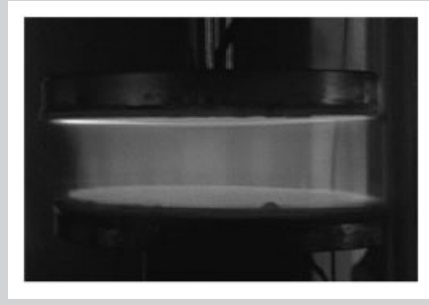
Summary: Low temperature, high pressure, non-equilibrium plasmas are now routinely used in several material processing applications, and in some cases are competing with low pressure plasmas in areas where these have historically been dominant. Etching and deposition are examples of such applications. Amongst the novel applications of non-equilibrium plasmas, biomedical applications such as electrosurgery, surface modification of biocompatible materials, and the sterilization of heat-sensitive medical tools are particularly interesting. In this paper, first a brief overview of recent research on reduced-pressure plasma-based sterilization/decontamination methods is given. Then a detailed review and discussion on the effects of atmospheric pressure non-equilibrium plasmas on the cells of bacteria is presented. This includes the evaluation of the inactivation kinetics and the roles of the various plasma agents in the inactivation process. Measurements of the plasma temperature, the UV emission, and concentrations of various reactive species for the case of air plasma are presented. Plasma sub-lethal effects are also briefly discussed, and the prospects of the use of ''cold'' plasmas in the biomedical field are outlined.



Low Temperature Plasma-Based Sterilization: Overview and State-of-the-Art

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I. Introduction

Non-equilibrium atmospheric pressure plasmas have recently been playing the role of an enabling technology in several applications such as UV and VUV sources,[1] surface treatment,[2] and the cleaning of flue gases.[3] Amongst the novel applications, the use of atmospheric pressure ''cold'' plasmas in the biomedical field is experiencing a heightened interest from both the plasma science research community and the biomedical research community. This is due to the relatively recent application of these plasmas in promising medical research such as wound healing,[4] tissue engineering,[5] surface modification of bio-compatible materials,[6] and the sterilization of reusable heat-sensitive medical instruments.[7] This paper first briefly reviews important recent work done under low-pressure conditions, then mostly focuses on the interaction of high-pressure, low temperature air plasmas with bacteria. The results of this line of research have direct implications in the areas of sterilization and decontamination of surfaces and media.

This paper is organized as follows: Section II presents a brief review of notable recent efforts on the inactivation of bacteria by low-pressure plasmas. Switching the focus to high-pressure plasmas, Section III briefly discusses the fundamental properties of non-equilibrium atmospheric pressure plasmas. In Section IV the effects of the various plasma-generated agents on the cells of bacteria will be presented. Section V delves on the germicidal properties and inactivation kinetics of atmospheric pressure plasmas. This is followed by an introductory section on the sub-lethal effects of plasmas, such as the alteration of the heterotrophic pathways of cells and the localized detachment of mammalian cells. Finally, in Section VII concluding remarks and the prospects of the use of plasmas in the biomedical field are outlined.

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II. Review of Recent Results of Low-Pressure Plasmas

Low-pressure plasmas have been considered for biological sterilization for some time. Some of the systems developed in the 1970s and 1980s were not really ''plasma-based'' sterilization systems. This is due to the use of gas mixtures that contain components with germicidal properties (such as H_2O_2 and aldehydes) before the plasma is ignited.[8,9] These are more correctly termed as ''plasma-assisted'' sterilization systems. Plasma-based sterilization uses gases that posses no germicidal property on their own. They become biocidal only when a plasma is ignited. Example of such gases or mixtures of gases are air, helium/air or helium/O_2, and N_2/O_2. In this section I will only review plasma-based systems. Most recently, many studies on the effects of low-pressure plasma on biological matter in plasma-based systems were conducted for various gas mixtures. Examples are low-pressure oxygen plasmas and O_2/N_2 plasmas.[10–12] These studies were motivated by specific objectives such as the decontamination of interplanetary space probes and the sterilization of medical tools. RF and microwave driven low-pressure plasmas were mostly used in these studies.

Recently Bol'shakov et al. published a detailed study of the effects of radio frequency (RF) oxygen plasma at reduced pressure on bacteria.[10] The study was carried out for two modes of operation, the inductively coupled mode and the capacitively coupled mode. The inductive mode was found to offer a better efficiency in destroying biological matter. This was due to higher electron and ion densities in this mode, which resulted in an enhancement of electron-impact processes.[10] High densities of atomic oxygen and perhaps O_2^* in synergy with UV photons induced chemical degradation of the biological materials followed by volatilization of the decomposition products (CO_2, CO, N_2,. . .). Plasmid DNA degradation was evaluated for both the inductive mode and the capacitive mode. It was found that at the same power the inductively coupled plasma destroyed over 70% of supercoiled DNA in 5 s while only 50% was destroyed by the capacitively coupled plasma.[10] Characterization of the decomposition of the byproducts was carried out during plasma exposure by emission spectroscopy. CO, N_2, N_2^+, OH, Na, K,. . .were amongst the detected species. CN, CH, and NO were not detected indicating that their concentrations were below 1 ppm.[10]

Moreau et al.[11] and Moisan et al.[12] carried out detailed studies on the effects of low pressure N_2/O_2 plasma on various bacteria. The biological samples were placed in the flowing after-glow of a plasma generated by a surfatron source.[13] The surfatron source was driven by microwave power with a frequency of 915 or 2 450 MHz. In their studies Moisan et al.[12] characterized the inactivation kinetics and correlated that with the physics and chemistry of the afterglow. Since this study was focused on the inactivation kinetics, I would like to briefly introduce the reader to some basic definitions:

One kinetics measurement parameter, which has been used extensively by researchers studying sterilization by plasma, is what is referred to as the ''D'' value (Decimal value). The D-value is the time required to reduce an original concentration of microorganisms by 90%. The D-value is therefore expressed in the unit of time. Since survivor curves are plotted on semi-logarithmic scales, the D-value is determined as the time for a one $\log_{10}$ reduction.

In their early studies on the inactivation of *Bacillus subtilis* spores by low-pressure plasmas, Moisan et al.[12] reported that survivor curves (colony forming units, CFUs, versus treatment time) exhibited three inactivation phases. Figure 1 shows a typical example of such survivor curves.[14] Moisan et al. claimed that the first phase, which exhibited the shortest D-value, was mainly due to the action of UV radiation on isolated spores or on the first layer of stacked spores. The second phase, which had the slowest kinetics, was attributed to a slow erosion process by active species (such as atomic oxygen, O). Finally the third phase was initiated after spores and debris had been cleared during phase 2, hence allowing UV to hit the genetic material of the

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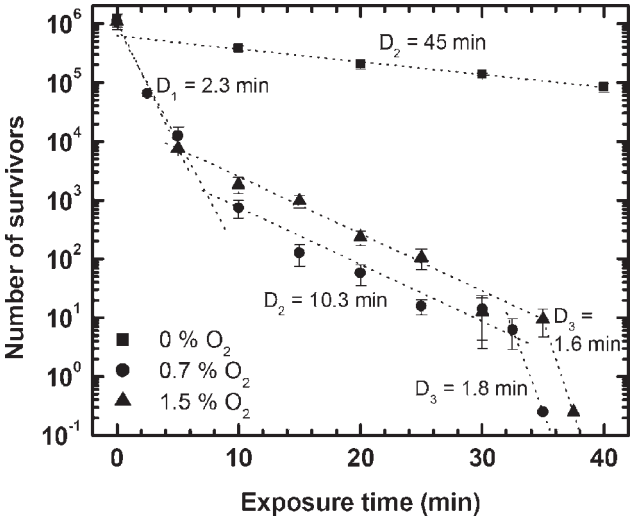

Figure 1. Survival curves for *B. subtilis* spores exposed to a N_2/O_2 discharge afterglow. Gas pressure in the sterilization chamber is 5 torr. Gas flow is one standard l/min plus *x*% of added O_2 and discharge field frequency is 2 450 MHz.[14,15]

still living spores. The D-value of this phase was observed to be close to the D-value of the first phase. However, in a more recent study, the same research group examined the inactivation process of *B. subtilis* spores exposed to the flowing afterglows of an N_2/O_2 mixture and of pure argon, and reported that UV radiation, not the radicals, played the dominant role.[15] The survivor curves were biphasic and consistent with UV inactivation. The second phase represented spores that were shielded by others and that needed more irradiation time to accumulate a lethal UV dose. This observation was further supported by the fact that at low UV intensity a lag time existed before inactivation. This was due to the requirement that a minimum UV dose had to be achieved before irreversible damage to the DNA strands occurred.[15] Since in pure argon, which would not contain oxygen radicals, inactivation was achieved for similar lengths of time, it was concluded that the role of oxygen in the N_2/O_2 plasma was mainly to provide oxygen atoms to form NO,[15] which was the main source of the UV photons.

III. Non-Equilibrium Atmospheric Pressure Plasmas

In addition to their practical side, design simplicity, and low operational cost, non-equilibrium atmospheric pressure plasmas exhibit unique features, which have provided the base for numerous applications. In these plasmas the electron energies are much higher than that of the heavy species, i.e. ions and neutrals. The energetic electrons collide with the background atoms and molecules causing enhanced level of dissociation, excitation and ionization. The attractive feature is the fact that these reactions occur without a substantial increase in the operating gas temperature. Because the ions and the neutrals remain relatively cold, the plasma causes no or just minimal thermal damage to articles it may come in contact with. This characteristic opened up the possibility to use these plasmas for the treatment of heat-sensitive materials including biological tissues. Several methods have been developed in the last two decades that allowed researchers to easily generate non-equilibrium plasmas at ''high-pressures'', up to one atmosphere.[16,17] One requirement is common to all the methods: the inhibition of the glow-to-arc transition. Dielectric barrier discharges (DBDs) achieve this requirement by covering at least one of two electrodes (separated by a gas gap) with a dielectric sheet.[18] In this case, the discharge current is self-limited by charge build-up on the dielectric surface. DBDs are generally driven by sine-wave high voltages with frequencies in the kHz range. This frequency range has recently been extended all the way down to DC by replacing the dielectric barrier by a resistive layer, which plays the role of a distributed ballast, thus limiting the discharge current.[19] Plasma discharges with metallic bare electrodes driven by RF voltages in the MHz range were also developed.[20] In this case a gas composed mainly of a noble gas (such as He) is flown at a rate of few liters/min in the gap between the electrodes. DC-driven microdischarges, such as the microhollow cathode discharge,[21] can also generate high-pressure non-equilibrium plasmas by reducing the diameter of a hollow cathode to the sub-millimeter range. Millimeter-size plasmas that can be used in microsurgery are well established.[22] A version allowing the generation of cold plasma uses a needle electrode driven by relatively low voltage RF sources (13.56 MHz).[23,24]

IV. Plasma Inactivation Agents

Biological materials can be exposed to plasma in two different methods: ''Direct exposure'' is when the sample to be treated is in direct contact with the plasma. All plasma-generated agents, including charged particles, come in contact with the sample. The second method is ''remote exposure''. In this case the sample is placed at a distance from the plasma volume or in an adjacent chamber. In this configuration, the amount of heat transmitted to the sample is reduced, the charged particles do not play a role since they recombine before reaching the sample, and many of the short-lived neutral reactive species also do not reach the sample. In the following section the contribution of the four main inactivation factors of a non-equilibrium high-pressure air plasma are reviewed.

Heat

Heat-based sterilization methods use either moist heat or dry heat. In the case of moist heat, such as in an autoclave, a

temperature of 121 °C at a pressure of 15 psi is used.[25] Dry heat sterilization requires temperatures close to 170 °C and treatment times of about 1 h.[25]

To assess the inactivation role of heat from a high pressure non-equilibrium air plasma, Laroussi and Leipold[26] determined the gas temperature in the discharge by comparing experimentally measured rotational bands structure of the 0–0 transition of the 2nd positive system of nitrogen with simulated spectra at different temperatures. Using a thermocouple probe, they also measured the temperature in a sample, placed 2 cm away from the discharge.

Figure 2 shows the measured and calculated rotational bands of the 0–0 transition of the 2nd positive system of N_2, for a power of 10 W. It indicates that the gas temperature remains close to room temperature. A variation in power from 2 W to 15 W showed no variation in the temperature. An air flow rate of 10 $l \cdot min^{-1}$ was used in these experiments. The gas temperature for various gas flow rates at a power consumption of 10 W was also investigated. The results are shown in Figure 3. Increasing the airflow causes the gas temperature to approach room temperature (300 K).

Figure 4 shows the increase in the temperature of the biological sample under treatment for various dissipated power levels, as measured by a thermocouple. A maximum increase of 21 K was observed. Therefore, based on these measurements no substantial thermal effects on bacterial cells are expected.

UV Radiation

UV radiation in the 200–300 nm wavelength range with doses of several $mW \cdot s \cdot cm^{-2}$ are known to cause lethal damage to cells. Amongst UV effects on cells of bacteria is the dimerisation of thymine bases in their DNA strands. This inhibits the ability of the bacteria to replicate properly.[27]

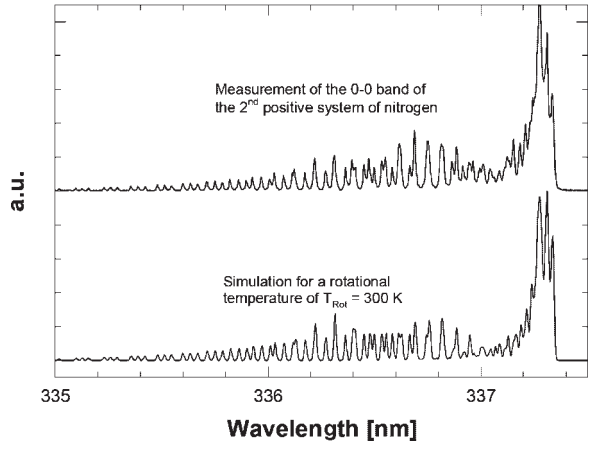

Figure 2. Measured and calculated rotational bands of the 0–0 transition of the second positive system of nitrogen. The spectra are intentionally shifted vertically for better comparison.[26]

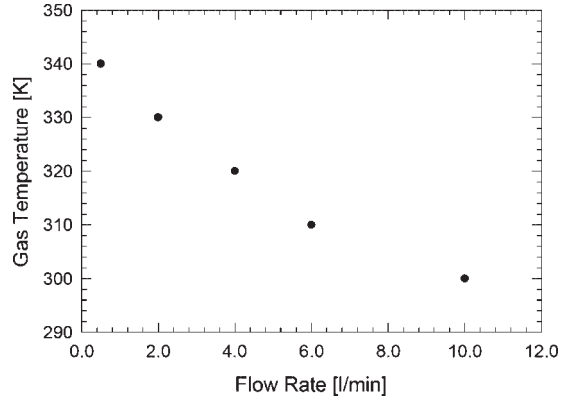

Figure 3. Gas temperature versus gas flow rate for a power of 10 W.[26]

To quantify the UV contribution to the inactivation process of non-equilibrium air plasma, spectroscopic and absolute power measurements were conducted by Laroussi and Leipold.[26] They reported that no significant UV emission occurs below 285 nm. This is illustrated in Figure 5. Power measurements with a calibrated UV detector in the 200–300 nm wavelength region revealed that the power density of the emitted UV radiation is below 50 $\mu W \cdot cm^{-2}$. At this power level the UV radiation does not play a significant direct role in the sterilization process by low temperature air plasmas.

Charged Particles

Mendis et al.[28] and Laroussi et al.[29] suggested that charged particles can play a very significant role in the rupture of the outer membrane of bacterial cells. They showed that the electrostatic force caused by charge accumulation on the outer surface of the cell membrane could overcome the tensile strength of the membrane and cause its rupture.

When charged, a body of the size of a bacterial cell (in the μm range) experiences an outward electrostatic force due to

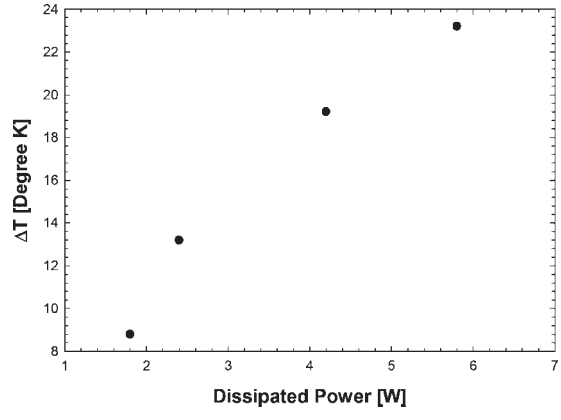

Figure 4. Increase of sample temperature versus plasma dissipated power.[26]

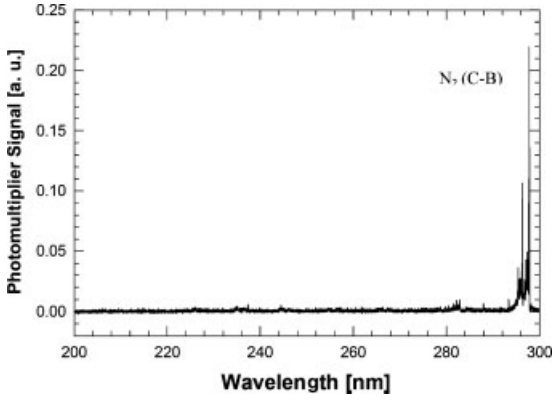

Figure 5. UV spectrum of a DBD in air in the 200–300 nm wavelength range.[26]

each charge being subjected to the repulsive forces of all the similar charges accumulated on the cell surface. This force is proportional to the square of the charging potential, Φ, and inversely proportional to the square of the radius of curvature of the surface, *r*. Therefore, the smaller the radius of curvature the stronger the electrostatic force. The charging potential Φ depends on the ratio of the ion mass to the electron mass. So gases with larger atomic mass lead to higher electrostatic forces. Mendis et al.[28] derived the condition for membrane disruption as:

$$|\Phi| > 0.2 \cdot (r \cdot \Delta)^{1/2} \cdot F_t^{1/2}$$

where r is the radius of curvature, Δ is the thickness of the membrane, and F_t its tensile strength.

The scenario described above is more likely to occur for gram-negative bacteria, the membrane of which possesses an irregular surface. These irregularities offer small radii of curvatures that cause localized high outward electrostatic forces. This conclusion was supported by experimental results obtained by direct exposure experiments carried out by Laroussi et al.[29] Figure 6 shows gross morphological damage to the cells of *Escherichia coli*, a gram-negative bacterium. The localized occurrence of openings or ''pores'' in the membrane due to the charging effect can also potentially be used towards other ends such as the introduction of macromolecules into cells (especially eukaryotic cells). However, this interesting possibility remains to be investigated.

Reactive Species

In high-pressure non-equilibrium plasma discharges, reactive species are generated through various collisional pathways, such as electron impact excitation and dissociation. Reactive species play an important role in all plasma-surface interactions. Air plasmas, for example, are excellent sources of reactive oxygen-based and nitrogen-based species, such as O, O_2^*, O_3, $OH^\bullet$, NO, NO_2, etc.

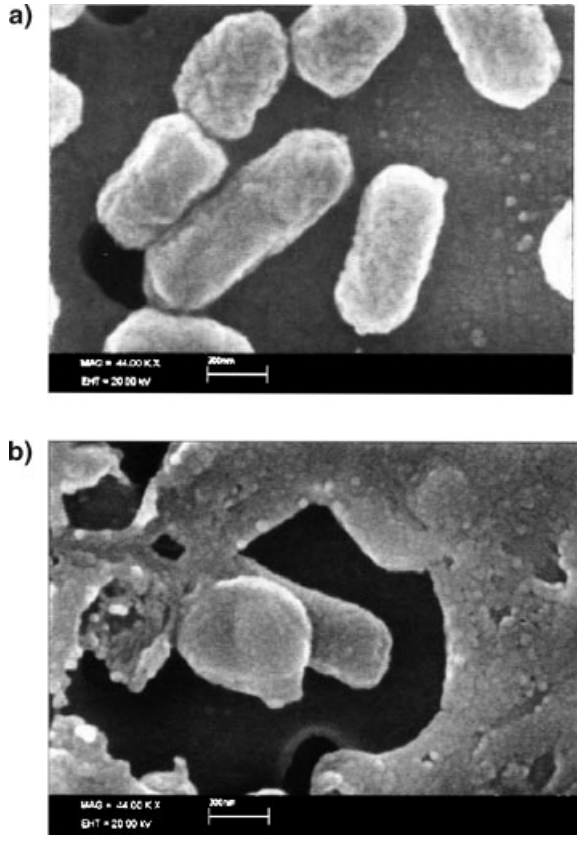

Figure 6. SEM micrographs of *E. coli* (a) control, (b) after exposure to plasma, showing gross morphological damage.[39]

Laroussi and Leipold[26] carried out measurements of the concentrations of hydroxyl and nitrogen dioxide produced by a DBD operated in air, at atmospheric pressure. The presence of $OH^\bullet$ was measured by means of emission spectroscopy, looking for the rotational band of $OH^\bullet$ A-X (0–0) transition. Figure 7 shows the relative concentration of $OH^\bullet$ in the discharge as a function of the air flow rate and dissipated power, assuming that the rotational band intensity represents the $OH^\bullet$ concentration. Nitrogen dioxide was measured as a function of the air flow rate and for different power levels by a calibrated gas detecting system and the results are shown in Figure 8. The ozone concentration was measured for varying flow rates and at various power levels by a chemical titration method by Minayeva and Laroussi.[30] The results are shown in Figure 9. The germicidal effects of Ozone are caused by its interference with cellular respiration.

Oxygen-based and nitrogen-based reactive species have strong oxidative effects on the outer structures of cells. Cell membranes are made of lipid bilayers, an important component of which is unsaturated fatty acids. The unsaturated fatty acids give the membrane a gel-like nature. This allows the transport of the biochemical by-products across the membrane. Since unsaturated fatty acids are susceptible to attacks by hydroxyl radical ($OH^\bullet$),[26] the presence of this

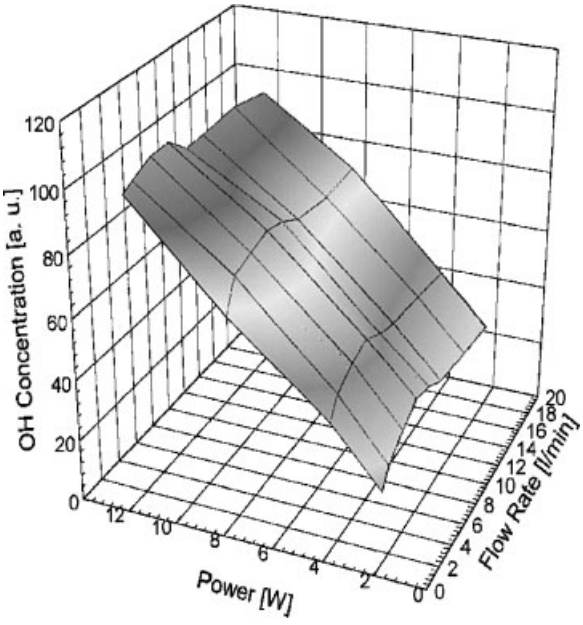

Figure 7. Relative $OH^{\bullet}$ concentration as a function of plasma dissipated power and air flow rate.[26]

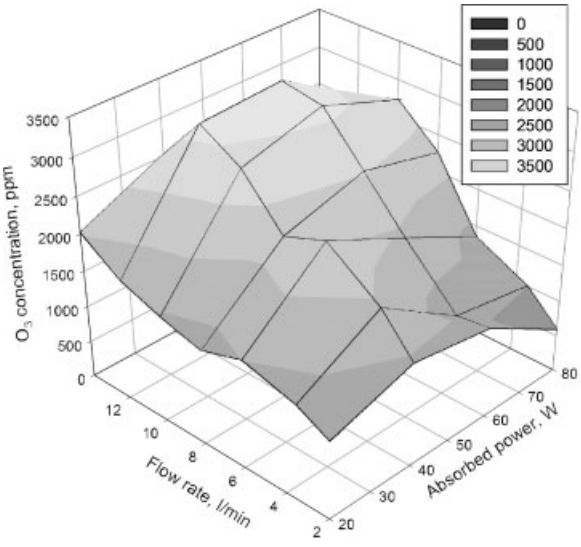

Figure 9. Ozone concentration in a DBD in air as a function of air flow rate and dissipated power.[30]

radical can therefore compromise the function of the membrane lipids whose role is to act as a barrier against the transport of ions and polar compounds in and out of the cells.[31] Protein molecules, which are basically linear chains of amino acids, are susceptible to oxidation by atomic oxygen or metastable oxygen molecules. Proteins also play the role of gateways that control the passage of various macromolecules in and out of cells. In the case when bacteria are of the gram-positive type, they are able to form spores, which are highly resistive states of cells. Spores are made of several coats surrounding a genetic core. These coats are also made of proteins susceptible to chemical attack. Therefore, the reactive species generated by air plasmas are expected to greatly compromise the integrity of the walls, coats, and membranes of the cells of microorganisms. As an example, Figure 10 shows how *B. subtilis* spores undergo erosion after exposure to a N_2/O_2 plasma.[15]

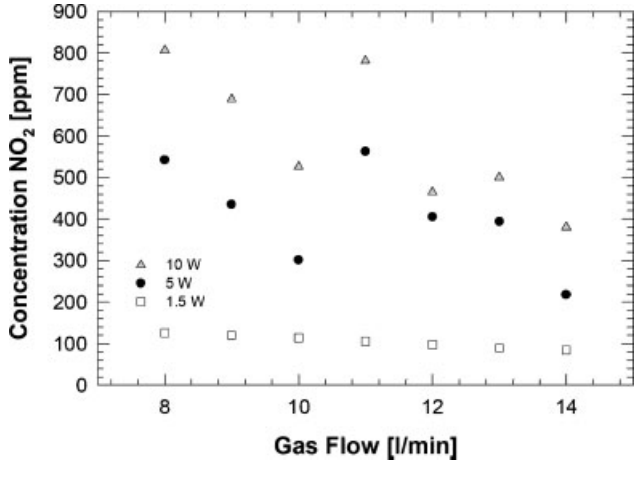

Figure 8. Concentration of nitrogen dioxide generated in a DBD in air as a function of air flow rate and at three power levels (1.5 W, 5 W, and 10 W).[26]

To study the release of proteins from spore coats during plasma treatment, Birmingham et al. used the ''Matrix-Assisted Laser Desorption Ionization-Time of Flight Spectrometry'' (MALDI-TOF) technique.[32] This technique allows analyzing the protein profile of spore coats by monitoring the sequence of proteins released as the plasma treatment time is increased. By correlating the inactivation kinetics, the morphological changes of the cells, and the sequence of protein release, a clear picture of events that lead to spore disruption may emerge. That is, it may be that after the plasma has caused the release of a specific protein from a spore undercoat, the spore becomes very vulnerable to the plasma and ultimately perishes. To check this hypothesis Birmingham et al.[32] prepared mutant spores of *B. subtilis* that are missing particular proteins from their coats. Then the inactivation kinetics of these spores were compared to normal spores to see which lack of protein has caused the spore to be more susceptible to plasma treatment. At this time, this work is still in progress and its results will certainly help elucidate why some spores are more resistant than others to plasma treatment.

V. Kinetics of Inactivation by Non-Equilibrium High Pressure Plasmas

Survivor curves are plots of the number of Colony Forming Units (CFUs) per unit volume versus treatment time. They

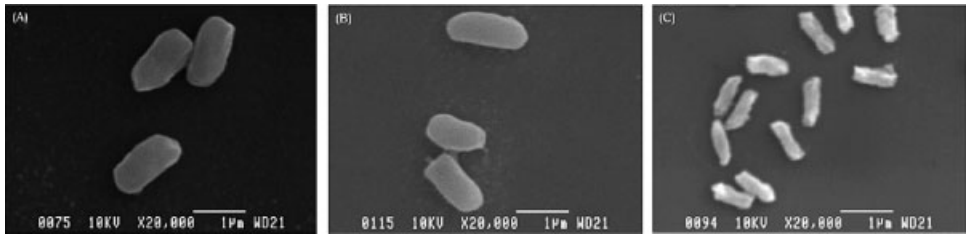

Figure 10. SEM micrographs showing (a) untreated *B. subtilis* spores, (b) spores inactivated under 0.7% added O_2, and (c) spores exposed to a 40-min treatment under 10% added O_2. Not all the spores are inactivated but they are visibly eroded.[14,15]

are plotted on a semi-logarithmic scale with the CFUs on the logarithmic vertical scale and time on the linear horizontal scale. A single line indicates that the relationship between the concentration of survivors and time is given by:

$$\log[N(t)/N_0] = -k \cdot t$$

where N_0 is the initial concentration and k is the ''death rate'' constant.

As already mentioned earlier in this paper the widely used kinetics measurement parameter is the ''D'' value (Decimal value). The D-value is the time required to reduce an original concentration of microorganisms by 90% (or the time for a one $\log_{10}$ reduction). It can be expressed as follows:

$$\mathrm{D} = t/(\log N_0 - \log N_s),$$

where t is the time to destroy 90% of the initial population, N_0 is the initial population, and N_s is the surviving population.[25]

To date, the experimental work on the germicidal effects of cold, atmospheric pressure plasmas has shown that survivor curves take different shapes depending on the type of microorganism, the type of the medium supporting the microorganisms, and the method of exposure, ''direct exposure'' or ''remote exposure''.

Herrmann et al., Laroussi et al., and Yamamoto et al.[33–35] reported ''single slope'' survivor curves (one-line curves) for the inactivation of some bacteria strains. The D-values ranged from 4.5 s to 5 min. Figure 11 shows an example of a single phase survivor curve. Two-slope survivor curves (two consecutive lines with different slopes) were reported by Kelly-Wintenberg[36] and by Laroussi.[37] The curves show that the D-value of the second line, D_2, was smaller (shorter time) than the D-value of the first line, D_1. Montie et al.[38] also reported the same type of survivor curve. They claimed that D_1 was dependent on the species being treated and that D_2 was dependent on the type of surface (or medium) supporting the microorganisms. The ''bi-phasic'' nature of the survivor curve was explained as follows. During the first phase, the active species in the plasma react with the outer membrane of the cells, inducing damaging alterations. After this process has sufficiently advanced, the reactive species can then quickly cause cell death, resulting in a rapid second phase. Multi-slope survivor curves (3 phases or more) were also reported in some cases (see the example of Figure 12). Each line has a different D-value. As mentioned earlier, 3-phase survivor curves were also reported by Moisan et al.[12] in their low-pressure studies. Moisan's interpretation of the three phases, as was described in Section II, claims that the first phase is mainly due to the action of UV radiation on isolated spores or on the first layer of stacked spores. The second phase is attributed to a slow erosion process by active species and the third phase is again due to UV photons hitting the genetic material of the still living spores which were cleared from covering debris during phase 2.

These inactivation kinetics studies reveal that bacteria inactivation by non-equilibrium high pressure plasmas is a complex process. Several factors can impact the killing process: the type of bacteria, the type of medium in/on which the cells are seeded, the number of cell layers in the sample, the type of exposure, contribution of UV or lack-thereof, operating gas mixture, etc. If UV plays an important or dominant role, the survivor curves tend to exhibit a first rapid phase (small D-value) followed by a second slower phase. When UV does not play a role, such as in the case of an air plasma, single phase survivor curves were mainly observed. However, in some cases, multi-slope curves have also been reported.

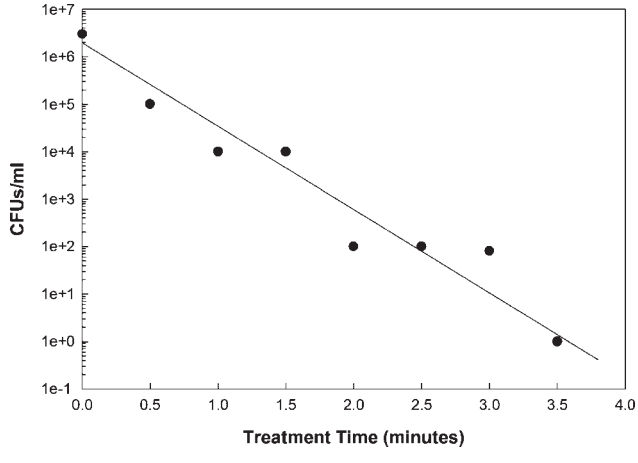

Figure 11. Example of a single phase survivor curve: *E. coli* on Luria-Bertani broth exposed to a He/air plasma generated by a DBD at atmospheric pressure.

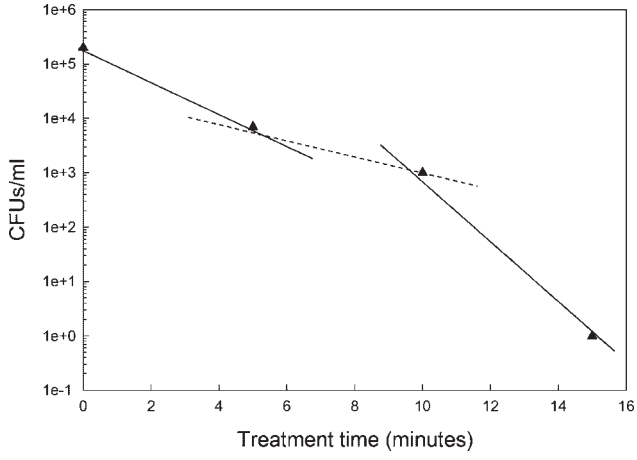

Figure 12. Example of a multiphase survivor curve: *Pseudomonas aeuroginosa* on filter exposed to a He/air plasma generated by a DBD at atmospheric pressure.[37]

VI. Sub-Lethal Experiments

Plasmas can also be used to affect biological materials in a non-lethal way. Laroussi et al.[39] reported that if a ''sub-lethal'' plasma exposure is administered to bacterial cells, a change in their metabolic behavior could occur. To demonstrate these effects Laroussi et al. addressed the biochemical impacts of plasma on *Escherichia coli* with sole carbon substrate utilization (SCSU) experiments, using Biolog (Hayward, CA) GN2^{TM} 96-well microtiter plates. The purpose of the SCSU experiments was to determine if exposure to plasma altered the heterotrophic pathways of the bacteria. It was presumed that any changes in metabolism would be indicative of plasma-induced changes in cell function. The Biolog GN2 plate was comprised of a control well and 95 other wells, each containing a different carbon substrate. Color development of a redox dye present in each well indicated utilization of that particular substrate by the inoculated bacteria. The 95 substrates were dominated by amino acids, carbohydrates, and carboxylic acids. The extent and rate of color development for plasma treated cells and control cells were determined over 5 d at 24-hour intervals, by measuring the optical densities of each well with a microplate reader. Laroussi et al.[39] observed that plasma exposure caused an increase of utilization of some substrates and a decrease of utilization in others. They therefore concluded that the plasma induced noticeable changes in the corresponding enzyme activities without causing any lethal impact on the cells.

Stoffels et al.[40] studied the effect of plasma on mammalian cells. As a model, they used Chinese hamster ovarian cells, CHO-K1. They exposed these cells to an RF driven, low power, small volume, cold plasma generated around the tip of a needle-shaped electrode (plasma needle). It was found that ''necrosis'' occurs for powers greater than 0.2 W and exposure times longer than 10 s. Necrosis is cell death

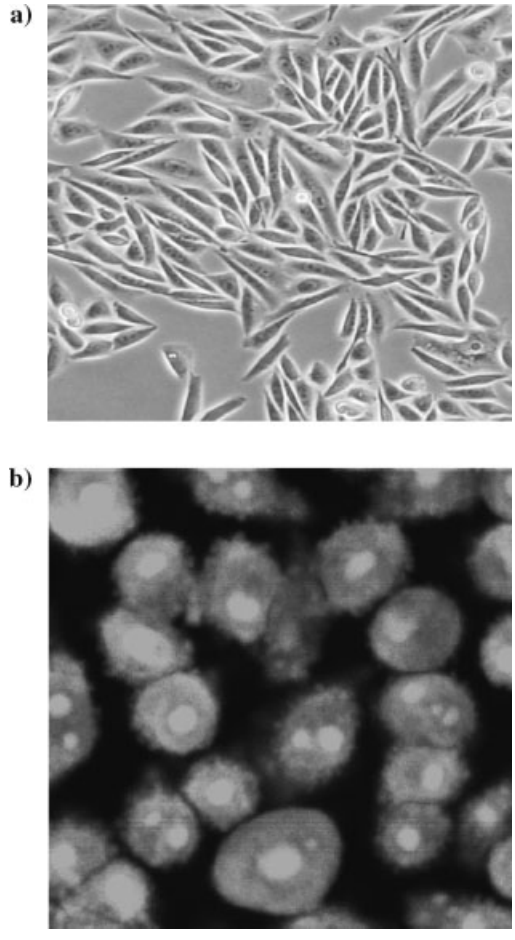

Figure 13. CHO-K1 cells before (a) and after (b) exposure to the low-power plasma needle. The treated cells have a more rounded shape and are detached but not necrotic.[40]

due to catastrophic injury. During necrosis the cell's membranes are damaged and the cytoplasm is released. Lower doses of exposure to the plasma can lead to ''apoptosis''. Apoptosis is an internally triggered cell mechanism of self-destruction. It is also referred to as ''programmed cell death''. Stoffels et al.[40] found that if the power level and exposure time were reduced to about 50 mW and 1 s, the cells partly detach from the sample, take a more rounded shape, and did not undergo apoptosis. In addition, no necrotic zone was observed in the exposed sample. Figure 13 illustrates this interesting finding by showing CHO-K1 cells before and after treatment by the plasma needle.

VII. Conclusion

During the last decade the plasma physics research community has witnessed a burst of research activity to investigate the germicidal effects of both low-pressure and high-pressure non-equilibrium plasmas. These research

efforts were motivated by both a desire to understand the mechanisms whereby plasma affects the cells of micro-organisms and by exciting prospects of using plasma in the biomedical field. In the medical field, the use of plasma to sterilize heat-sensitive re-usable tools in a rapid, safe, and effective way will potentially replace the present method, which relies on the use of ethylene oxide, a toxic gas. Plasma-assisted surgery for blood coagulation and/or cutting and welding of tissues without sutures could be a standard in the near future. In the food industry, the use of plasmas to sterilize packaging will lead to a safer food with longer shelf life. In space applications, plasma is considered as a potential method to decontaminate life-searching probes of spacecraft on planetary missions. Non-equilibrium high-pressure plasma is also a potential technology that can be used for the destruction of biological contaminants (including biological warfare agents) on high-value objects such as equipment and gear. In this particular application, atmospheric pressure air plasmas have been shown to possess very effective germicidal characteristics. Their relatively simple and inexpensive designs, as well as their non-toxic nature can make them the plasma technology of choice in other applications too.

On the more basic scientific side, the understanding of the interaction of plasma with living cells in general, and with bacterial cells in particular, requires a careful investigation which probes the effects on the cellular and sub-cellular levels. Since plasma is a source of charged particles, radicals, and radiation, it is expected that physical as well as biochemical effects enter into play. To a certain degree the role of various plasma agents can be separated and studied. However, when all of the agents are present simultaneously, as is in fact the case, one can expect some level of synergy to exist. To date, an in-depth or systematic study on the specific effects of the plasma on sub-cellular components such as nucleoid, cytoplasm, ribosome, etc. have yet to emerge. Therefore, a lot of careful work requiring collaborative efforts between plasma physicists, microbiologists, and biochemists remains to be done.

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