Low-Temperature Plasmas for Medicine?

Mounir Laroussi, *Fellow, IEEE*

(Invited Paper)

***Abstract*—Can low-temperature plasma technology play a role in medicine? This is a question that many investigators today are trying hard to give a positive answer to. It did not quite start out this way. Almost two decades ago, few "curious" electrical engineers and physicists with the help of few "brave" biologists/ microbiologists asked themselves more basic questions: What happens to biological cells if they were exposed to low-temperature plasma? Will they die? Will they survive? If they survive, will they come out the same or somehow "injured"? If injured, will they be able to repair the damage and recover? What kind of damage? Which plasma agent causes the damage? etc. As will be shown in this paper, some of these fundamental questions have been partially or fully answered, but until today, a complete picture has yet to emerge. This is good and not so good. It is good because if we already knew all the answers, we would not be looking forward to a more exciting research. It is not so good because after all these years, we are still quite a ways from an implementable medical application. In this review paper, the present state of knowledge regarding the effects of cold plasma on bacteria cells (prokaryotes) and on eukaryotic cells (such as mammalian cells) will be presented. As medical applications where low-temperature plasma is showing signs of success, blood coagulation and wound healing will be described.**

***Index Terms*—Atmospheric pressure, bacteria, biomedical, blood coagulation, cold plasma, discharge, medicine, plasma, reactive species, sterilization, wound healing.**

I. Introduction

ONE of the attractive features of nonequilibrium plasmas is the ability to achieve enhanced gas phase chemistry without the need for elevated gas temperatures [1], [2]. This is because these plasmas exhibit electron energies much higher than that of the ions and the neutral species. The energetic electrons enter into collision with the background gas, causing enhanced level of dissociation, excitation, and ionization. Because the ions and the neutrals remain relatively cold, the plasma does not cause any thermal damage to articles it comes in contact with. This characteristic opened up the possibility to use these plasmas for the treatment of heat-sensitive materials including biological matter such as cells and tissues [3], [4].

Low-temperature high-pressure nonequilibrium plasmas are already routinely used in material processing applications. Etching and deposition, where low-pressure plasmas have historically been dominant, are examples of such applications. More recently, the biological and medical applications of high-pressure plasmas have witnessed a great interest from the plasma and medical research communities. This is due to promising applications in medical research such as electrosurgery [5], tissue engineering [6], surface modification of biocompatible materials [7], and the sterilization of heat-sensitive materials and instruments [8]. This burst of research activities resulted in the organization of several workshops and conferences worldwide, the latest being the "International Conference on Plasma Medicine." Some of the papers presented at that conference were published in a special issue of the "Plasma Processes and Polymers" (Vol. 5, No. 6, August 2008). The following statement in the editorial of that special issue summarized the excitement built around a new multidisciplinary field that bridges engineering, physics, and biology: "Recent demonstrations of plasma technology in treatment of living cells, tissues, and organs are creating a new field at the intersection of plasma science and technology with biology and medicine—Plasma Medicine. This fascinating field poses many technological challenges and brings to the forefront many fundamental questions regarding the mechanisms of interaction between living organisms and plasma." [9].

This paper is organized as follows. First, and for the benefit of the reader with only plasma physics background, a brief description of the two types of cells considered in this paper will be presented. These are the prokaryotic cells (such as bacteria cells) and the eukaryotic cells (such as mammalian cells). Next, the known effects of plasmas on bacterial cells will be outlined and discussed. This includes the inactivation kinetics as well as the effects of the various plasma agents on this type of cells. This section will be followed by a coverage of what is presently known regarding the effects of plasma on some eukaryotic cells, particularly in relation to specific medical applications, namely, wound healing and blood coagulation.

II. Prokaryotic Cells Versus Eukaryotic Cells

A. Prokaryotes

Prokaryotic cells lack a cell nucleus and do not have membrane-bound organelles. Bacterial cells are prokaryotic. The chromosomal DNA of prokaryotes is a single loop and is contained in the nucleoid, an area not bound by an envelope. Prokaryotes have a relatively large surface-to-volume ratio, a high metabolic rate, and a high growth rate (they multiply quite fast). Fig. 1 shows a schematic of a prokaryotic cell.

Manuscript received December 5, 2008; revised January 16, 2009. First published April 17, 2009; current version published June 10, 2009.

The author is with the Laser and Plasma Engineering Institute, Old Dominion University, Norfolk, VA 23529-0246 USA (e-mail: mlarouss@odu.edu).

Color versions of one or more of the figures in this paper are available online at http://ieeexplore.ieee.org.

Digital Object Identifier 10.1109/TPS.2009.2017267

0093-3813/$25.00 © 2009 IEEE

Authorized licensed use limited to: Old Dominion University. Downloaded on June 8, 2009 at 14:03 from IEEE Xplore. Restrictions apply.

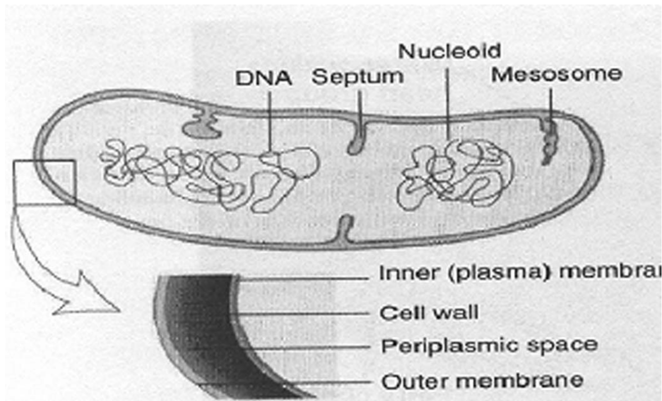

Fig. 1. Schematic of a bacterial cell (prokaryote) [10], [11].

Bacteria cells are either gram positive or gram negative. The gram-positive cells can exist in one of two states, namely, the vegetative state and the spore state. In the vegetative state, cells can uptake nutrient, divide, form colonies, etc. The spore state is a state under which the bacterium can overcome extremely harsh and unfavorable conditions that would otherwise kill the vegetative cell. In the spore state, the genetic material of the cell is located in a compact core that has small water content and is protected by a wall. Around this structure, several protein-based wall-like layers or "coats" are formed. These coats include the inner and outer coats. Under these coats (and around the core) is located the cortex, a region made of several concentric rings composed of peptidoglycan. With this structure and morphological configuration, the cell can go in a dormant state for extended lengths of time. When the surrounding conditions become favorable, the spore can germinate and revert to a vegetative cell.

Gram-negative cells do not have the ability to form spores. However, it is important to remember that this lack of ability to form spores does not render gram-negative bacteria less pathogenic than their gram-positive counterpart. *Escherichia coli*, for example, is a gram-negative bacterium.

B. Eukaryotes

The term "eukaryote" comes from the Latin "eu" meaning true and "karyon" meaning nucleus. Organisms possessing a nuclear membrane are called eukaryotes. Eukaryotic cells are therefore cells where the genetic material is contained within a well-defined region surrounded by a membrane. As shown in Fig. 2, eukaryotes exhibit membrane-bound organelles, the description of some is shown next [10], [11].

1) *Plasma membrane*: It is located inside the cell wall and forms a sort of a bag that completely surrounds the cell. It is about 5 nm thick and is a bilayer of phospholipids and proteins. The phospholipids are formed by water-insoluble fatty acids and water-soluble phosphate esters. One of the functions of the plasma membrane is control of the transport of nutrients and ions $(\mathrm{Na}^+, \mathrm{K}^+, \ldots)$ in and out of the cell.
2) *Nucleus*: The nucleus is the largest organelle. It is spherical in shape with a diameter of a few micrometers. It is bound by an envelope that includes an inner and an outer membrane. This membrane contains pores that allow the interior of the nucleus to communicate to the rest of the cell. The nucleus contains the DNA and an inner organelle, the nucleolus, which produces ribosomes $(\mathrm{RNA} + \mathrm{proteins})$.
3) *Mitochondria*: Mitochondria are usually rod-shaped structures, about 1 μm in diameter and 2–3 μm long. Mitochondria are membrane bound, and they are the sites of cellular respiration. Organs with high-energy demands have cells with large number of mitochondria, whereas organs with low-energy demands have cells with only a few mitochondria. Mitochondria produce adenosine triphosphate (ATP), which is the cellular energy mediator. Energy of the ATP is used in biosynthetic reactions in the cell.
4) *Lysosome*: Lysosomes are single-membrane-bound structures. They contain various enzymes involved in intracellular digestive activities.

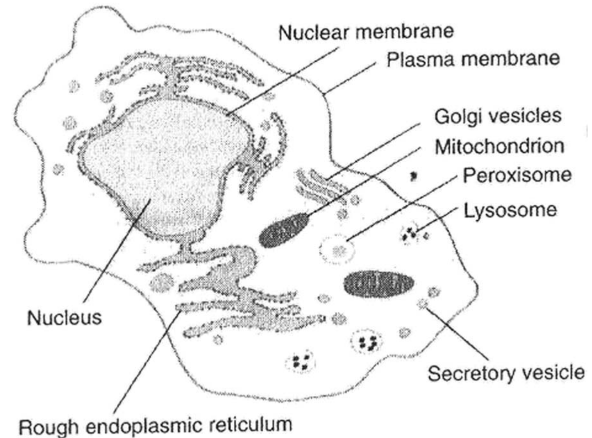

Fig. 2. Schematic of a eukaryotic cell [10], [11].

III. Effects of Low-Temperature Plasma on Bacterial Cells

To reach a general understanding of the effects that plasma has on the cells of bacteria, one has to study the time evolution of the inactivation process (kinetics) and to elucidate the potential physical and/or chemical effect that each plasma-generated inactivation agent has on the cells. This section discusses these two important aspects of the study of plasma-based bacterial inactivation.

A. Kinetics

Survivor curves are plots of the number of colony-forming units (CFUs) per unit volume versus treatment time. They are plotted on a semilogarithmic 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

$$\mathrm{Log}[N(t)/N_0] = -k \cdot t \quad (1)$$

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

Authorized licensed use limited to: Old Dominion University. Downloaded on June 8, 2009 at 14:03 from IEEE Xplore. Restrictions apply.

A widely used kinetics measurement parameter 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% (or the time for a one $\log_{10}$ reduction). It can be expressed as follows:

$$D = t/(\log N_0 - \log N_s) \quad (2)$$

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 [12].

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, namely, "direct exposure" or "remote exposure." Direct exposure is when the sample under treatment comes in direct contact with the plasma. In this method of exposure, the sample is subject to all possible agents generated by the plasmas. These include heat, charged particles, reactive neutrals, and electromagnetic radiation (such as ultraviolet (UV) photons). Remote or indirect exposure is when the sample does not come in direct contact with the plasma, e.g., the sample is placed at some distance from the plasma. In this method, the charged particles do not affect the sample under treatment as they recombine before reaching it. In addition, the heat flux and the possible radiation flux are greatly reduced. This leaves mainly the long-lived radicals to directly interact with the biological sample.

Single-slope survivor curves (one-line curves) for the inactivation of some bacteria strains have been reported [13]–[15]. The D-values ranged from 4.5 s to 5 min. Two-slope survivor curves (two consecutive lines with different slopes) were reported by various investigators [14], [16]–[18]. The curves show that the D-value of the second line, i.e., D_2, was smaller (shorter time) than the D-value of the first line, i.e., D_1. Montie *et al.* [19] 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 "biphasic" 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. Multislope survivor curves (three phases or more) were also reported in some cases, each line having a different D-value (for more information on this, the reader is referred to [3] and the references therein).

These inactivation kinetics studies reveal that 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 (see Fig. 3). However, in some cases, multislope curves have also been reported.

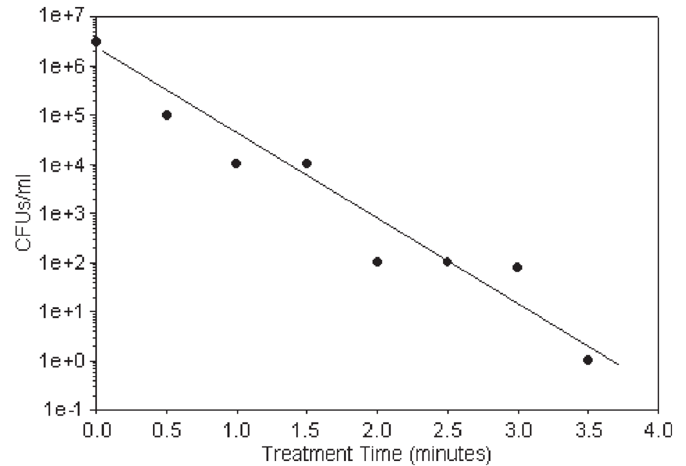

Fig. 3. Example of a single-phase survivor curve. *E. coli* on Luria-Bertani broth exposed to a helium/air plasma [3].

B. Inactivation Agents

As mentioned earlier, biological materials can be exposed to plasma in two different methods: direct exposure and remote (or indirect) exposure. In the case of direct exposure, all plasma-generated agents, including charged particles, come in contact with the sample. In the case of remote exposure, the amount of heat transmitted to the sample is reduced and the charged particles and short-lived species recombine before reaching the sample. In the following section, the contribution of the four main inactivation factors of a nonequilibrium high-pressure air plasma are reviewed.

1) Heat: As nonequilibrium plasmas do not produce high temperatures, heat is not an important factor in the inactivation process of bacterial cells. Indeed, temperature measurements in various cold plasma sources showed that the temperature of the biological samples exposed to the plasma did not rise substantially. Laroussi and Leipold [20] determined the gas temperature in their plasma discharge (DBD in air) by comparing experimentally measured rotational band structure of the 0–0 transition of the second positive system of nitrogen with simulated spectra at different temperatures. By using a thermocouple probe, they also measured the temperature in a sample, placed 2 cm away from the discharge. Their measurements showed a minimal temperature increase, and they concluded that thermal effects do not play an important role.

2) UV Radiation: UV radiation has been known to be germicidal for quite a long time. In fact, various kinds of UV lamps have been developed and are routinely used in biology laboratories to sterilize the surfaces of some tools and instruments. Low-pressure mercury lamps are an example of such UV sources. UV radiation in the 200–300-nm wavelength range with doses of several milliwatt–seconds per square centimeter are known to cause lethal damage to cells. Among the UV effects on cells of bacteria is the dimerization of thymine bases in their DNA strands. This inhibits the ability of the bacteria to replicate properly.

Depending on the operating gas, plasmas generate UV radiation in several wavelength ranges including vacuum UV and up to 380 nm. Although they produce energetic photons, wavelengths below 200 nm will not propagate at atmospheric

Authorized licensed use limited to: Old Dominion University. Downloaded on June 8, 2009 at 14:03 from IEEE Xplore. Restrictions apply.

pressures. Therefore, if such a wavelength range is to be used, the target sample has to be located in a low-pressure vessel. Low-pressure plasmas using N_2/O_2 gas mixture or argon have been shown to produce large enough UV doses to inactivate bacterial cells [21]–[23]. However, atmospheric-pressure air plasma, which is likely to be the most effective type of cold plasmas for sterilization, produces very little UV [20]. Most of the UV radiation in air plasma is emitted from N_2 and NO bands but with insufficient power densities, below 50 μW/cm^2. Therefore, UV does not play a major role in the inactivation process by atmospheric-pressure air plasma. Gas mixtures may of course be tailored to optimize UV emission since UV photons do increase the inactivation efficiency. In this context, gases that can form UV-emitting excimers can be used alone or in combination with a second discharge that provides the additional chemical action necessary to enhance the inactivation process.

3) Charged Particles: Mendis *et al.* [24] and Laroussi *et al.* [25] 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 micrometer range) experiences an outward electrostatic force due to 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. Therefore, gases with larger atomic mass lead to higher electrostatic forces. Mendis *et al.* [24] derived the condition for membrane disruption as

$$|\Phi| > 0.2 \cdot (r \cdot \Delta)^{1/2} \cdot F_t^{1/2} \tag{3}$$

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

The scenario described earlier 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.* [25].

4) Reactive Species: In high-pressure nonequilibrium 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, NO, NO_2, etc.

Laroussi and Leipold [20] 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 was measured by means of emission spectroscopy, looking for the rotational band of OH A–X (0–0) transition. Nitrogen

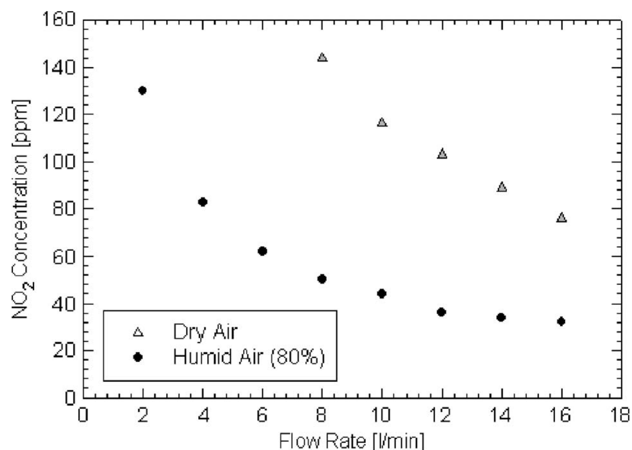

Fig. 4. NO_2 concentrations generated by a humid and dry air plasma [20].

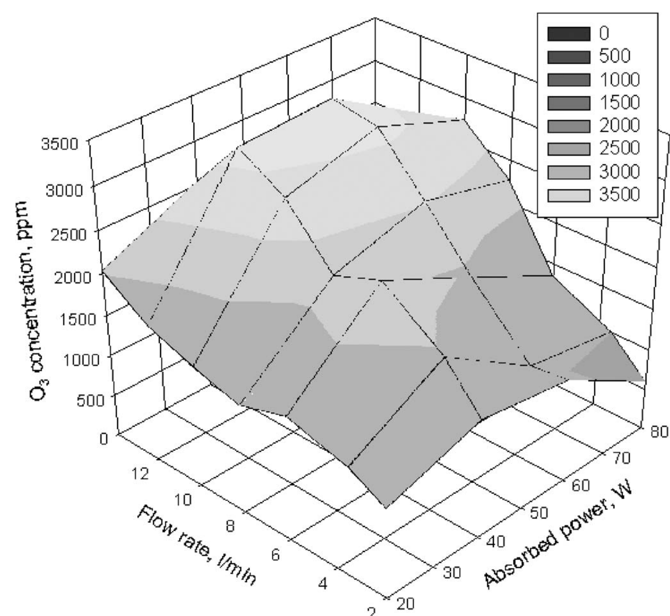

Fig. 5. Ozone concentration generated by air plasma [26].

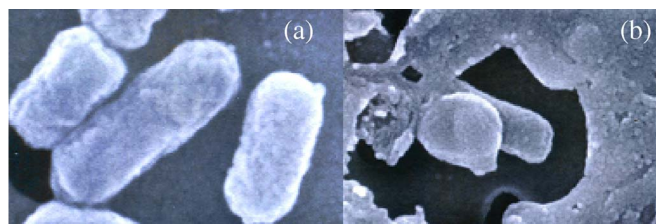

Fig. 6. SEM micrographs of *E. coli* cell. (a) Control. (b) Plasma treated [27].

dioxide was measured as a function of the airflow rate and for different power levels by a calibrated gas detecting system (see Fig. 4). The ozone concentration was measured for varying flow rates and at various power levels by chemical titration method by Minayeva and Laroussi [26] (see Fig. 5). Ozone germicidal effects are caused by its interference with cellular respiration.

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

Authorized licensed use limited to: Old Dominion University. Downloaded on June 8, 2009 at 14:03 from IEEE Xplore. Restrictions apply.

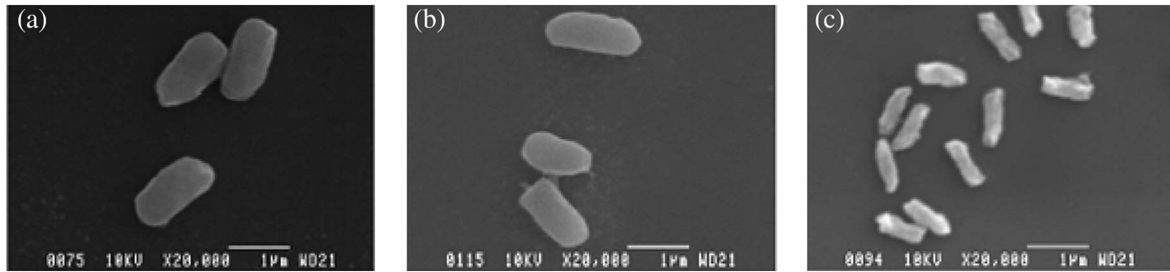

Fig. 7. SEM micrographs showing (a) untreated *B. subtilis* spores, (b) spores inactivated under 0.7% added O_2, and (c) spores exposed to a 40-min treatment under 10% added O_2. Not all spores are inactivated, but they are visibly eroded [22], [23].

hydroxyl radical (OH), the presence of this 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. Protein molecules 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.

IV. Morphological Observation

Investigators have used scanning electron microscope (SEM) to visually inspect the impact that plasma exposure may have on cell morphology. Any visible change in outer structure of cells after exposure to plasma was attributed to the impact of a specific plasma agent. By using SEM images, Laroussi *et al.* [27], [28] showed that after exposure to plasma, *E. coli* cells underwent severe morphological changes such as lysis (splitting of cells). Fig. 6 shows SEM micrographs of lysed *E. coli* cells. Laroussi *et al.* [27] attributed such damage to one of two processes: membrane rupture due to charge buildup on the cells or to chemical attack by radicals such as O and/or OH.

By using SEM, Moisan *et al.* [21], [22] showed that cells of *B. subtilis* that were exposed to low-pressure N_2/O_2 afterglow were subject to severe etching that eroded the cells. Atomic oxygen was identified as the possible player responsible for this cell erosion. Fig. 7 shows a SEM micrograph illustrating this scenario.

V. Inactivation of Biofilms

Cells of microorganisms exhibit two forms of behaviors. The "planktonic" form is when the cells are free floating more or less independently. The second form is when cells become attached to each other and to the surface of a host material. It is the latter form that can lead to the formation of a dense microbial community embedded in an adhesive gluelike matrix that anchors itself to, generally, a solid surface. This complex community with its protective matrix is referred to as "biofilm." Biofilms can be found on solid surfaces and in pores in contact with aqueous solutions or as films floating on liquid surfaces. Because cells in a biofilm closely interact with each other within the protective environment of the film, they exhibit different characteristics than free-floating planktonic cells. One of the key differences is their enhanced resistance to adverse conditions and external stresses. This makes biofilms very resistant to chemicals found in detergents and even to antibiotics. Therefore, if not controlled, biofilms could represent serious

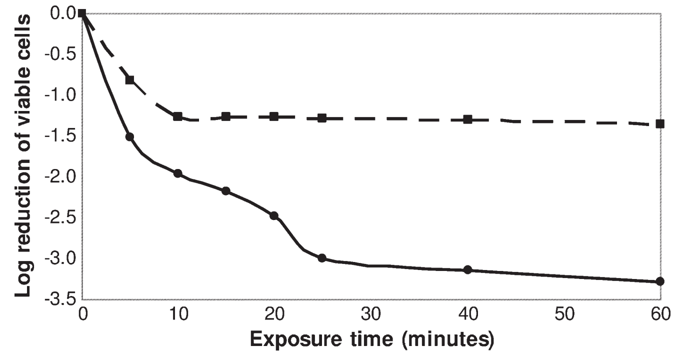

Fig. 8. Inactivation kinetics of (solid line) a 12-h and (dashed line) a 24-h-old *P. agglomerans* biofilm [29].

health hazards in many situations. In addition, biofilms can cause damage, such as corrosion, to the surfaces of the materials they attach to, adding an economical cost to their health risk.

Recently, some attempts were made to evaluate the effects of nonequilibrium plasmas on biofilms. Vleugels *et al.* [29] studied the efficacy of an atmospheric-pressure glow discharge to destroy biofilms formed on fresh food products. In their tests, a helium/oxygen gas mixture was used. The biofilm-forming bacterium *Pantoea agglomerans* was grown on synthetic membranes to simulate plant tissues. After allowing a period of growth, a biofilm developed with a polysaccharide matrix, shielding an extensive network of cells. The samples were then placed downstream (remote exposure) of a plasma generated between two parallel electrodes driven by a high ac voltage at a frequency of 460 kHz. Fig. 8 shows the inactivation results. It is clear that as more time was allowed for the biofilm to grow, both the inactivation kinetics and efficiency exhibited a different behavior. For a 12-h curve, there are three distinct inactivation phases, while for a 24-h curve, there are only two phases. In addition, the 24-h-old biofilm showed a much increased resistance to plasma exposure.

Brelles-Marino *et al.* [30], [31] used a helium-stabilized atmospheric-pressure jet driven by a 100-W 13.56-MHz RF source to inactivate biofilm-forming bacteria such as *Rhizobium gallicum* and *Chromobacterium violaceum*. A small admixture of nitrogen was added to the gas flow, and the biofilm samples were located 7 mm away from the plasma. Fig. 9 shows a biphasic survivor curve [31]. The first phase is relatively short, while the second phase is quite slow. A three-log reduction in CFUs was achieved in about 1-h exposure time duration. This is a much slower inactivation process that could generally be achieved on planktonic cells, hence illustrating the inherent difficulty in destroying biofilms.

Authorized licensed use limited to: Old Dominion University. Downloaded on June 8, 2009 at 14:03 from IEEE Xplore. Restrictions apply.

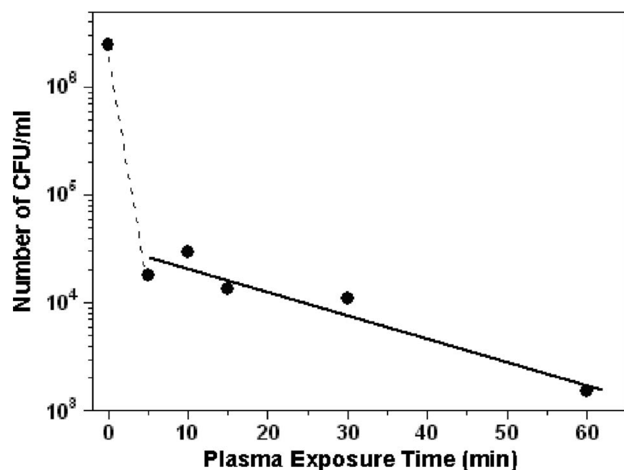

Fig. 9. Inactivation kinetics of *C. violaceum*, a biofilm-forming bacteria [31].

VI. Inactivation of Bacteria and Protein by Plasma Jets

Nonthermal atmospheric-pressure plasma jets/plumes are playing an increasingly important role in various plasma processing applications. This is because of their practical capability in providing plasmas that are not spatially bound or confined by electrodes [32]. This capability is very desirable particularly in biomedical applications. Various types of "cold" plasma jets have therefore been developed by several investigators [32], and their potential use to inactivate bacteria on surfaces including skin/tissue was tested [33]–[35]. Laroussi *et al.* [33], [34] reported that the plasma pencil, a pulsed device that emits a plume several centimeters long, was capable of destroying bacteria (*E. coli*, *Vibrio cholera*, *Staph epidermis*, etc.) in a targeted and localized manner. Fig. 10 is a photograph showing a treated zone for different operating conditions. Lu *et al.* [36] showed similar efficacy results on inactivating *Staphylococcus aureus* with their plasma jet. They also showed that with an He/O_2 mixture, the charged particles in the plasma plume/jet play a significant role in the inactivation process. Weltmann *et al.* [18] reported on the antimicrobial effects of miniaturized atmospheric-pressure plasma jets. Like Laroussi *et al.* and Lu *et al.*, they showed that their jets are capable of inactivating *E. coli*, *Bacillus atrophaeus*, and *S. aureus* at exposure times of several minutes.

Another interesting recent development is the destruction of proteins by atmospheric-pressure plasma jets. This is important for the sterilization of medical tools, which are frequently contaminated by both microorganisms, and proteinaceous matter. By using a plasma jet operated with a helium/oxygen gas mixture, Deng *et al.* [37], [38] achieved substantial destruction of proteins deposited on stainless steel. Protein reduction of 4.5 logs was achieved, which corresponded to a minimum surface protein of 0.36 fmol/mm^2. Deng *et al.* [37] attributed this result to protein degradation by excited atomic oxygen and excited nitride oxide. The hope of this study was that potentially plasmas could be used to inactivate prions, which are regarded as the etiological agent of spongiform neurodegenerative diseases [37].

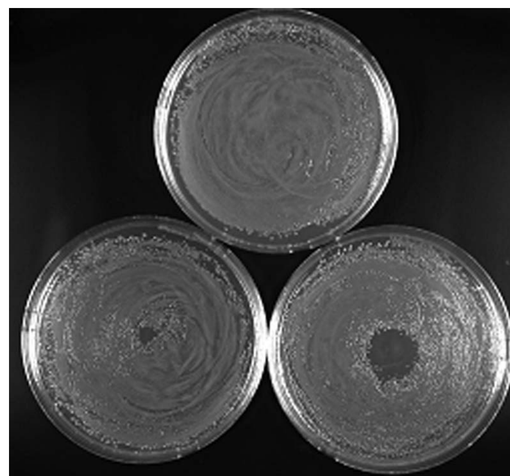

Fig. 10. Photographs of petri dishes showing the effects of the cold plume, generated by the plasma pencil, on *E. coli* cells. Operating gas is helium. Top petri dish is control; bottom petri dishes were treated for (left) 30 s and (right) 120 s [34].

VII. Nonlethal Studies

Some investigators asked the following question: Does exposure to plasma affect bacteria even if the cells "survive" and remain culturable? This question was partially addressed by asking a simpler and more specific question: Does exposure to plasma change the metabolism of bacteria? To investigate this eventuality, sole-carbon-source-utilization profiles of bacteria (*E. coli* and *Bacillus globiggi*) were compared to profiles of bacteria that survived and grew following exposure to an air plasma [27], [39]. The tool used to assess these profiles was Biolog's GN2 plate, a 96-well microtiter plate. The Biolog GN2 plate is comprised of 95 different carbon substrates, each contained in a separate well to which a minimal growth medium and tetrazolium violet are added. The redox dye turns purple in the presence of electron transfer, indicating that the substrate has been utilized by the inoculated bacteria. The 95 substrates are dominated by amino acids, carbohydrates, and carboxylic acids [27]. A control well containing no substrate is also included. Color development for both control and plasma-treated cells was then compared. The amount of color development in the wells was determined at 24-h intervals by measuring the optical densities with an EL 800 Universal Microplate Reader. Any change in the rate of utilization of a particular substrate by the exposed cells relative to the control cells would indicate that the biochemical pathways associated with that substrate have been altered.

In the *E. coli* [27] case, it was found that some small changes in the utilization of some substrates did occur. Fig. 11 shows an increased utilization of D-sorbitol in the plasma-treated cells, and Fig. 12 shows a decreased utilization of D,L-lactic acid. These changes are presumed indicative of plasma-induced alterations in enzyme activity.

In the case of *B. globiggi*, Laroussi *et al.* [39] analyzed the sole-carbon-utilization profile of "descendent" of cells that survived plasma exposure to that of their "parent" cells, and they found the following: The utilization of the sole-carbon substrates is sufficiently different between parent cells and

Authorized licensed use limited to: Old Dominion University. Downloaded on June 8, 2009 at 14:03 from IEEE Xplore. Restrictions apply.

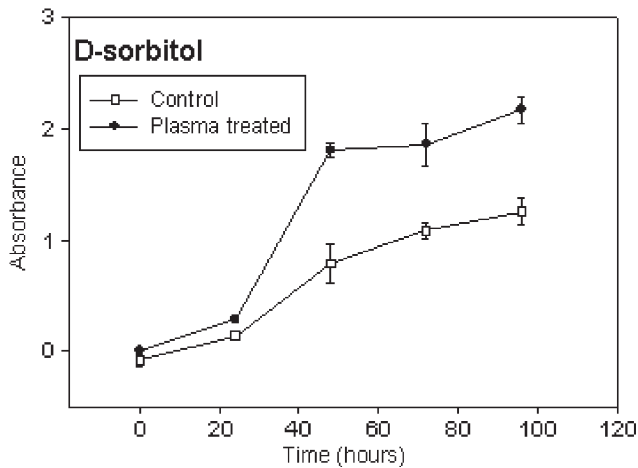

Fig. 11. Heterotrophic pathway changes after plasma treatment: Increased utilization of substrate by *E. coli* exposed to plasma [27].

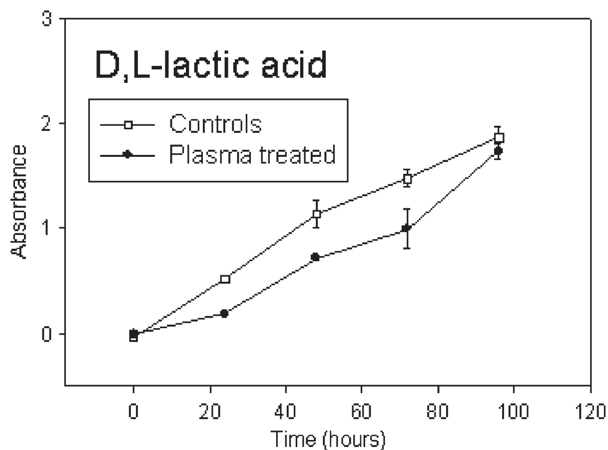

Fig. 12. Heterotrophic pathway changes after plasma treatment: Decreased utilization of substrate by *E. coli* exposed to plasma [27].

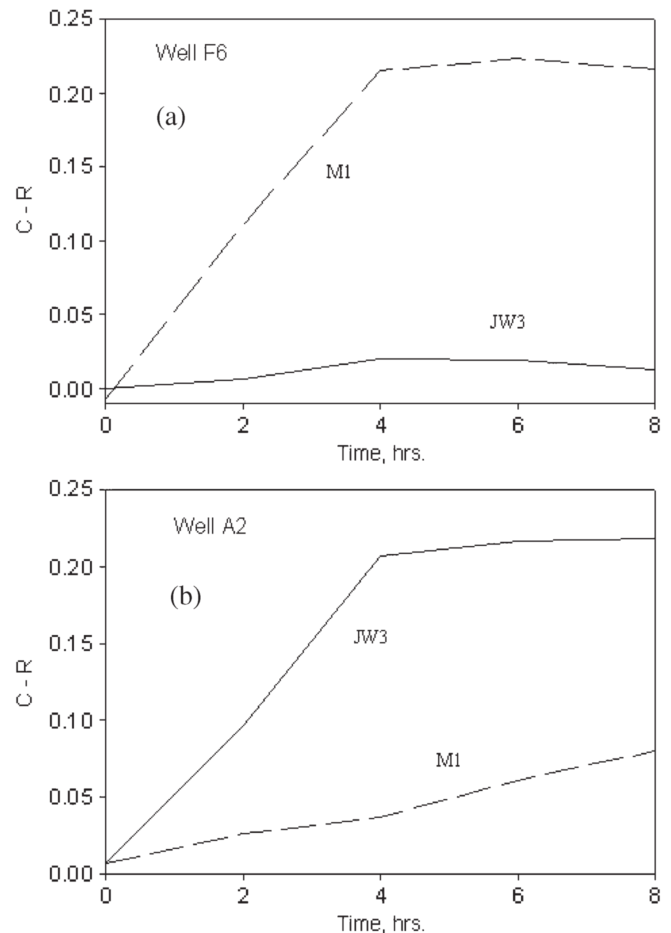

Fig. 13. Examples of sole-carbon-utilization data comparing the patterns of "parent" (M1) cells of *B. globiggi* (no plasma treatment) and descendents (JW3) of plasma-treated cells. Cells were in suspensions, and the plasma treatment time was 180 s. The selected wells [F6 for (a) and A2 for (b)] contained, respectively, L-alanine and α-cyclodextrine [39].

descendent cells to be of consequence (see Fig. 13(a) and (b) as an example). These differences indicate that although the plasma treatment did not result in the inactivation of the cells, it did cause some substantial changes in their metabolic behavior. Temporary and maybe permanent changes in some biochemical pathways of cells can result. These changes may also be the precursor to cell death.

VIII. Effects of Low-Temperature Plasma on Eukaryotic Cells

Until recently, not much effort has been dedicated to study the effects of nonequilibrium plasmas on "eukaryotic" cells, such as mammalian cells. In many respects, eukaryotic cells are different and have a more complex structure than prokaryotic cells. As mentioned earlier in this paper, unlike the case with prokaryotes, cellular functions in eukaryotic cells are localized and the cells exhibit membrane-bound subcellular structures (organelles). The effects of plasma on eukaryotic cells are therefore expected to be somehow different from those on the cells of bacteria. Preliminary work has shown that at low power, short exposures of mammalian cells to "cold" plasma can lead to cell detachment without causing necrotic effects. Under certain conditions, short exposures can lead to "apoptosis," or programmed cell death. Death of cells by apoptosis is not accompanied by lysis, which is a source of inflammation in affected areas. These very preliminary results, although in need of more in-depth investigations, point out that cold plasma may elicit some beneficial changes and therefore might play a role in practical medical applications, such as the removal of dead tissue and the acceleration of wound healing.

In the following, we first start by describing the effects of cold air plasma on eukaryotic microalgae, which can be a source of contamination in water. Then, we will cover preliminary studies that were done on few mammalian cells. This is followed by describing promising medical applications where cold plasma may potentially play a successful role. These are wound healing and blood coagulation.

A. *Effects of Plasma on Pathogenic Microalgae*

Studies on the inactivation of pathogenic microorganisms by low-temperature plasmas have focused mainly on bacteria [40], [41], but very little work has considered effects of plasma on eukaryotic pathogens.

Recently, Tang *et al.* [42] reported on the effects of nonthermal air plasma on cell motility, viability staining, and morphology in a variety of eukaryotic microalgae (*Akashiwo*

Authorized licensed use limited to: Old Dominion University. Downloaded on June 8, 2009 at 14:03 from IEEE Xplore. Restrictions apply.

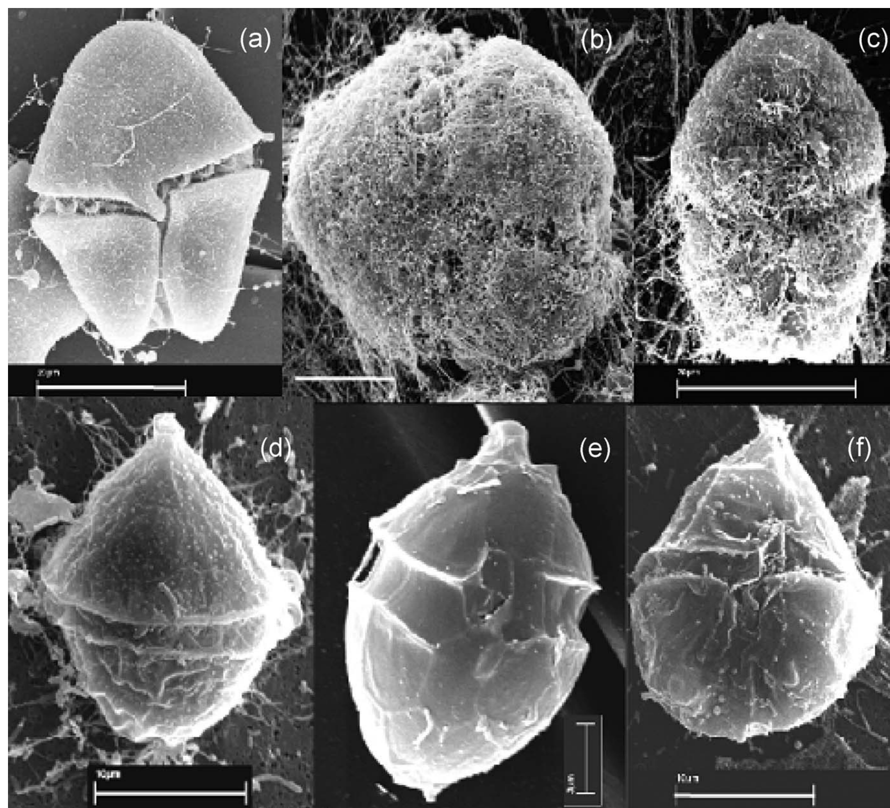

Fig. 14. Scanning electron micrographs showing effects of plasma exposure and pH decrease on cellular morphology of (a)–(c) *Akashiwo sanguinea* and (d)–(f) *Scrippsiella trochoidea*: (a) Control, (b) cell exposed to plasma for 320 s, (c) cell in medium with pH decreased to 3.0, (d) control, (e) cell exposed to plasma for 480 s, and (f) cell in medium with pH decreased to 2.7. Scale bars: [(a), (c)] 20 μm; [(b), (d), and (f)] 10 μm; and (e) 3 μm [42].

sanguinea, *Scrippsiella trochoidea*, *Heterocapsa triquetra*, and *Corethron hystrix*) in aqueous environments. These algae, and others like them, can cause a variety of concerns for human populations, ranging from taste and odor problems in drinking-water supplies to noxious blooms of so-called "red tides," responsible for fish deaths, beach closures, and shellfish poisoning. Based on systematic measurements of pH and comparative observations of algal cell morphology using SEM, Tang *et al.* [42] concluded that a strong pH decrease in samples following air plasma exposure was the principal mechanism responsible for its deleterious effects on algal cells. Fig. 14 shows SEM micrographs illustrating the comparable effects of low pH suspension (acidy) and air plasma on algal cells. Since O•, NO•, NO_2, O_3, and OH• are the major reactive components of air plasma, the pH decrease may be attributed to acid-forming reactions such as follows: $2NO + H_2O + O_3 \rightarrow 2HNO_3$; $NO + O_3 \rightarrow NO_2 + O_2$; and $NO_2 + OH\bullet \rightarrow HNO_3$. Following exposure to air plasma, therefore, the algal cultures could be considered a dilute nitric acid.

B. Effects of Plasma on Select Mammalian Cells

Until now, only limited work has been done on the cellular level to elucidate some of the effects of low-temperature plasma on mammalian cells. Preliminary work was carried out by Stoffels *et al.* [43]–[47] and Yonson *et al.* [48] on few types of cells. In one study by Stoffels, Chinese hamster ovarian cells, CHO-K1, were used as a model. They exposed these cells to an RF-driven low-power small-volume cold plasma generated around the tip of a needle-shaped electrode. It was found that "necrosis" occurs for powers greater than 0.2 W and exposure times longer than 10 s. Necrosis is cell death due to catastrophic injury. During necrosis, the cell's membranes are damaged and the cytoplasm is released, causing inflammation in the affected areas. Lower doses of exposure to the plasma were found to result in "apoptosis." Apoptosis is an internally triggered cell mechanism of self-destruction. It is also referred to as "programmed cell death." During apoptosis, the cell internal contents remain within the cell wall, therefore avoiding inflammation. It was found that if the power level and exposure time were reduced to about 50 mW and 1 s, respectively, the cells partly detach from the sample, take a more rounded shape, and did not undergo apoptosis [43]. In addition, no necrotic zone was observed in the exposed sample. Fig. 15 shows this interesting finding by showing CHO-K1 cells before and after plasma exposure. Stoffels *et al.* were also able to induce cell detachment in two other cell types, i.e., endothelial cells and smooth muscle cells. These were Bovine aortic endothelial cells and rat aortic smooth muscle cells. It was found that exposure as short as 10 s induced cell detachment generally without necrosis. By using a miniature atmospheric-pressure glow discharge plasma torch, Yonson *et al.* [48] also showed detachment of human hepatocytes (HepG2). Details of this group's work on cell detachment, reattachment, and surface functionalization can be found in [48]. By using a cold plasma atmospheric jet, Shashurin *et al.* also observed detachment in mouse dermal fibroblast cells, under what they termed "medium" plasma treatment levels [49]. Finally, Kieft *et al.* [47]

Authorized licensed use limited to: Old Dominion University. Downloaded on June 8, 2009 at 14:03 from IEEE Xplore. Restrictions apply.

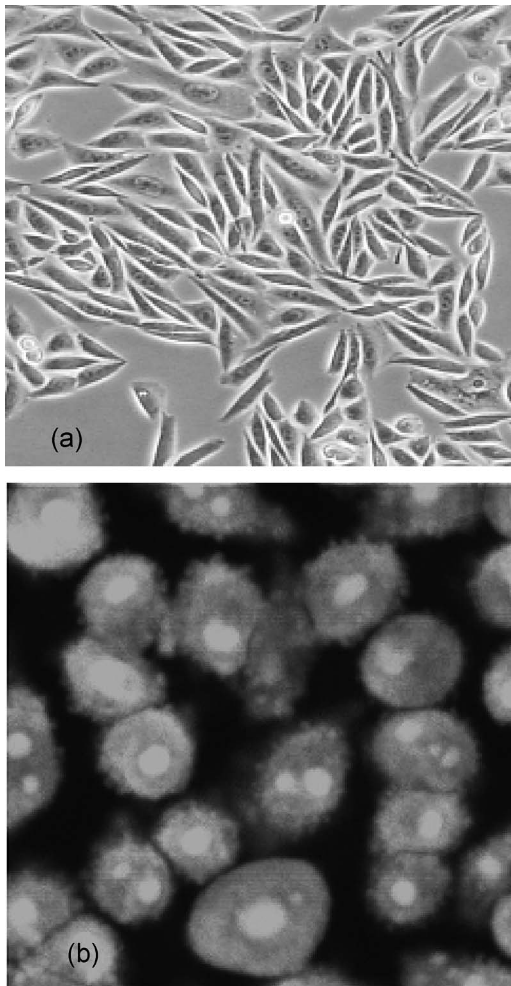

Fig. 15. CHO-K1 cells (a) before and (b) after exposure to the low-power plasma needle. The treated cells have a more rounded shape and are detached but not necrotic [43].

were able to induce apoptosis in 3T3 mouse fibroblast cells, and Fridman *et al.* [50] reported that low doses of plasma promote apoptotic behavior in human Melanoma skin cancer cell lines, *in vitro*. High doses, on the other hand, were found to cause necrosis of the cells. It is not clear to the author of this paper that the biochemical pathways and chain of events that plasma is able to trigger so as to cause apoptosis are well understood at the present time. Therefore, no further comments regarding this rather interesting and important result will be added here. Hopefully, future investigations will shed enough light on this process, which can open the door for the use of plasma in the treatment of some types of cancer.

C. Wound Healing

Wound healing follows a complex and highly orchestrated series of events. Three distinct main phases can be identified in the course of the healing of a dermal and epidermal tissue after an injury. Failure to complete all chain of events described hereinafter can lead to chronic wounds which may not heal at all.

1) The inflammatory phase: During this first phase, bacteria and debris are phagocytized. It is also during this phase that clotting occurs to achieve hemostatis and stop blood loss. The inflammation is due to the secretion of inflammatory factors from blood platelets. Other processes involving cell aggregation through the cross-linking of fibrin also occur.
2) The proliferative phase: This phase exhibits several stages. First new blood vessels are grown from preexisting vessels, a process referred to as "angiogenesis." This is followed by collagen deposition, collagen being a fibrous structural protein of connective tissue. After this stage, granulation tissue made of a variety of cells is grown to fill the volume of the wound. Next to occur are epithelialization followed by wound contraction.
3) The remodeling phase: During this phase, reorganization or realignment of the connective tissue occurs. Cells that remain and are not needed subsequently undergo apoptosis.

What role can low-temperature plasma play to help or enhance the process of wound healing? First, it is important to note that conventional treatments of wounds (such as using antibiotics) are limited by the bacteria's ability to develop resistance against antibiotics. This appears not to be the case for plasma-based treatments. However, before answering the question on the role, if any, plasma can play in wound healing, researchers had to first investigate the potential cytotoxicity of cold plasma. Studies performed but not published by few investigators showed that when used at low power, cold plasmas had no irreversibly detrimental effects on the DNA of mammalian cells. Gel electrophoresis techniques were used in these tests. This was a welcome news to researchers interested in investigating plasma for wound healing. Another study looked at the possible damage that plasma exposure could inflict on animal skin. Fridman *et al.* [51], [52] used hairless mice and pig models to clarify this issue. They came up with what they called "maximum acceptable prolonged treatment" levels. A dose of 10 min at 0.6 $\mathrm{W \cdot cm^{-2}}$ was deemed acceptable for the hairless mouse case. Histological studies showed no microscopic damage to the treated skin.

Laroussi and co-workers looked at another model to test toxicity and to see if nonthermal plasma affects cell regeneration [53]. They used planaria (flatworms, *Dugesia tigrina* species), known for more than two centuries to have remarkable regenerative capabilities and currently a focus of studies, hoping to identify a molecular strategy for metazoan wound healing [54]. These organisms are easily held in culture and provide an abundant inexpensive experimental model. A postpharyngeal cut (the tails of the worms were sliced off) was carried out, such that the anterior section of the worm could feed. To maximize potential for regeneration, the worms were fed twice per week. Worms were exposed to plasma for 0, 10, 20, and 40 s. Nearly all the worms survived the exposure and most lived throughout the following 14 days. Laroussi *et al.* saw no evidence that regeneration of worms exposed to plasma treatment differed from regeneration in control worms. Thus, it was concluded that exposure to nonthermal plasma does not hinder (nor accelerates) growth or cell regeneration in planaria. Fig. 16 shows the progress of an injured worm in regenerating its cut tail.

Authorized licensed use limited to: Old Dominion University. Downloaded on June 8, 2009 at 14:03 from IEEE Xplore. Restrictions apply.

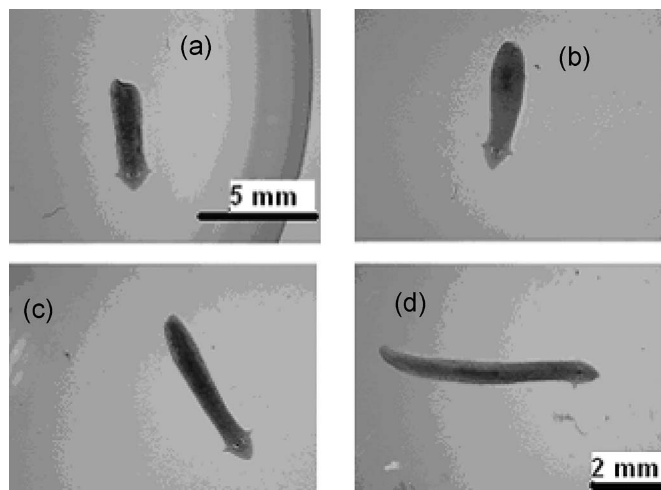

Fig. 16. Photographs of a worm having part of its tail cut, exposed to plasma for 40 s, and then left in conditions to grow back. (a) After 1 day, (b) after 4 days, (c) after 7 days, and (d) after 14 days. The photos show that the worm was able to grow back in a normal way [53].

Assuming that low-temperature plasma poses no cytotoxicity, the question is what evidence can be produced to show that plasma can indeed help or accelerate the healing of wounds. By using MTT assays (measuring mitochondrial activity), Stoffels showed that proliferation of fibroblasts does occur [55] when exposed to low-power density plasma. As described earlier, fibroblast proliferation is an important step in the cascade of events necessary for wound healing. Referring to a prior work done by Shekhter *et al.* [56], [57], Fridman *et al.* [52] claimed that fibroblast proliferation is induced by the exogenic NO generated by the plasma. NO is also known to help in the regulation of immune deficiencies, induction of phagocytosis, proliferation of keratinocytes, regulation of collagen synthesis, etc. [52], all of these being processes that play important roles in wound healing. In addition, plasma plays a role in greatly lowering wound infection by inactivating a large number of bacteria inhabiting the wound. It is therefore safe to conclude that low-temperature plasma not only reduces infection by inactivating bacteria but also, at the same time, seems to enhance key events necessary for proper wound healing, such as the proliferation of fibroblasts. Recently, a clinical study conducted by Stolz *et al.* [58] showed that low-temperature argon plasma treatment of chronic wounds was well tolerated by patients, without side effects. However, the uneven surfaces of the wounds presented challenges that limited the success of the procedures.

D. Blood Coagulation

Blood is a fluid medium through which nutrients and oxygen are delivered to the cells of a body. It is composed of a fluid called "plasma" in which three types of cells are in suspension. These are the red blood cells, the white blood cells, and platelets. The fluid plasma is mostly water with dissolved minerals, hormones, glucose, proteins, and carbon dioxide. The red blood cells, called erythrocytes, contain an oxygen-binding biomolecule called hemoglobin. The red blood cells collect oxygen at the lungs and release it throughout the body. The white blood cells, called leukocytes, play a key role in the immune system by defending the body against invasion by foreign cells, such as pathogenic bacteria. Platelets, also called thrombocytes, are disk-shaped cells without a nucleus. They are involved in blood coagulation, an important part of hemostasis. Blood coagulation occurs via a complex cascade of events that start with platelet activation, the formation of a plug, and the triggering of clotting factor (a protein in the blood plasma) to form fibrin.

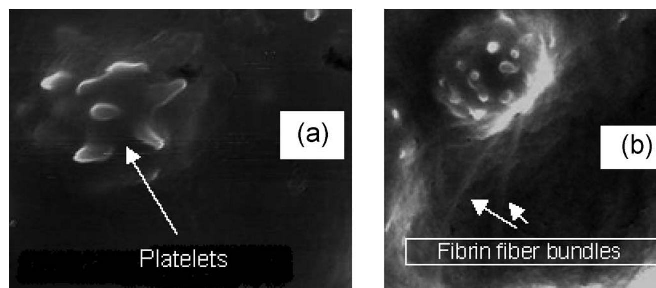

Fig. 17. SEM micrographs of platelets in anticoagulated blood. (a) Blood (platelets) not treated by plasma. (b) Formation of fibers in the plasma-treated blood [61].

Historically, excessive blood loss from a cut or a wound was stopped by applying pressure or by "cauterization." Cauterization involves the use of heat to "burn" the wounded area so as to coagulate the blood and stop further bleeding. This technique was used since ancient times and was first described in medical/scientific terms by the Andalusian scientist Abu Al-Qasim Al-Zahrawi about a millennium ago (10th Century) in his medical encyclopedia entitled Kitab al-Tasrif [59]. In modern times, cauterization is achieved by electrical means and is referred to as electrocautery or electrosurgery. Electrosurgery is used to cut soft tissues and/or to arrest blood flow out of a wound/cut. Because of its elevated temperature, thermal plasma has also been used successfully as an electrosurgical method to help coagulate blood [60]. Argon beam coagulator is an example of a plasma device used for cauterization. However, cauterization, in general, is accompanied by damage to surrounding tissues and may further complicate or delay the healing process. Therefore, investigations to employ low-temperature plasma have recently been conducted. Fridman's group at Drexel University demonstrated that nonthermal plasma could also quickly stop bleeding by triggering platelet activation, formation of fibrin mesh, and platelet aggregation [52], [61]. They also claimed that the mechanism of blood coagulation by nonthermal plasma does not involve the release of calcium ions or induce substantial change in the pH level of blood, factors previously thought to initiate coagulation. Instead, they proposed that nonthermal plasma has highly selective effects on blood proteins. As evidence, they found that plasma helps the conversion of fibrinogen into fibrin without affecting albumin. Fig. 17 shows the presence of a fiber mesh in a plasma-treated blood sample [52], [61].

IX. Conclusion

In this paper, the known effects of nonthermal plasma (also known as nonequilibrium, low-temperature, or cold plasma) at atmospheric pressure on both prokaryotes and eukaryotes have been described. Two potential applications in medicine

Authorized licensed use limited to: Old Dominion University. Downloaded on June 8, 2009 at 14:03 from IEEE Xplore. Restrictions apply.

were presented. These were wound treatment and blood coagulation. Both processes play significant roles in the healing of wound or cuts. Plasma was found to reduce infection, activate platelets, and enhance fibroblast proliferation without damage to living tissue. These results are very promising and point to the possibility of success in the use of nonthermal plasma in the wound-care arena. Chronic wounds affect millions of Americans, with tens of thousands of amputations taking place every year because of diabetic ulcers. It is therefore understandable to realize how attractive the idea of using low-temperature plasma to help the healing of chronic wounds can be. However, in my opinion, the results obtained so far are of preliminary nature, and much more careful work still needs to be done before plasma can establish itself as a technology used routinely and effectively in wound care. Another possible application of plasma is the treatment of some types of cancers by inducing apoptosis in cancer cells. However, it is the author's opinion that, here, also more duplication of the preliminary results needs to occur and more work aimed at elucidating the mechanisms whereby plasmas induce apoptosis is required. In the meantime, it is of note to mention that preliminary results on successful treatments of skin diseases (Melanoma, Cutaneous Leishmaniasis) as well as other under the skin- or organ-related diseases have recently been reported (for more information, [52] and references therein can be consulted).

There are other equally important biomedical applications of low-temperature plasmas that were not covered in this paper: Their use to address material-related issues such as biocompatibility, self-cleaning surfaces, hydrophilicity/hydrophobicity, and the coating with antibacterial films. Plasmas offer an attractive technological solution to prepare surfaces presenting selective functions such as primary amine functions or carboxylic groups. This is done by either surface grafting or by depositing organic coatings by plasma polymerization. Here, plasmas are replacing multistep wet processes. Interesting studies have recently been carried out to determine the critical number of functional groups (NH_2 or COOH) per square centimeter required for the adhesion of biomolecules, the mechanisms by which proteins adhere to the plasma-treated surfaces, and how the treatments by different plasma reactors compare.

Other uses of low-temperature plasma in the medical field are the sterilization of heat- and/or moisture-sensitive medical instruments, the sterilization of the inside of small-diameter plastic tubes (capillaries) such as those used in catheters, the inactivation and cleaning of proteins that may still remain on the metallic surface of medical tools such as scalpels, etc. To conclude, there is no question that low-temperature plasma is likely to play some kind of role in medicine in the near future; however, how much of a role and how many diseases will it help cure are hard to predict. Hopefully, more than we now expect.

Acknowledgment

The author would like to thank the many colleagues who, at one time or another, provided him with some of the figures used in this paper. The author would also like to thank the Guest Editor, K. Becker, for inviting him to write this review paper for this special issue of TPS, dedicated to E. Kunhardt. E. Kunhardt has had profound contributions to the field of discharge physics. The author is honored to have had the opportunity to contribute to this special issue.

References

[1] E. E. Kunhardt, "Generation of large volume atmospheric pressure non-equilibrium plasmas," *IEEE Trans. Plasma Sci.*, vol. 28, no. 1, pp. 189–200, Feb. 2000.

[2] U. Kogelschatz, "Filamentary, patterned, and diffuse barrier discharges," *IEEE Trans. Plasma Sci.*, vol. 30, no. 4, pp. 1400–1408, Aug. 2002.

[3] M. Laroussi, "Low temperature plasma-based sterilization: Overview and state-of-the-art," *Plasma Process. Polym.*, vol. 2, no. 5, pp. 391–400, Jun. 2005.

[4] E. Stoffels, A. J. Flikweert, W. W. Stoffels, and G. M. W. Kroesen, "Plasma needle: A non-destructive atmospheric plasma source for fine surface treatment of biomaterials," *Plasma Sources Sci. Technol.*, vol. 11, no. 4, pp. 383–388, Nov. 2002.

[5] K. R. Stalder, G. Nersisyan, and W. G. Graham, "Spatial and temporal variation of repetitive plasma discharges in saline solutions," *J. Phys. D, Appl. Phys.*, vol. 39, no. 16, pp. 3457–3460, Aug. 2006.

[6] E. A. Blakely, K. A. Bjornstad, J. E. Galvin, O. R. Monteiro, and I. G. Brown, "Selective neuron growth on ion implanted and plasma deposited surfaces," in *Proc. IEEE Int. Conf. Plasma Sci.*, 2002, p. 253.

[7] F. S. Sanchez-Estrada, H. Qiu, and R. B. Timmons, "Molecular tailoring of surfaces via RF pulsed plasma polymerization: Biochemical and other applications," in *Proc. IEEE Int. Conf. Plasma Sci.*, 2002, p. 254.

[8] M. Laroussi, "Plasma-based sterilization," in *Proc. Int. Conf. Phenom. Ionized Gases*, Greifswald, Germany, Jul. 2003, vol. 4, pp. 11–12.

[9] M. Laroussi, A. Fridman, and R. M. Satava, "Plasma medicine," *Plasma Process. Polym.*, vol. 5, no. 6, pp. 501–502, 2008.

[10] M. T. Madigan, J. M. Martinko, and J. Parker, *Brock Biology of Microorganisms*, 10th ed. Upper Saddle River, NJ: Prentice-Hall, 2003.

[11] R. F. Boyd, *Basic Medical Microbiology*, 5th ed. Boston, MA: Little Brown, Co., 1995.

[12] S. S. Block, *Sterilization*, vol. 4. New York: Academic, 1992, pp. 87–103.

[13] H. W. Herrmann, I. Henins, J. Park, and G. S. Selwyn, "Decontamination of chemical and biological warfare (CBW) agents using an atmospheric pressure plasma jet," *Phys. Plasmas*, vol. 6, no. 5, pp. 2284–2289, May 1999.

[14] M. Laroussi, I. Alexeff, and W. Kang, "Biological decontamination by non-thermal plasmas," *IEEE Trans. Plasma Sci.*, vol. 28, no. 1, pp. 184–188, Feb. 2000.

[15] M. Yamamoto, M. Nishioka, and M. Sadakata, "Sterilization using a corona discharge with H_2O_2 droplets and examination of effective species," in *Proc. 15th Int. Symp. Plasma Chem.*, Orleans, France, 2001, vol. II, pp. 743–751.

[16] K. Kelly-Wintenberg, T. C. Montie, C. Brickman, J. R. Roth, A. K. Carr, K. Sorge, L. C. Wadworth, and P. P. Y. Tsai, "Room temperature sterilization of surfaces and fabrics with a one atmosphere uniform glow discharge plasma," *J. Ind. Microbiol. Biotechnol.*, vol. 20, no. 1, pp. 69–74, Jan. 1998.

[17] M. K. Boudam, M. Moisan, B. Saoudi, C. Popovici, N. Gherardi, and F. Massines, "Bacterial spores inactivation by atmospheric pressure plasmas in the presence or absence of UV photons as obtained with the same gas mixture," *J. Phys. D, Appl. Phys.*, vol. 39, no. 16, pp. 3494–3507, Aug. 2006.

[18] K.-D. Weltmann, R. Brandenburg, T. von Woedtke, J. Ehlbeck, R. Foest, M. Stieber, and E. Kindel, "Antimicrobial treatment of heat sensitive products by miniaturized atmospheric pressure plasma jets (APPJs)," *J. Phys. D, Appl. Phys.*, vol. 41, no. 19, p. 194 008, Oct. 2008.

[19] T. C. Montie, K. Kelly-Wintenberg, and J. R. Roth, "An overview of research using the one atmosphere uniform glow discharge plasma (OAUGDP) for sterilization of surfaces and materials," *IEEE Trans. Plasma Sci.*, vol. 28, no. 1, pp. 41–50, Feb. 2000.

[20] M. Laroussi and F. Leipold, "Evaluation of the roles of reactive species, heat, and UV radiation in the inactivation of bacterial cells by air plasmas at atmospheric pressure," *Int. J. Mass Spectrom.*, vol. 233, no. 1–3, pp. 81–86, Apr. 2004.

[21] M. Moisan, J. Barbeau, S. Moreau, J. Pelletier, M. Tabrizian, and L. H. Yahia, "Low temperature sterilization using gas plasmas: A review of the experiments, and an analysis of the inactivation mechanisms," *Int. J. Pharm.*, vol. 226, no. 1/2, pp. 1–21, Sep. 2001.

[22] N. Philip, B. Saoudi, M. C. Crevier, M. Moisan, J. Barbeau, and J. Pelletier, "The respective roles of UV photons and oxygen atoms in plasma sterilization at reduced gas pressure: The case of N_2-O_2 mixtures," *IEEE Trans. Plasma Sci.*, vol. 30, no. 4, pp. 1429–1436, Aug. 2002.

Authorized licensed use limited to: Old Dominion University. Downloaded on June 8, 2009 at 14:03 from IEEE Xplore. Restrictions apply.

[23] S. Moreau, M. Moisan, J. Barbeau, J. Pelletier, and A. Ricard, "Using the flowing afterglow of a plasma to inactivate bacillus subtilis spores: Influence of the operating conditions," *J. Appl. Phys.*, vol. 88, no. 2, pp. 1166–1174, Jul. 2000.
[24] D. A. Mendis, M. Rosenberg, and F. Azam, "A note on the possible electrostatic disruption of bacteria," *IEEE Trans. Plasma Sci.*, vol. 28, no. 4, pp. 1304–1306, Aug. 2000.
[25] M. Laroussi, D. A. Mendis, and M. Rosenberg, "Plasma interaction with microbes," *New J. Phys.*, vol. 5, pp. 41.1–41.10, 2003.
[26] O. Minayeva and M. Laroussi, "Molecular absorption spectroscopy of atmospheric pressure air DBD," in *Proc. IEEE Int. Conf. Plasma Sci.*, Baltimore, MD, 2004, p. 122.
[27] M. Laroussi, J. P. Richardson, and F. C. Dobbs, "Effects of non-equilibrium atmospheric pressure plasmas on the heterotrophic pathways of bacteria and on their cell morphology," *Appl. Phys. Lett.*, vol. 81, no. 4, pp. 772–774, Jul. 2002.
[28] M. Laroussi, "Non-thermal decontamination of biological media by atmospheric pressure plasmas: Review, analysis, and prospects," *IEEE Trans. Plasma Sci.*, vol. 30, no. 4, pp. 1409–1415, Aug. 2002.
[29] M. Vleugels, G. Shama, X. T. Deng, E. Greenacre, T. Brocklehurst, and M. G. Kong, "Atmospheric pressure plasma inactivation of biofilm-forming bacteria for food safety control," *IEEE Trans. Plasma Sci.*, vol. 33, no. 2, pp. 824–828, Apr. 2005.
[30] G. Brelles-Marino, J. C. Joaquin, J. Bray, and N. Abramzon, "Gas discharge plasma as a novel tool for biofilm destruction," in *Proc. Int. Workshop Cold Atmos. Pressure Plasmas*, Bruges, Belgium, 2005, pp. 69–72.
[31] K. Becker, A. Koutsospyros, S. M. Yin, C. Christodoulatos, N. Abramzon, J. C. Joaquin, and G. Brelles-Mariño, "Environmental and biological applications of microplasmas," *Plasma Phys. Control. Fusion*, vol. 47, no. 12B, pp. B513–B523, Dec. 2005.
[32] M. Laroussi and T. Akan, "Arc-free atmospheric pressure cold plasma jets: A review," *Plasma Process. Polym.*, vol. 4, no. 9, pp. 777–788, Nov. 2007.
[33] M. Laroussi and X. Lu, "Room temperature atmospheric pressure plasma plume for biomedical applications," *Appl. Phys. Lett.*, vol. 87, no. 11, p. 113 902, Sep. 2005.
[34] M. Laroussi, C. Tendero, X. Lu, S. Alla, and W. L. Hynes, "Inactivation of bacteria by the plasma pencil," *Plasma Process. Polym.*, vol. 3, no. 6/7, pp. 470–473, Aug. 2006.
[35] J. Goree, B. Liu, D. Drake, and E. Stoffels, "Killing of S. mutans bacteria using a plasma needle at atmospheric pressure," *IEEE Trans. Plasma Sci.*, vol. 34, no. 4, pp. 1317–1324, Aug. 2006.
[36] X. Lu, T. Ye, Y. G. Cao, Z. Y. Sun, Q. Xiong, Z. Y. Tang, Z. Xiong, J. Hu, Z. H. Jiang, and Y. Pan, "The roles of various plasma agents in the inactivation of bacteria," *J. Appl. Phys.*, vol. 104, no. 5, p. 053 309, Sep. 2008.
[37] X. T. Deng, J. J. Shi, and M. G. Kong, "Protein destruction by a helium atmospheric pressure glow discharge: Capability and mechanisms," *J. Appl. Phys.*, vol. 101, no. 7, p. 074 701, Apr. 2007.
[38] X. T. Deng, J. J. Shi, H. L. Chen, and M. G. Kong, "Protein destruction by atmospheric pressure glow discharge," *Appl. Phys. Lett.*, vol. 90, no. 1, p. 013 903, Jan. 2007.
[39] M. Laroussi, O. Minayeva, F. C. Dobbs, and J. Woods, "Spores survivability after exposure to low-temperature plasmas," *IEEE Trans. Plasma Sci.*, vol. 34, no. 4, pp. 1253–1256, Aug. 2006.
[40] M. Laroussi, "Sterilization of contaminated matter with an atmospheric pressure plasma," *IEEE Trans. Plasma Sci.*, vol. 24, no. 3, pp. 1188–1191, Jun. 1996.
[41] M. Laroussi, "The biomedical application of plasma: A brief history of the development of a new field of research," *IEEE Trans. Plasma Sci.*, vol. 36, no. 4, pp. 1612–1614, Aug. 2008.
[42] Y. Z. Tang, X. P. Lu, M. Laroussi, and F. C. Dobbs, "Sublethal and killing effects of atmospheric pressure, non-thermal plasma on eukaryotic microalgae in aqueous media," *Plasma Process. Polym.*, vol. 5, no. 6, pp. 552–558, Aug. 2008.
[43] E. Stoffels, "Plasma needle: Treatment of living cells and tissues," in *Proc. Gaseous Electron. Conf.*, San Francisco, CA, 2003, p. 16.
[44] E. A. Sosnin, E. Stoffels, M. V. Erofeev, I. E. Kieft, and S. E. Kunts, "The effects of UV irradiation and gas plasma treatment on living mammalian cells and bacteria: A comparative approach," *IEEE Trans. Plasma Sci.*, vol. 32, no. 4, pp. 1544–1550, Aug. 2004.
[45] I. E. Kieft, D. Darios, A. J. M. Roks, and E. Stoffels, "Plasma treatment of mammalian vascular cells: A quantitative description," *IEEE Trans. Plasma Sci.*, vol. 33, no. 2, pp. 771–775, Apr. 2005.
[46] E. Stoffels, Y. A. Gonzalvo, T. D. Whitmore, D. L. Seymour, and J. A. Rees, "A plasma needle generates nitric oxide," *Plasma Sources Sci. Technol.*, vol. 15, no. 3, pp. 501–506, Aug. 2006.
[47] I. E. Kieft, M. Kurdi, and E. Stoffels, "Reattachment and apoptosis after plasma-needle treatment of cultured cells," *IEEE Trans. Plasma Sci.*, vol. 34, no. 4, pp. 1331–1336, Aug. 2006.
[48] S. Yonson, S. Coulombe, V. Leveille, and R. Leask, "Cell treatment and surface functionalization using a miniature atmospheric pressure glow discharge plasma torch," *J. Phys. D, Appl. Phys.*, vol. 39, no. 16, pp. 3508–3513, Aug. 2006.
[49] A. Shashurin, M. Keidar, S. Bronnikov, R. A. Jurjus, and M. Stepp, "Living tissue under treatment of cold atmospheric jet," *Appl. Phys. Lett.*, vol. 93, no. 18, p. 181 501, Nov. 2008.
[50] G. Fridman, A. Shereshevsky, M. M. Jost, A. D. Brooks, A. Fridman, A. Gutsol, V. Vasilets, and G. Friedman, "Floating electrode dielectric barrier discharge plasma in air, promoting apoptotic behavior in melanoma skin cancer cell lines," *Plasma Chem. Plasma Process.*, vol. 27, no. 2, pp. 163–176, Apr. 2007.
[51] G. Fridman, "Medical applications of floating electrode dielectric barrier discharge FE-DBD," in *Proc. 1st Int. Conf. Plasma Med.*, Corpus Christi, TX, Oct. 2007, p. 16.
[52] G. Fridman, G. Friedman, A. Gutsol, A. B. Shekhter, V. N. Vasilets, and A. Fridman, "Applied plasma medicine," *Plasma Process. Polym.*, vol. 5, no. 6, pp. 503–533, Aug. 2008.
[53] M. Laroussi, "Interaction of low temperature plasma with prokaryotic and eukaryotic cells," in *Proc. 61st Gaseous Electron. Conf.*, Dallas, TX, Oct. 2008, p. 20.
[54] P. A. Newmark and A. Sánchez Alvarado, "Not your father's planarian: A classic model enters the era of functional genomics," *Nat. Rev. Genet.*, vol. 3, no. 3, pp. 210–219, Mar. 2002.
[55] E. Stoffels, "Wound treatment in wound care: Efficacy and risk assessment," in *Proc. Int. Conf. Plasma Med.*, Corpus Christi, TX, Oct. 2007, p. 14.
[56] A. B. Shekhter, V. A. Serezhenkov, T. G. Rudenko, A. V. Pekshev, and A. F. Vanin, "Beneficial effect of gaseous nitric oxide on the healing of skin wounds," *Nitric Oxide-Biol. Chem.*, vol. 12, no. 4, pp. 210–219, Jun. 2005.
[57] A. B. Shekhter, R. K. Kabisov, A. V. Pekshev, N. P. Kozlov, and Y. L. Perov, "Experimental and clinical validation of plasmadynamic therapy of wounds with nitric oxide," *Bull. Exp. Biol. Med.*, vol. 126, no. 8, pp. 829–834, Aug. 1998.
[58] W. Stolz, M. Georgi, H.-U. Schmidt, K. Ramrath, R. Pompl, T. Shimizu, B. Steffes, W. Bunk, B. Peters, F. Jamitzky, and G. Morfill, "Low temperature argon plasma for sterilization of chronic wounds: From bench to bedside," in *Proc. 1st Int. Conf. Plasma Med.*, Corpus Christi, TX, 2007, p. 15.
[59] F. Ramen, *Albucasis (Abu Al-Qasim Al-Zahrawi): Renowned Muslim Surgeon of the Tenth Century*. New York: Rosen Publishing Group, 2006.
[60] K. R. Stalder, "Plasma characteristics of electrosurgical discharges," in *Proc. Gaseous Electron. Conf.*, San Francisco, CA, 2003, p. 16.
[61] S. Kalghatgi, G. Fridman, G. Nagaraj, M. Cooper, M. Balasubramanian, A. Brooks, V. Vasilets, A. Gutsol, A. Fridman, and G. Friedman, "Mechanism of blood coagulation by nonthermal cold atmospheric pressure dielectric barrier discharge plasma," in *Proc. Int. Conf. Plasma Med.,* Corpus Christi, TX, Oct. 2007, p. 79.

Mounir Laroussi (S'84–M'86–SM'97–F'09) received the Ph.D. degree in electrical engineering from the University of Tennessee, Knoxville, in 1988.

From 1995 to 1998, after a few years of teaching, he joined the Microwave and Plasma Laboratory, University of Tennessee, as a Research Assistant Professor. In 1998, he then joined the Applied Research Center, Old Dominion University (ODU), Norfolk, VA, as a Research Scientist. From 2002 to 2007, he was an Associate Professor with the Department of Electrical and Computer Engineering, ODU. Since January 2007, he has been the Director of the Laser and Plasma Engineering Institute, ODU, and, in 2008, he was promoted as a Full Professor with the Department of Electrical and Computer Engineering, ODU. He has more than 100 publications in journals and conference proceedings. He is the holder of four patents. His research interests include the physical electronics area and particularly in the physics and applications of nonequilibrium gaseous discharges, including the biomedical applications of nonthermal plasmas.

Dr. Laroussi served as an elected member of the Administrative Committee (2002–2005) and the Plasma Science and Applications Executive Committee (2005–2007) of the IEEE Nuclear and Plasma Science Society (NPSS). He has also served as a Guest Editor of the IEEE TRANSACTIONS ON PLASMA SCIENCE, as the Session Organizer with the International Conference on Plasma Science for many years, and as a Guest Editor of *Plasma Processes and Polymers*. In January 2008, he was named as an IEEE-NPSS Distinguished Lecturer.

Authorized licensed use limited to: Old Dominion University. Downloaded on June 8, 2009 at 14:03 from IEEE Xplore. Restrictions apply.