, 2008; Beckman and Koppenol, 1996; Shintani, 2015), but sterilization or disinfection functions have not been reported. Nitric monoxide (NO) radicals detected by us (Shintani et al., 2014, Japan patent) combine with superoxide ions (O₂⁻) and produce peroxynitrite anion (OONO⁻) on the biological indicator spores or bioburden to sterilize them. The life period of the peroxynitrite anion, nitric monoxide radical and superoxide anion is a few s, 3-6 s and 5 s, respectively, which are relatively long periods compared with most radicals with µs life periods such as the OH radical.

As the outer layers of gram-negative and gram-positive bacteria have been charged, charged contributors cannot penetrate into the interior of the bacteria to attack interior DNA or RNA. In that sense, the nitric monoxide radical can be understood as a contributor due to its neutral nature, but it is difficult to understand the superoxide anion in the same light due to its minus charge. As a whole, speculation regarding peroxynitrite anion as a contributor is problematic and requires consideration of other reasonable factors. As indicated, charged factors can be neglected as contributors. This indicates that metastables of N or O may be the major contributors due to a few s of life period, their neutrality and abundant energy produced from the excited state to the ground state to destroy bacteria. Radicals may be candidates as they are not charged, but their life period is too short such as a few µs, so radicals with relatively long life period could be considered. That means the NO radical may also be a candidate, but unfortunately the NO radical has no sterilization or disinfection function.

UV leads to very effective sterilization when spores are not clumped on the materials. However, UV or VUV alone is not efficient for inactivating bacterial spores, endotoxins or prion proteins. This indicates that although gas plasma produce UV or VUV (Figures 3 and 5), they are not major contributors and not even minor contributors.

2. 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 and their limited penetration at 10-20 nm in high aspect ratio holes and trenches (1ow-pressure plasma discharges, Shintani et al., 2007: Shintani 2012). 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 decreases the sterilization effects.

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, which means gas plasma sterilization is not carried out like γ -ray or e-beam irradiation sterilization. This is a serious obstacle in the application of gas plasma sterilization for practical use.

3. Treatment of biological pathogens in an aqueous environment

Another important aspect is the possibility of treating biological pathogens in an aqueous environment by means of atmospheric pressure plasma discharges. Bacteria can be effectively sterilized not only in a moist environment, when a minute amount of a non-liquid form of water is present (Dobrynin et al., 2009), but also when completely suspended in liquid (Liu et al., 2010; Oehmigen et al., 2010). This effect was attributed to a gradual acidification of the water caused by the reactions of NOx produced in plasma with water and the subsequent oxidation of the bacterial fatty acids by perhydroxyl radicals (OOH•) at low pH produced by nitrous acid or nitric acid formed (Liu et al., 2010). This is not the plasma effect, but acid effect.

Another explanation assumes that there is synergy between reactive oxygen and nitrogen species, particularly the OH radical, NO radical and OONO− (peroxynitrite anion). In this reaction scheme, NO releases iron ions from intracellular metalloproteins; the Fe2+ content then catalyses the Fenton reaction to produce toxic OH radicals if hydrogen peroxide (H2O2) happens to be present together with Fe2+. In reality, it is quite rare to have hydrogen peroxide present together with Fe2+. Thus, the above explanation would describe quite a rare event.

4. Treatment of biological items

The possibility of using atmospheric pressure plasma discharges for the treatment of living tissue is currently the subject of research. It has been demonstrated that different plasma sources are capable of effectively killing bacteria, but are non-destructive to human tissues, which may be the shallow penetration depth of gas plasma at 10-20 nm level (Shintani et al., 2007; Shintani 2012). Bioburden can be destroyed by gas plasma, but contributors of gas plasma sterilization may not penetrate to attack the interior DNA or RNA in humans. Other possible explanations have been proposed:

1. Mammalian cells have a defense mechanism against oxidative stress. For instance, the presence of NO radicals induces cellular synthesis of antioxidative enzymes in cells. This mechanism, which is absent or considerably lower in bacteria, then counterbalances the increased production of Fe2+ or Cu+ ions responsible for the formation of toxic OH radicals through the Fenton reaction if hydrogen peroxide (H2O2) happens to be present together with Fe2+ in this case.

2. There is a size effect. The bacteria are much smaller than mammalian cells and thus the dose of the 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.

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 (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. It can be used for wound healing, tissue regeneration, blood coagulation or even for killing of cancer cells (Vandamme et al., 2010). If these effects are reproducible, plasma discharges can be used not only in sterilization procedures, but also in medical applications.

CONCLUSIONS

This review article summarized the work carried out to date on low-pressure and atmospheric-pressure sterilization. The main mechanisms of plasma action on various biological pathogens have not well been identified and it has partly been shown that plasma treatment is a process for sterilization at low temperatures (Shintani et al., 2007; Shintani 2012). However, several points still need to be clarified before plasma technologies can be applied for practical use.

For example, the sterilization mechanism has not yet been completely clarified and the contributing factors of sterilization have still not been identified. At this point, therefore, it may be too early to use plasma discharge for real-life sterilization procedures. For medical applications, official approval is required beforehand; however, with what is not known about plasma technology, approval may be quite difficult to obtain.

Apart from issues related to the general process, there is an issue linked to the variability in efficiency of the sterilization mechanisms, which leads to difficulties in homogeneously treating large loads with different shapes and sizes. Another issue is linked to the matrix effect, which underlines the need to integrate plasma technology in a complete washing/cleaning process. The economics of the whole process including plasma discharge will need to be defined, otherwise practical
application of gas plasma cannot be widely utilized in future.

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