Journal of Industrial Microbiology & Biotechnology (1998) 20, 69–74

Room temperature sterilization of surfaces and fabrics with a One Atmosphere Uniform Glow Discharge Plasma

K Kelly-Wintenberg[1], TC Montie[1], C Brickman[1], JR Roth[2], AK Carr[2], K Sorge[2], LC Wadsworth[3] and PPY Tsai[3]

[1]*Department of Microbiology, University of Tennessee, Knoxville, TN 37996;* [2]*UTK Plasma Science Laboratory, Department of Electrical Engineering, University of Tennessee, Knoxville, TN 37996;* [3]*Department of Textiles and Interior Design, and UTK Textiles and Nonwovens Development Center (TANDEC), University of Tennessee, Knoxville, TN 37996, USA*

We report the results of an interdisciplinary collaboration formed to assess the sterilizing capabilities of the One Atmosphere Uniform Glow Discharge Plasma (OAUGDP). This newly-invented source of glow discharge plasma (the fourth state of matter) is capable of operating at atmospheric pressure in air and other gases, and of providing antimicrobial active species to surfaces and workpieces at room temperature as judged by viable plate counts. OAUGDP exposures have reduced log numbers of bacteria, *Staphylococcus aureus* and *Escherichia coli*, and endospores from *Bacillus stearothermophilus* and *Bacillus subtilis* on seeded solid surfaces, fabrics, filter paper, and powdered culture media at room temperature. Initial experimental data showed a two-$\log_{10}$ CFU reduction of bacteria when 2×10^2 cells were seeded on filter paper. Results showed $\geq$3 $\log_{10}$ CFU reduction when polypropylene samples seeded with *E. coli* (5×10^4) were exposed, while a 30 s exposure time was required for similar killing with *S. aureus*-seeded polypropylene samples. The exposure times required to effect $\geq$6 $\log_{10}$ CFU reduction of *E. coli* and *S. aureus* on polypropylene samples were no longer than 30 s. Experiments with seeded samples in sealed commercial sterilization bags showed little or no differences in exposure times compared to unwrapped samples. Plasma exposure times of less than 5 min generated $\geq$5 $\log_{10}$ CFU reduction of commercially prepared *Bacillus subtilis* spores (1×10^6); 7 min OAUGDP exposures were required to generate a $\geq$3 $\log_{10}$ CFU reduction for *Bacillus stearothermophilus* spores. For all microorganisms tested, a biphasic curve was generated when the number of survivors *vs* time was plotted in dose-response cures. Several proposed mechanisms of killing at room temperature by the OAUGDP are discussed.

Keywords: One Atmosphere Uniform Glow Discharge Plasma; sterilization; *Staphylococcus aureus*; *Escherichia coli*; bacterial spores

Introduction

Sterilization is the process of killing or removing microorganisms, including vegetative cells, spores and viruses, from an item or material of interest. Common sterilization methods include dry heat, moist heat (boiling and autoclaving), filtration, radiation, and chemical means such as gaseous ethylene oxide (EtO) sterilization. The method of sterilization used depends in large part on the materials involved and factors of safety, handling or disposal requirements, and expense. Time requirements also impose an important limitation on the choice of sterilization methods used in certain applications. To address the need for a broad application and relatively inexpensive method of reducing log number of cells, a new process at room temperature using a One Atmosphere Uniform Glow Discharge Plasma (OAUGDP) has been developed.

Partially ionized glow discharges in air, whether under vacuum conditions below one torr or at one atmosphere, produce active species that are potential sterilizing agents. These active species include strong oxidizing agents such as atomic oxygen and ozone, as well as ultraviolet photons, energetic ions, and energetic electrons, which can break molecular bonds and denature organic proteins. Recent work by Korzec *et al* [6] has shown that low pressure (circa 0.5 torr) RF oxygen glow discharge plasmas can oxidize and remove layers of hydrocarbon oils several microns thick in exposure times of only a few minutes. These results suggest a potential killing mechanism for microorganisms, which are approximately of the same dimensions as the hydrocarbon film thickness removed by plasma exposure in the experiments of Korzec *et al* [6]. Glow discharge plasmas operated in vacuum below one torr were used to sterilize test samples as early as the 1960s [9], and more recently, some of these techniques have been commercially employed. Although introduction of vacuum plasmas can be viewed as a significant improvement over existing systems, limitations of vacuum plasma sterilization include the need for batch processing, time requirements, and the expense associated with vacuum systems.

Glow discharge plasmas were operated at one atmosphere in hydrogen and in air in 1933 [15], but they had to be initiated at low pressures and raised to atmospheric pressure gradually. These discharges were too unstable with respect to the glow-to-arc transition at one atmosphere for industrial use. More recently, filamentary dielectric barrier discharges have been operated at one atmosphere in argon, and argon with an admixture of acetone [4,5], but these plasmas require special gases, are non-uniform, and their filaments tend to burn holes in fabrics and thin films.

Correspondence: K Kelly-Wintenberg or TC Montie, Department of Microbiology, University of Tennessee, Knoxville, TN 37996, USA
Received 6 June 1997; accepted 1 November 1997

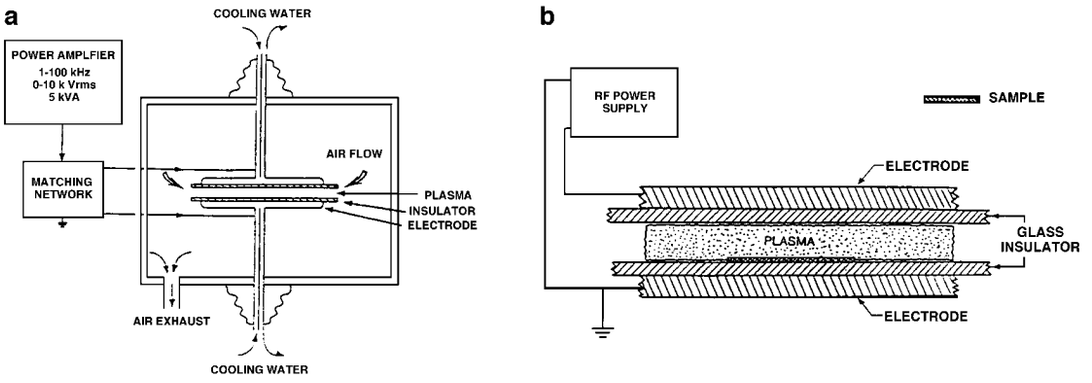

Figure 1 (a) Schematic of the One Atmosphere Uniform Glow Discharge Plasma reactor. Dimensions of the reactor: $40 \times 35 \times 35$ cm, width, height, and depth respectively. (b) Schematic of the plane parallel plasma sterilization configuration.

The One Atmosphere Uniform Glow Discharge Plasma (OAUGDP) recently developed at the UTK Plasma Science Laboratory [12] can produce a uniform, steady state glow discharge plasma in air and other gases at one atmosphere of pressure, as the result of the proprietary (to the University of Tennessee Research Corporation [13,14]), ion trapping mechanism [10]. The OAUGDP operating in air has an electron number density of approximately 10^{10} electrons per cubic centimeter, and produces a variety of oxidizing species which include ozone and nitrogen oxides. In this study we report on the effectiveness of OAUGDP as a sterilization process, and suggest possible mechanisms of killing generated during plasma exposure.

Materials and methods

The OAUGDP reactor

A schematic drawing of the MOD IV OAUGDP reactor at the UTK Plasma Science Laboratory is shown in Figure 1. For sterilization research, ordinary atmospheric air was normally used as the working gas. The reactor was made of stainless steel, and had interior dimensions of $40 \times 35 \times 35$ cm in width, height, and depth, respectively. One or both of the water-cooled electrodes were covered with a glass or plastic insulator to inhibit arcing. A characteristic pair of rectangular copper electrodes had dimensions of 18×15 cm. The RF power supply consisted of a power amplifier which works over the range 1–100 kHz and at RMS voltages up to 6.0 kV. Characteristic operating conditions during sterilization runs are listed in Table 1.

Table 1 Characteristic operating and plasma parameters for the OAUGDP process

RF frequency:	1~8 kHz
RMS voltage:	2~6 kV
RMS power (reactive):	10~150 Watts
RMS electric field:	10 kV cm^{-1}
Approximate electron number density	10^{10} cm^{-3}
Plasma power density:	tens of milliwatts cm^{-2}
Electrode gap separation:	5 mm or less
Working gas used:	air
Pressure:	760 + 15, –5 torr
Treatment time:	5 s to 7 min

Atmospheric air plasmas were used for all but a few experimental runs with helium, which were designed initially to assess the antimicrobial effect of any active species present.

All samples were laid flat and taped down on the surface of the insulator covering the bottom electrode. Airflow was maintained to promote plasma uniformity, and the samples were exposed for defined times. For a given gap separation, the RF frequency and RMS voltage were adjusted to produce an optimum uniform glow discharge plasma, where the oscillating electric field traps ions, but not electrons between the electrodes. When electrons are also trapped, the plasma polarizes, and filamentation instability develops.

Microorganisms and culturing conditions

Escherichia coli K-12 was maintained on LB (Luria-Bertani) agar plates or slants and *Staphylococcus aureus* was maintained on tryptic soy agar (TSA) plates or slants. *Bacillus stearothermophilus* spores were obtained from Fisher Scientific (Atlanta, GA, USA) and *Bacillus subtilis* spores were purchased from NAmSA (Northwood, OH, USA). The spores were germinated on standard methods media (SMA). Chemical reagents and culture media were obtained from Fisher Scientific. All described experiments were done in duplicate and plating was done at least in duplicate unless otherwise noted. The values represented in the dose-response curves were an average of two experiments performed separately.

OAUGDP exposure of bacteria on Whatman filters

To prepare seeded filter paper for OAUGDP exposure, exponentially growing *S. aureus* cells were spread on tryptic soy agar (TSA) plates or exponentially growing *E. coli* cells were spread on LB plates. Sterile filter paper was immediately pressed onto the surface of the plate, removed, and placed in sterile petri plates until plasma exposure. The seeded filters (2×10^2 cells) were exposed to the plasma cell side up for 0, 5, 15, 30, 60, or 120 s with operating conditions of 4 kV and 5.5 kHz. Following exposure, the filters were incubated cell side up on sterile TSA plates at 37°C for 18–36 h. The numbers of colonies growing on the surface of the plasma-exposed filter paper were compared to non-exposed controls, and a dose-response kill curve was made. For all experiments described, control plates were strategically placed in the plasma reactor to determine if

bacterial cells were being removed from the test material by air circulation in the plasma reactor. In every instance, no colonies were detected on these control plates.

Bacteria embedded in tryptic soy agar

Exponential *S. aureus* cells were diluted and mixed with sterile tryptic soy broth containing 2% agar. Thin agar slabs (3–5 mm) containing 2×10^2 cells were exposed to OAUGDP for 60 s under reactor operative conditions at 4.5 kV and 7 kHz. After exposure the slabs were incubated at 37°C for 18–36 h and the number of survivors was determined. To test the effects plasma had on the capacity of nutrient agar to sustain bacterial growth, agar slabs without cells were exposed to air plasma as described above. Approximately 2×10^2 *S. aureus* cells were spread onto the surface of exposed agar. After incubation at 37°C, the plates were examined for growth and the colonies were enumerated.

Bacteria on polypropylene melt blown webs

One ounce polypropylene (PP) melt blown webs (fabric) were designed and fabricated on the UTK campus in collaboration with the Textiles and Non-Woven Development Center (TANDEC). At least 2 weeks prior to use, the PP samples were ethylene oxide sterilized. No residual ethylene oxide was present on the fabric judged by the presence of the predicted number of viable cells on the sterile non-exposed (control) fabrics. To examine the antimicrobial effects on the fabric generated by plasma exposure, one ounce PP samples were seeded with 5×10^3 to 5×10^7 mid-log *S. aureus* or *E. coli* cells and exposed to the OAUGDP under operative conditions ranging from 4.5–5.5 kV and 5.0–7.0 kHz. The time exposures were 0, 5, 15, 30, or 60 s. Cells seeded on exposed PP samples and non-exposed control PP samples were removed by washing in osmotically stable phosphate-buffered saline (14 mM NaH_2PO_4, 36 mM Na_4HPO_4, with 0.05% Triton X-100) for 5 min. The cells were serially diluted and plated on TSA plates, and incubated for 18–36 h at 37°C. After incubation, the number of survivors was determined by colony counts from non-exposed and exposed PP samples, and data were plotted as a dose-response kill curve.

Bacteria on polypropylene melt blown webs in semi-permeable bags

Fabrics seeded with 5×10^3 to 1×10^7 *S. aureus* cells were placed in conventional medical sterilization bags. The bag was expressed at one atmosphere and heat-sealed before plasma exposure. Fabrics were exposed to the air plasma for 0, 5, 15, 30, or 60 s under operating conditions of 5 kV and 5.5 kHz. After exposure the samples were washed, serially diluted and plated on TSA as described above. The numbers of survivors from exposed fabrics enclosed in the bags were compared to both open exposed fabrics and control non-exposed fabrics by colony counts. The data were plotted as a dose-response curve. A related experiment involved placing the seeded fabrics in the bags, and replacing the O_2 in the bag with helium to assess the role of toxic O_2 species generated within the plasma.

Preparation and exposure of bacterial endospores

Prepared *B. stearothermophilus* endospores (4×10^4) were filtered onto a Millipore HA membrane, and the filters were exposed to plasma for various times, measured in minutes. The filters were removed and placed on a TSA plate and incubated at 55°C for 18–36 h. Any viable spores that germinated were recorded by counting colonies on the surface of the filter. *B subtilis* spores (1×10^6) were commercially prepared on paper strips. After plasma exposure, the spores were recovered by macerating the strips for 2 min in sterile phosphate-buffered water (0.4% NA_2HPO_4, 0.05% Tween 80, pH 7.0) using a Stomacher® laboratory blender as described in a NAmSA technical protocol [8]. The recovered spores were serially diluted and plated on SMA. After incubating 18–36 h at 37°C the number of survivors was determined by colony counts.

Results

OAUGDP exposure of bacterial-seeded Whatman filter paper

Initial experiments examined the antimicrobial effects of OAUGDP exposure on bacterial seeded Whatman filter paper (2×10^2 cells). The results in Figure 2 showed that within 15 s of plasma exposure, 80% (1.6×10^2 cells) of *S. aureus* were killed and within a 30 s plasma exposure no viable colonies were detected on the filters after incubation on TSA plates for 36 h. Identical experiments were performed with *E. coli*, and no colonies grew on the filters after a 15 s plasma exposure, while 90% of the cells (1.8×10^2) were rendered nonviable after a 5 s exposure (Figure 2).

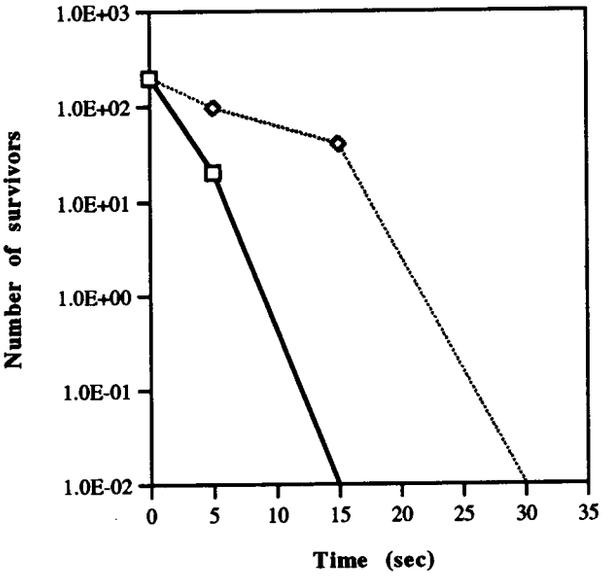

Figure 2 OAUGDP exposure of bacteria seeded on filter paper (2×10^2 cells filter^{-1}). Bacteria were seeded on filter paper and exposed to plasma for defined times. The numbers of survivors, as determined by plate counts, were plotted logarithmically *vs* time. The D_1 value was calculated from the slope of the data accumulated from 0–5 s for *E. coli*, and the D_2 value from the 5–30 s data. For *S. aureus*, the D_1 value was calculated from the slope of the data collected from 0–15 s, while the D_1 value was calculated from the 15–30 s data. *Escherichia coli* cells (□), *Staphylococcus aureus* cells (◇).

After a 5 s OAUGDP exposure, the majority of the remaining viable *E. coli* colonies had a distinct alteration in morphology and grew more slowly than non-exposed cells. These observations suggested that the surviving cells were mutants or injured by exposure to the plasma.

Based upon these data, the dose-response curve for *E. coli* and *S. aureus* was a biphasic plot (Figure 2). The D value (D_1) for the first phase of the *E. coli* dose-response curve was 7 s, and based upon the data it was not clear that the second mechanism was exponential, however, an approximate D value (D_2) for the second phase was 2 s. The D value (D_1) for the first phase of the dose-response curve of *S. aureus* was calculated to be 30 s, while the D value (D_2) for the second phase was calculated to be 2 s (Figure 2).

A possible explanation for differences in exposure times necessary for the antimicrobial effects of plasma on *S. aureus* and *E. coli* cells was the innate differences in the lipid configuration of Gram-positive and Gram-negative cell envelopes. One possible mechanism of bacterial cell destruction by OAUGDP was oxidation of the lipid moieties of the cell that may initiate membrane alteration.

When filter paper seeded with *S. aureus* or *E. coli* cells (2×10^2 cells) were placed cell side down in the plasma reactor, no increase in exposure times was required for killing. These results indicated that the active species generated by the plasma were penetrating through the filter paper to kill the bacterial cells.

OAUGDP-treated solid growth medium

To determine if the plasma active species could penetrate porous materials, thin agar slabs (3–5 mm) containing 2×10^2 *S. aureus* cells were exposed to OAUGDP for 60 s then incubated at 37°C. After 36 h of incubation, no colonies were seen in the agar. More recent experiments with 10^7 *E. coli* cells embedded in TSA slabs required no more than 3 min exposure times for a ≥ 6 $\log_{10}$ CFU reduction. These data suggested that longer exposure times were required for the active species to penetrate the porous medium to generate killing compared to filter paper and polypropylene fabric. Similar experiments with TSA slabs sterilized by the OAUGDP process for 60 s were subsequently able to support normal cell growth of *S. aureus*. These data suggested that organic nutrients in the plasma exposed growth medium was not altered or damaged in such a way as to limit or inhibit growth of microorganisms.

OAUGDP experiments with polypropylene melt blown webs

Since polypropylene (PP) is an important material in the medical industry, bacterial seeded one-ounce PP fabrics and plasma exposure were investigated. Experimental results showed that in 30 s or less, OAUGDP treatment of PP samples seeded with 5×10^4 *S. aureus* cells or *E. coli* cells routinely produced a ≥ 3 $\log_{10}$ CFU reduction (Figure 3). Recently, 10^7 mid-log *S. aureus* cells or *E. coli* cells seeded on one-ounce PP samples were exposed. Subsequent results from these experiments showed a ≥ 6 $\log_{10}$ CFU reduction on fabrics following plasma exposures of 30 and 60 s (Table 2). Experiments are currently underway to generate

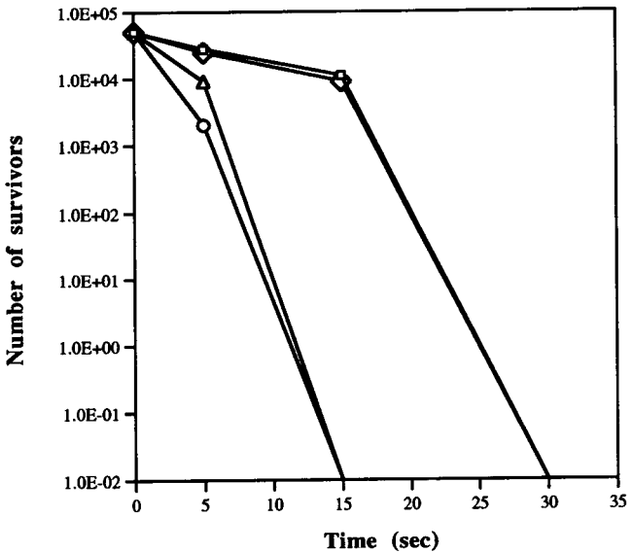

Figure 3 OAUGDP treatment of *S. aureus* or *E. coli* cells seeded on polypropylene samples exposed in air or in semi-permeable bags. *S. aureus* or *E. coli* cells (5.0×10^4) were seeded on polypropylene and exposed to plasma for various times. The number of survivors, as determined by plate counts, was plotted logarithmically *vs* time (s). For *S. aureus*, the D_1 value was calculated from the slope of the data accumulated from 0–15 s, and the D_2 value from the 15–30 s data. For *E. coli* cells there was a slight difference in the D values when the cells were exposed in air or exposed in bags. The D_1 value was calculated from the slope of the data accumulated from 0–6 s, and the D_2 value from the 6–30 s data. *S. aureus* cells (◇), *S. aureus* cells exposed in bags (□), *E. coli* cells (O), *E. coli* cells exposed in bags (△).

Table 2 Comparison of OAUGDP exposed *S. aureus* and *E. coli* cells (10^7) on polypropylene fabric packaged in semi-permeable bags or air exposed[a]

Exposure conditions	Organism 1×10^7	Plasma exposure time (s)	Number of survivors (% kill)[b]
Unexposed	*S. aureus*	0	1×10^7 (0%)
Air exposed – without bags	*S. aureus*	30	$\leq 1 \times 10^1$ (100%)
Exposed in semi-permeable bags	*S. aureus*	30	$\leq 1 \times 10^1$ (100%)
Unexposed	*E. coli*	0	1×10^7 (0%)
Air exposed	*E. coli*	30	$\leq 1 \times 10^1$ (100%)
Exposed in semi-permeable bags	*E. coli*	30	$\leq 1 \times 10^1$ (100%)
Exposed in semi-permeable bags in helium	*S. aureus*	60	4×10^6 (60%)

[a]Actively growing cells were transferred to one ounce polypropylene fabric as described in Materials and Methods, and exposed at plasma conditions of 5.0 kV and 5.5 kHz.
[b]The number of survivors was determined by colony counts. Plating techniques allowed enumeration of survivors to 10^1. In these experiments duplicate experiments were done and for each experiment duplicate plates were done. The number of survivors reflects an average of the duplicate plates and duplicate experiments.

additional data for dose-response curves with 10^7 number of cells.

OAUGDP experiments with packaged polypropylene (PP) melt blown webs

To determine if the OAUGDP technology is applicable for packaged materials, bacterial seeded PP samples of *S. aureus* or *E. coli* (5×10^4 cells) were placed in conventional heat-sealed medical sterilization bags and exposed to plasma for 0, 5, 15, 30, or 60 s. Seeded PP samples enclosed in bags were compared to non-packaged, exposed filters and packaged and non-packaged control filters. The dose-response kill curves showed packaged PP samples seeded with *S. aureus* or *E. coli* cells (initially 5×10^4 cells) were reduced by ≥ 3 $\log_{10}$ within 30 s exposures (Figure 3). Within a 5 s exposure, more than 80% (4.1×10^4) of the *E. coli* cells were non-viable when samples were placed in bags, while 96% (4.8×10^4) of the cells were non-viable when the samples were air plasma exposed. A plasma exposure time of 15 s rendered approximately 80% of *S. aureus* cells non-viable when the samples were placed in packages, or left open for plasma exposure. In these experiments, killing appears to be as efficient or more efficient with samples placed in sealed commercial bags as compared to samples exposed openly. These experiments indicated the active species killing the bacterial cells are small enough to enter the porous sterilization bags, which are intended for use with ethylene oxide gas. The calculated D values from the dose-response curves (Figure 3) gave values very similar to earlier calculated values for *E. coli* and *S. aureus* (Figure 2). Data from the *S. aureus* dose-response curve generated a D_1 value of 30 s and a D_2 value of 2 s in both the non-bagged and bagged samples. The *E. coli* dose-response curve data generated a D_1 value of 7 s and a D_2 value of 2 s in both the samples tested. OAUGDP exposure experiments with *E. coli* (10^7) cells generated the same D_2 value when data points were collected within the second phase of the dose-response graph (data not shown). These are preliminary D values, more data points will be necessary to determine the D values more precisely. However, the consistency of the data taken together reinforces the D value validity.

Experiments were performed with larger, initial numbers of bacteria seeded onto one-ounce PP filters. Exposed *S. aureus* or *E. coli* seeded filters (10^7) enclosed in bags were compared to non-packaged, exposed filters and control non-exposed filters. The results showed a $\geq 10^6$ $\log_{10}$ reduction within a 30 s exposure time of the packaged and air-exposed seeded filters (Table 2). It appeared that the plasma exposure times necessary to kill the microorganisms were not dependent upon cell number since the exposure time necessary to kill 2×10^2 cells was about the same as the time to kill 10^7 cells.

For comparison, PP samples were seeded with *S. aureus* cells (1×10^7), and placed in conventional sterilization bags. The air in the bags was flushed out and refilled with helium. When the OAUGDP was generated with helium a reduction in antimicrobial effectiveness was seen. When the exposure time of the seeded PP samples in helium was doubled to 60 s, the number of survivors was 4×10^6. Only a 60% reduction in cell number was obtained compared to 100% killing in 30 s when air was used to generate the plasma (Table 2).

OAUGDP experiments with bacterial endospores

An important criterion of any sterilization process is its effectiveness in killing bacterial endospores, since they are considered one of the most resistant cells to any lethal agent. Experimental data showed within 7 min of exposure that the OAUGDP process was capable of generating ≥ 3 $\log_{10}$ reduction of *B. stearothermophilus* spores (4×10^4) on HA filters. In experiments using *B. subtilis* var *niger* spores on commercially prepared strips (1×10^6), exposure times of less than 5 min were required to generate ≥ 5 $\log_{10}$ reduction in CFU after germination on standard method agar. Currently, dose-response kill curves for *B. stearothermophilus*, *B. subtilis* var *niger*, and *B. pumilus* are being completed.

Discussion

With this new technology, plasma is generated under ambient conditions, in air at standard pressure and temperature. The OAUGDP process kills microorganisms, including spores, at room temperature by a relatively simple, safe, and fast process. The OAUGDP process offers several advantages. Namely, this technology does not require batch processing, does not expose materials to high temperature and pressure like steam sterilization or use strong chemicals and potentially toxic gases such as ethylene oxide or use high doses of radiation. Nor does the OAUGDP appear to degrade materials tested, eg polypropylene used in many medical products is degraded by gamma radiation, which generates radicals on methyl side groups [11]. Another major advantage of the OAUGDP is that it kills vegetative cells and spores in seconds to minutes.

Two low-pressure plasma sterilizers are commercially available [3]; one is based on hydrogen peroxide as a working gas, and the other on paracetic acid, rather than air. These units can sterilize materials in a period of from 1–2 h at pressures below about one torr. These plasma sterilization units are an improvement over autoclaving and ethylene oxide for some sterilization needs, but require batch processing in a vacuum system.

The OAUGDP unit is composed of a RF power supply and a pair of parallel plate electrodes between which a uniform glow discharge plasma is generated in a volume of many liters, using gases such as helium, carbon dioxide, and most importantly, air (Figure 1). Sterilization of surfaces is a result of the interaction of one or more active species from the plasma with the contaminated surface of interest. These active species are more chemically reactive and more energetic than the species associated with conventional chemical processing. The active species may include: ultraviolet photons, which are capable of breaking chemical bonds; charged particles, which may be produced by ionization events or charge exchange in the plasma; and free radicals or other charged molecular fragments such as OH resulting from plasma-chemical reactions. Neutral particles are another group of active species present in the plasma, which can include very reactive atoms such as monoatomic oxygen, or other atomic species [10]. The

experiments in which wrapped samples were exposed to the plasma suggest that UV is not a significant contribution to lethality since the bag would have blocked much of the UV. A study of the plasma effects on microorganisms is complex and probably multi-factorial. The dose-response curves of *E. coli* and *S. aureus* (Figures 2 and 3) showed biphasic plots. These curves indicate that an initial slow exponential killing curve occurs, which varies with the microorganism, followed by a second discrete phase exhibiting a much more rapid killing response. This shift in response profile suggests that a second killing mechanism has become dominant. The first phase of killing is slower than the second phase as reflected by longer D_1 values of 7 s for *E. coli* and approximately 30 s for *S. aureus* cells. Based upon the data presented in this paper, it cannot be clearly concluded that the second mechanism is exponential, however the D_2 value calculated was 2 s for either *E. coli* or *S aureus* cells. More recent plots made from additional data phase two data points from tests with several bacteria have confirmed that the same biphasic curve was generated. Thus the first phase is longer (larger D_1 values), while the second phase is much shorter with an approximate D_2 value of 2 s for each microorganism tested (data not shown).

We hypothesize that during the first killing phase, toxic active species are concentrating and generating alterations at the membrane level. Hence the differences in D_1 values of different organisms reflect the differences in surface and membrane structure coinciding with varied resistance to these active species. Once the concentration of active species is sufficient for lethality the second phase is very rapid resulting in irreversible damage and lysis of the cells. This is reflected in the extremely short D_2 value of 2 s. There are several possible sites on, and within the cell, which could react with the plasma, ultimately leading to cell death. Although oxygen is a requirement for respiratory processes of cells, any excess oxygen becomes a toxic compound to them. This occurs as a result of diatomic oxygen becoming partially reduced to produce highly reactive free radicals (superoxide, O_2^-, hydroxyl, OH^-) and hydrogen peroxide [2]. Another more recently recognized oxidant is NO^-, a nitric oxide radical. Ozone is another related toxicant that results from excessive oxidation [10]. Plasma generated in air is known to produce relatively high levels of these compounds, which can overwhelm the natural enzyme defenses of microorganisms. These defenses include destruction of DNA by oxygen adduct formation of nucleotide bases [7], protein oxidation imparting enzyme and structural damage, and lipid peroxidation resulting in membrane damage [1]. Although this might not be the only mechanism for killing, we noted that with samples exposed to plasma with helium gas instead of oxygen, killing is slower and less efficient.

Experimental work is necessary to define the plasma reactions and their effect on cell death. Preliminary electron micrographs of *E. coli* cells exposed to sub-lethal doses of plasma showed possible damage at the membrane level. Future experimental work in this area will involve scanning electron microscopy and membrane lipid analyses to determine specific lipid modification occurring during plasma exposure. It is important to determine the mechanism of killing of microorganisms generated by the OAUGDP. By understanding the mechanism of killing, modifications can be made to the operating parameters of the OAUGDP reactor for example, gas, voltage, and frequency to optimize the plasma exposure times.

Acknowledgements

This work was supported in part by the Center for Materials Processing and TANDEC, University of Tennessee. We are grateful for the assistance and support of Helen M Roth, for supplying sterilization bags, and information on current sterilization practice in hospitals. We also thank Kristie Tosh for technical work.

References

1 Bielski BHJ, RL Arudi and MW Sutherland. 1983. A study of the reactivity of perihydroxy radical/superoxide ion with unsaturated fatty acids. J Biol Chem 258: 4759–4761.
2 Fridovich I. 1995. Superoxide radicals and superoxide dismutases. Ann Rev Biochem 64: 97–112.
3 Jacobs P. 1994. A New Technology for Instrument Sterilization. Sterrad Sterilization System Technical Report.
4 Kanazawa S, M Kogoma, T Moriwaki and S Okazaki. 1988. Stable glow plasma at atmospheric pressure. J Physics D 21: 838–840.
5 Kanda N, M Kogoma, H Jinno, H Ychiyama and S Okzazki. 1991. Atmospheric pressure glow plasma and its application to surface treatment and film deposition, Paper 3.2-20. Proc 10th International Symposium on Plasma Chemistry. Vol 3, pp 3-2-201–204.
6 Korzec D, J Rapp, D Theirich and J Engemann. 1994. Cleaning of metal parts in oxygen radio frequency plasma: process study. J Vac Sci Technol A 12: 369–378.
7 Minton KW. 1994. DNA repair in the extremely radioresistant bacterium *Deinococcus radiodurans*. Microbiology 13: 9–15.
8 NAmSA Technical Protocol. 1992. Population Verification for SPORTROL® Biological Indicator Strips. NAmSA, Northwood, Ohio.
9 Ratner BD, A Chilkoti and GP Lopez. 1990. Plasma deposition and treatment for biomaterial applications. In: Plasma Deposition and Treatment and Etching of Polymers (d'Agostino R, ed), pp 464–476, Academic Press, Boston.
10 Roth JR. Industrial Plasma Engineering: Volume I, Principles. Institute of Physics Publishing, Bristol and Philadelphia, 1995.
11 Roth JR, Y Ku, PP Tsai, LC Wadsworth, CQ Sun, TC Montie and K Kelly-Wintenberg. 1996. A study of the sterilization of nonwoven webs using one atmosphere uniform glow discharge plasma. March'96 TAPPI Nonwovens Conference and Technology Exposition, Charlotte, NC.
12 Roth JR, C Liu and M Laroussi. 1992. Experimental generation of a steady-state glow discharge at atmosphere pressure, Paper 5p21. 19th IEEE International Conference on Plasma Science, Tampa, FL, June 1–3 1992.
13 Roth JR, PP Tsai and C Liu. 1995. Steady-state, glow discharge plasma. US Patent 5 387 842, February 7 1995.
14 Roth JR PP Tsai, C Liu, M Laroussi and PD Spence. 1995. One atmosphere, uniform glow discharge plasma. US patent 5 414 324, May 9 1995.
15 Von Engle A, R Seeliger and M Steenbeck. 1933. On the glow discharge at high pressure. Zeit Fuer Physik 85: 144–160.