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Use of a low-pressure plasma discharge for the decontamination and sterilization of medical devices*

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Abstract: Nonequilibrium low-pressure plasma discharges are extensively studied for their applications in the field of decontamination and sterilization of medical devices. The aim of this contribution is to discuss and demonstrate feasibility of oxygen low-pressure inductively coupled plasma (ICP) discharges for removal of various kinds of biological contamination. We demonstrate the ability of ICP discharges for the sterilization of bacterial spores and the removal of biological contamination from proteins and pyrogens.

Keywords: sterilization; decontamination; bacterial spores; endotoxins; proteins; oxygen ICP discharge.

INTRODUCTION

During surgery, various medical devices (e.g., surgical instruments or implants) are coming into direct contact with the patients' tissues and thus interact with their first immune defense system. According to the existing guidelines, all these devices have to be cleaned, decontaminated, and sterilized to prevent inflammation and to exclude the possibility of transmission of diseases connected with the presence of microorganisms or other substances of biological origin. This is especially true for the cases of reused instruments, such as endoscopes, dental drills, or surgical tools whose insufficient cleanness has been reported to be potentially the cause of post-intervention problems.

However, many of the instruments mentioned above cannot be effectively sterilized or even decontaminated with current sterilization methods. This is, for example, due to their thermal degradation in the case of articles made from plastic materials or because of the high resistance of certain biomolecules toward the commonly used sterilization/decontamination techniques. Therefore, new methods that overcome these limitations are urgently desired. From this perspective, the application of nonequilibrium plasma discharges currently appears as an interesting option. This is mainly due to their capacity of maintaining low temperatures during operations as well as the use of nontoxic gases reducing both environmental impacts and safety risks, while maintaining a high efficiency, as will be demonstrated below. These key advantages naturally result in increased interest in the investigations of the plasma interactions with microorganisms as well as with miscellaneous substances of biological origin. Indeed, many pathogens can be destroyed or eliminated by employing nonequilibrium plasma discharges [1–3]. However, the nature of plasma interactions with microorganisms or biomolecules still re-

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mains unclear. This is primarily due to the complexity of plasma discharges that are capable of producing high fluxes of various neutral or ionized active species as well as energetic photons interacting with treated substances. The exact role and contribution of each of these mechanisms naturally depends on many parameters such as, e.g., process conditions (pressure, applied power, gas flow, excitation frequency in the case of high-frequency sustained discharges), geometry of the system, and on the kind of target to be inactivated [2]. This complicates the comparison of results obtained in different experimental configurations and leads to controversy concerning the results published and the mechanisms playing a crucial role during the process. Nevertheless, on the basis of the published studies, two principal pathways of sterilization and decontamination employing nonequilibrium plasma discharges have been suggested:

- The first one relies on the modifications of the chemical or structural properties of the treated substances resulting in suppression of their biological activity. A typical example is the alteration of the DNA of bacterial spores by UV photons emitted by the plasma discharges [4]. A second example is the inactivation of bacterial endotoxins either by impact of hydrogen atoms or vacuum UV (VUV) radiation that leads to the modifications of the chemical structure responsible for their bioactivity [5,6].
- The second pathway is based on the physicochemical removal of the pathogens either by etching induced by chemically active species, or sputtering by energetic ions or, as recently suggested, via chemically assisted etching that combines the effects of ion sputtering and of reactive radicals [7].

Both approaches, of course, entail certain advantages as well as drawbacks. On the one hand, the fast physicochemical removal of pathogens is efficient but represents a risk of degradation of the treated objects: this is especially true in the case of polymer-based instruments whose properties and integrity can be affected by extensive etching. Conversely, the processes based on the inactivation of pathogens by UV photons usually have a mild effect on the sterilized article, but this is often counterbalanced by a substantial slowing down of the treatment efficiency. Moreover, the removal of pyrogens or protein residues is generally not addressed by these UV methods, as will be discussed in detail below. This is why we have selected the decontamination route based on etching whose action on bacterial spores, pyrogens, and protein residues is going to be described in the subsequent text.

PRINCIPLES OF LOW-PRESSURE, PLASMA-BASED STERILIZATION AND DECONTAMINATION

Bacterial spore sterilization and depyrogenation of bacterial endotoxins

The main mechanisms of spore destruction or deactivation by plasma discharges have been the object of several reviews (e.g., [1,2,3,8,9]) that the reader should refer to for more details. In brief, two main mechanisms can be invoked for spore inactivation or destruction employing low-pressure plasma discharges: The first one is related to the inactivation of spores by interaction of the UV photons, emitted by the discharge, with the DNA (e.g., [10–12]). It was found that the maximum efficiency of the UV photons is typically in the wavelength range 200–300 nm [13,14], and that there exists a minimum dose necessary for preventing DNA repair [15], leading to inactivation of the microorganisms. The second mechanism is linked to spore etching by the radicals and active species produced by the plasma discharge (e.g., atomic oxygen, atomic nitrogen, OH radicals, or fluorine atoms [16–20]). This can lead either to the direct killing of spores, or to the removal of the material that shields the spores from the direct view of UV radiation, resulting in an acceleration of the sterilization process. It is, nevertheless, clear that in common situations, both of these mechanisms may act simultaneously during the treatment and thus contribute to the killing/deactivation of spores. However, the rates of the two mechanisms are often markedly different, which can explain the two or three phase kinetics reported in the literature [1].

Regarding this last point, it was found [21] that in remote plasma processes, etching is the slowest mechanism of inactivation and thus controls the overall efficiency of sterilization. Furthermore, basing sterilization on the action of UV only has also two other principal drawbacks. The first one is connected with the organization of the spores on the surface. In many cases, the spores are stacked and mixed with protein residues or biological film. The UV photon flux decreasing exponentially with the thickness of the film they go through, the time needed for reaching the minimum dose necessary for spore inactivation increases exponentially: this makes the inactivation difficult to achieve for thick deposits.

The second aspect, often overlooked, is related to the presence of pyrogens on the surface to be decontaminated. The outer coats of the spores are composed among others by bacterial endotoxins (e.g., lipopolysaccharides (LPS), peptidoglycans (PGNs), or lipoteichoic acids, etc.) which are an important class of pyrogenic substances. They act as potent modulators of the human immune system, and their presence in the blood stream leads to physiological events such as fever, swelling, or sepsis, and at higher doses to death [22]. UV radiation affects minimally such substances, as we have already demonstrated [5]. Therefore, after a plasma treatment based on UV radiation only, the surface treated can still exhibit pyrogenic activity due to the endotoxins that are present in the spore membranes, which can consequently provoke serious immunological effects when brought into contact with the blood stream [23].

Elimination of proteins

Another group of possibly pathogenic agents of biological origin is constituted by proteins. This is particularly true for infectious prions, i.e., proteins responsible for the transmission of severe neurodegenerative diseases like Creutzfeld–Jacob disease. They have been found, however, to be extremely resistant toward the conventional sterilization and decontamination techniques (e.g., [24,25]) because of their unique and stable secondary and ternary structures that cannot be easily altered.

This, alongside with the high risks connected with their presence on the surfaces of the medical instruments, represents a serious problem in the medical praxis, and, therefore, alternative methods that will be capable of removing prions and proteins are urgently needed. Although the possibility of removing prions by means of nonequilibrium plasma discharges was already demonstrated by Baxter et al. [26], the studies focusing on the removal mechanisms of infectious prions are rather limited. This is primarily because of the necessity of high safety precautions due to the biohazard connected with such proteins. Therefore, in order to understand the mechanisms of plasma–prion interactions, as well as to evaluate favorable conditions for their destruction, the effects of nonequilibrium plasma discharges were predominantly studied on nonpathogenic model proteins (e.g., [27–34]) using different methods for monitoring the process of their elimination. The results of these studies reveal important facts regarding the action of low-pressure plasma discharges, and show the possibility of removing them by oxygen-containing discharges, to induce their fragmentation or to alter their chemical structure. Nevertheless, there is still a lack of systematic analysis related to the protein removal efficiency under different experimental conditions.

To conclude, a plasma discharge enabling a high etching rate is in many cases an interesting option for the successful sterilization/decontamination process. The main objective of the current contribution is to show that this can be achieved with a direct plasma process—in our case, an inductively coupled plasma (ICP) discharge sustained in pure oxygen—on selected examples of bacterial spores, bacterial endotoxins, and protein coatings.

EXPERIMENTAL

Plasma treatment

The schematic of the experimental set-up used for sterilization and decontamination tests is presented in Fig. 1. It consists of a rectangular stainless-steel vacuum chamber ($3 \times 65 \times 35$ cm) equipped with two large quartz windows, one on the top, and the other on the bottom. Two radio frequency (RF) coils serving as antennas for RF power coupling are placed above and under the quartz windows, which act as dielectric separators. The coils are connected in parallel through a matching network (Advanced Energy) to the 13.56 MHz RF power supply.

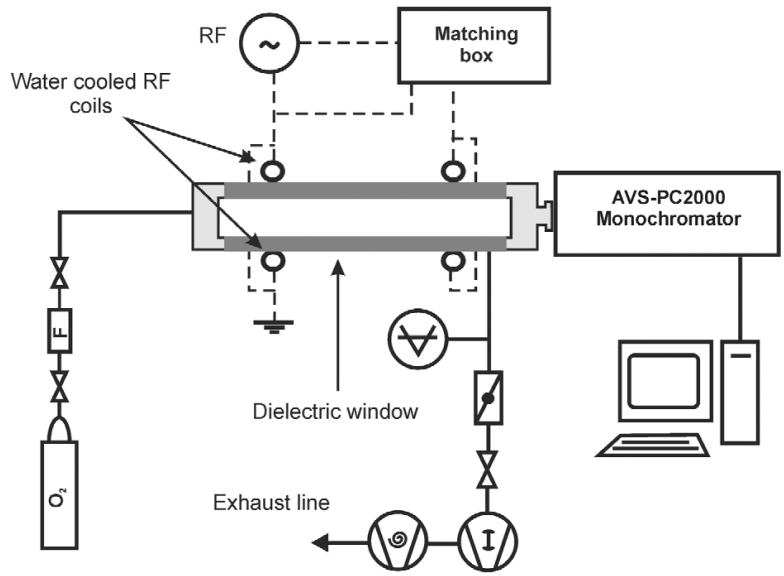

Fig. 1 Experimental set-up.

The discharge chamber is pumped down by a primary system equipped with a roots blower and is connected to the inlet system composed of mass flow controllers attached to the gas lines. The pressure in the chamber during plasma operation is manually regulated by a butterfly valve.

In the present contribution, the plasma treatment was performed in pure oxygen at pressure 13.3 Pa with an applied RF power up to 500 W in a gas flow of 10 sccm. In order to maintain a low temperature during the inactivation of bacterial spores, i.e., in order to exclude thermal effects leading to their destruction, RF power was pulsed with a 20 % duty cycle. In these conditions, the substrate temperature did not exceed 60 °C. In other experiments performed with proteins and bacterial endotoxins, a continuous mode of operation was used, leading to a temperature of 110 °C after 10 min of plasma duration, which is still far below the temperature needed for dry heat inactivation of both proteins [35] and bacterial endotoxins [36].

Bacterial spores

Spores of *Geobacillus stearothermophilus* deposited on stainless-steel disks provided by Raven Biological Laboratories, Inc. with declared spore population 2.5×10^6 were employed as bacterial indicators. After the plasma treatment, a thin gold layer (~10 nm) was deposited on the samples, which were examined by a scanning electron microscope (SEM) (LEO 435VP) in order to evaluate the degree of the spores' destruction induced by the plasma treatment.

Coating of bacterial endotoxins and protein

The efficiency of the plasma treatment in terms of etching of biomolecules has been studied on PGN, representative of highly resistant bacterial endotoxins [37], on lipid A, which constitutes pyrogenic entities of lipopolysaccharides, as well as on bovine serum albumin (BSA) that has been selected as a model protein. The samples were prepared by spotting small droplets of 0.1 % aqueous solution of these substances on polished Si wafers. Afterwards, the samples were dried at ambient temperature in a common flow hood and plasma treated. Due to the drying of the droplet, the deposit obtained is composed of an external region of a few μm of thickness, surrounding a thin film of about 100 nm. Both treated and untreated samples were subsequently examined by various surface diagnostic methods. In this contribution, only the results obtained by imaging ellipsometry (EP3, Nanofilm Surface Analysis GmbH), stylus profilometry (Alpha-step IQ, KLA-Tencor), atomic force microscopy (AFM type Solver P47H, NT-MDT Co.), and time-of-flight/secondary ion mass spectroscopy (TOF/SIMS) (TOF.SIMS IV, IONTOF) will be presented.

RESULTS AND DISCUSSION

Bacterial spores and bacterial endotoxins

Figure 2 presents SEM images of untreated spores and spores exposed to the O_2 ICP discharge. As can be seen, the spores' sizes are significantly reduced after 2 min of plasma treatment. According to the analysis of a statistically relevant number of spores done on the SEM picture, it has been found that, in these conditions, the mean length of spores is reduced from about 1.73 to 1.35 μm (see Fig. 3). Such length reduction corresponds to an etching rate close to 100 nm/min and is substantially higher than the ones obtained using other pure gases (data not shown).

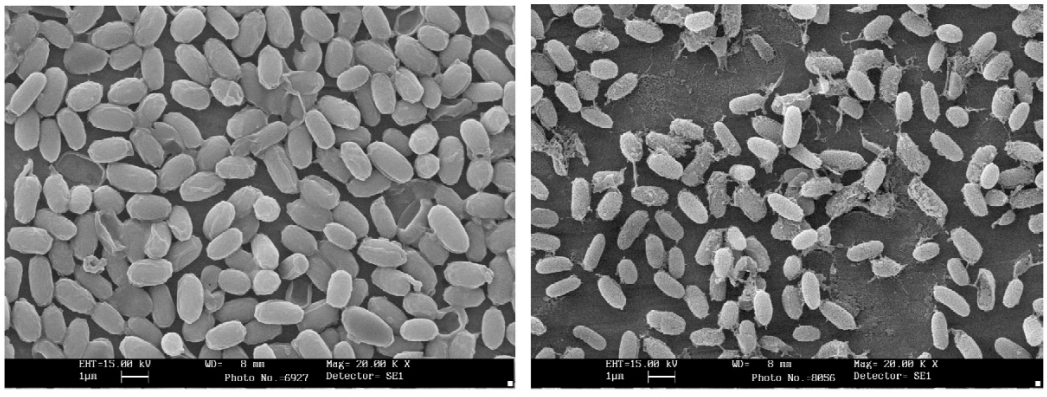

Fig. 2 SEM images of (A) untreated and (B) plasma-treated spores (magnification 20 KX).

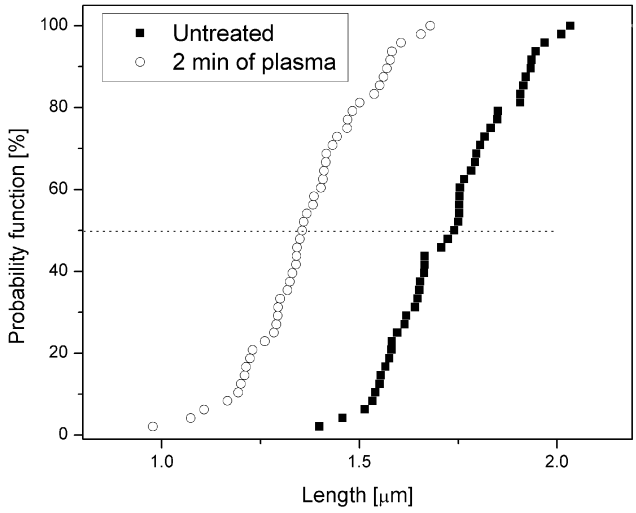

Fig. 3 Probabilities graph of spore lengths before and after plasma (plasma treatment 2 min, 500 W, 10 sccm, 13.3 Pa).

Nevertheless, it has to be noted that subsequent plasma treatment does not lead to further substantial decrease of the spores' sizes. This effect, observed also by other groups (e.g., [11,17]), is most likely connected with the increasing density of nonvolatile compounds (e.g., calcium, sodium, etc.) on the spores' surfaces, forming a resistant layer, a phenomenon that we have also found on protein films as will be shown later.

In order to demonstrate the capabilities of our O_2 low-pressure discharge not only to affect bacterial spores but also to remove bacterial endotoxins, further experiments were performed using PGN and lipid A. PGN was chosen in this work due to its high resistance to plasma treatment. In former experiments with a remote microwave post discharge, it had been impossible to inactivate PGN films even for a duration treatment of 30 min while other pyrogens (LPS or lipid A) obtained from different bacterial strands could be inactivated or removed easily in 10 min or less [37]. However, the results of imaging ellipsometry depicted in Fig. 4 showing 2D maps of variations of the phase shift (Δ angle) reveals that it is possible to significantly decrease the amount of both lipid A and PGN deposited on the surface within 2 min of plasma treatment employing oxygen ICP discharge, i.e., treatment duration demonstrated previously to be sufficient for the complete inactivation of bacterial spores [18]. This finding is of a great significance since it indicates the possibility of removing also bacterial endotoxins in treatments compatible with a sterilization process.

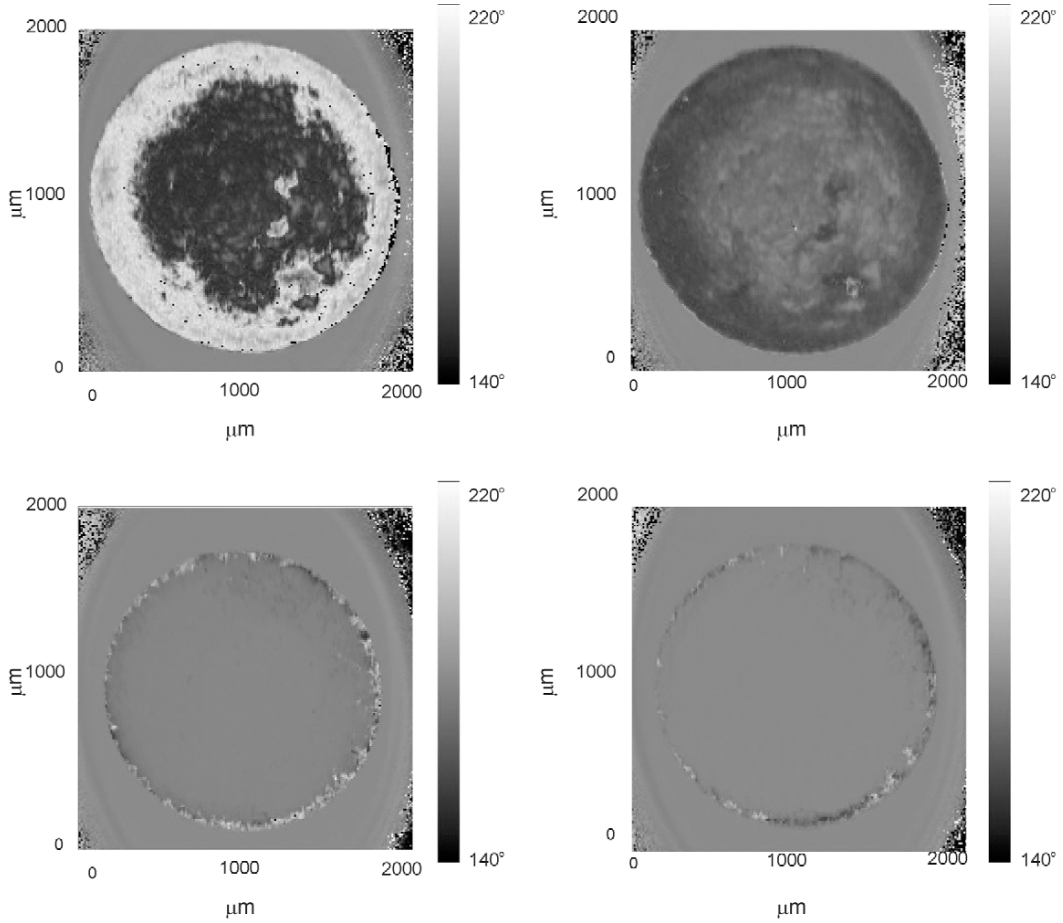

Fig. 4 Δ maps of lipid A (upper) and PGN (lower row) samples before (left panel) and after 2 min of plasma treatment (right panel) in pure O_2 (13.3 Pa, 10 sccm, 500 W).

Proteins

On the as-deposited samples, a well-defined structure (the coffee-ring effect) was observed by ellipsometry on the surface. As can be seen in Fig. 5A, two different regions can be clearly distinguished. At the border of the dried droplet, a relatively thick ring is formed, whereas in the central region, the coating is much thinner. Stylus profilometry showed that the ring has a height of approximately 1 μm, whereas the central region is less than 100 nm thick. After the plasma treatment, the ellipsometric images are markedly altered, which is especially true for the Δ values, as demonstrated in Fig. 5B. These modifications are clear indications that the deposit thickness decreases during the plasma treatment.

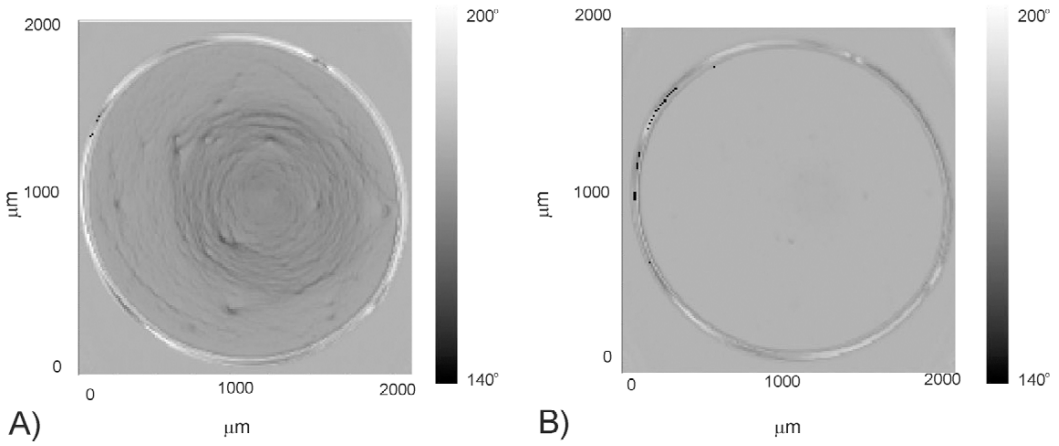

Fig. 5 2D maps of Δ angles of BSA sample (A) before and (B) after 2 min of plasma treatment (13.3 Pa, 10 sccm, 500 W).

In order to confirm removal of the protein from the surface, energy-dispersive X-ray (EDX) spectra were taken at the protein ring at different treatment times, and the results are shown in Fig. 6. It can be seen that while the signals of C, N, and O decrease with treatment time, the Si signal originating from the underlying substrate increases, which is indicative of a progressive removal of the protein layer.

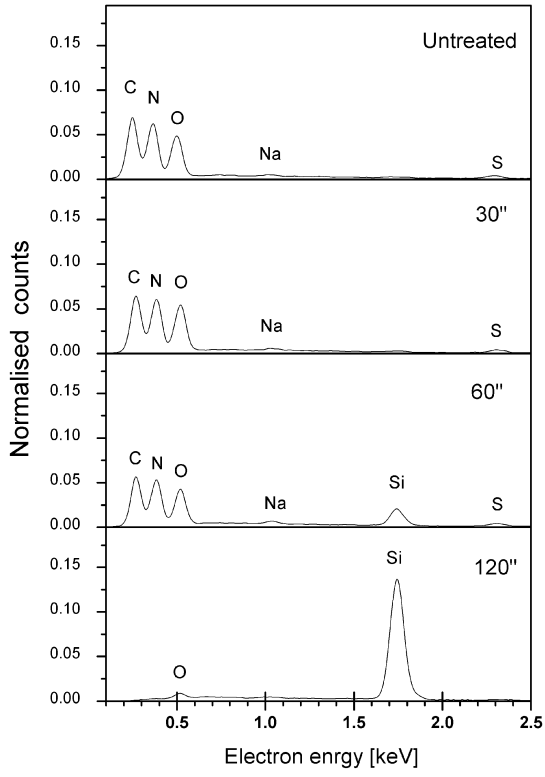

Fig. 6 EDX spectra taken on the protein film for different treatment duration (13.3 Pa, 10 sccm, 500 W).

Nevertheless, both imaging ellipsometry and EDX can provide us only with qualitative description of the protein removal. In order to obtain a quantitative estimate of the rate of this process depending on the operational conditions, stylus profilometry has been used for the evaluation of the height of the BSA ring after different treatment times. The results are summarized in Figs. 7 and 8. In Fig. 7, it can be seen that the removal rate increases with power, showing two regions exhibiting markedly different power dependences. These regions can be attributed to the E and H modes of ICP discharges.

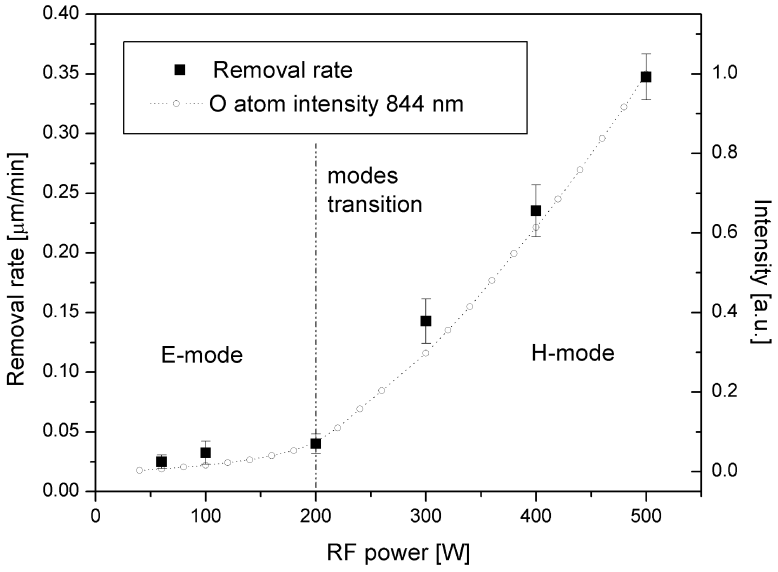

Fig. 7 Removal rate of the BSA film as a function of power (13.3 Pa, 10 sccm). The etching rates are measured on the ring of the droplet (initial thickness 1.3 μm).

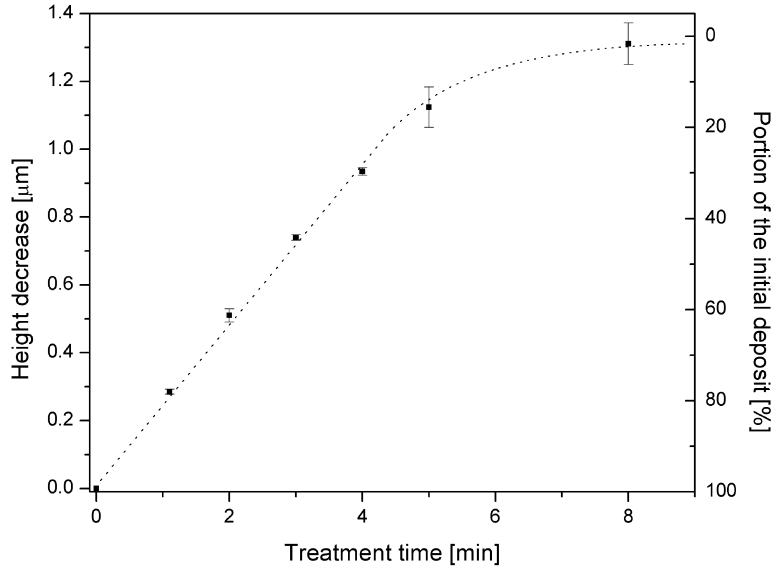

Fig. 8 Stylus profilometry measurement of thickness of the protein film as a function of time (13.3 Pa, 10 sccm, 500 W).

This observation is confirmed by the optical emission spectroscopy (OES) measurements of the O line at 844 nm, which is marked by the same trend (Fig. 7). The values of the removal rates observed in the E mode are, however, of the order of 150–350 nm/mn, i.e., larger than the one evaluated on the spores (100 nm/mn). A large range of removal rate values for biological material has already been observed in other works and is linked to the variation of composition of the materials treated [3].

Regarding the temporal evolution of the protein ring thickness, its linear decrease with treatment duration is observed until a certain point where a substantial slowing down of the process is observed (Fig. 8).

The effect of oxygen plasma treatment on protein samples was also evaluated by means of AFM. AFM pictures of the BSA films at different times of treatment are presented in Fig. 9. A strong change in the surface topography of the film can be observed as the plasma treatment proceeds. It can be seen that while the untreated sample is very smooth, with a roughness lower than 1 nm, after a short plasma treatment, the surface exhibits profound peaks and valleys, indicating a nonhomogenous etching of the protein film. For example, after 2 min etching, the peak-to-valley roughness is about 150 nm, while the overall decrease of protein ring measured for these conditions is about 700 nm. This suggests that the protein films are composed of different regions, with strongly different removal rates. Studies are ongoing to identify the protein components (e.g., α–helix, β-sheets…) which can account for these dif-

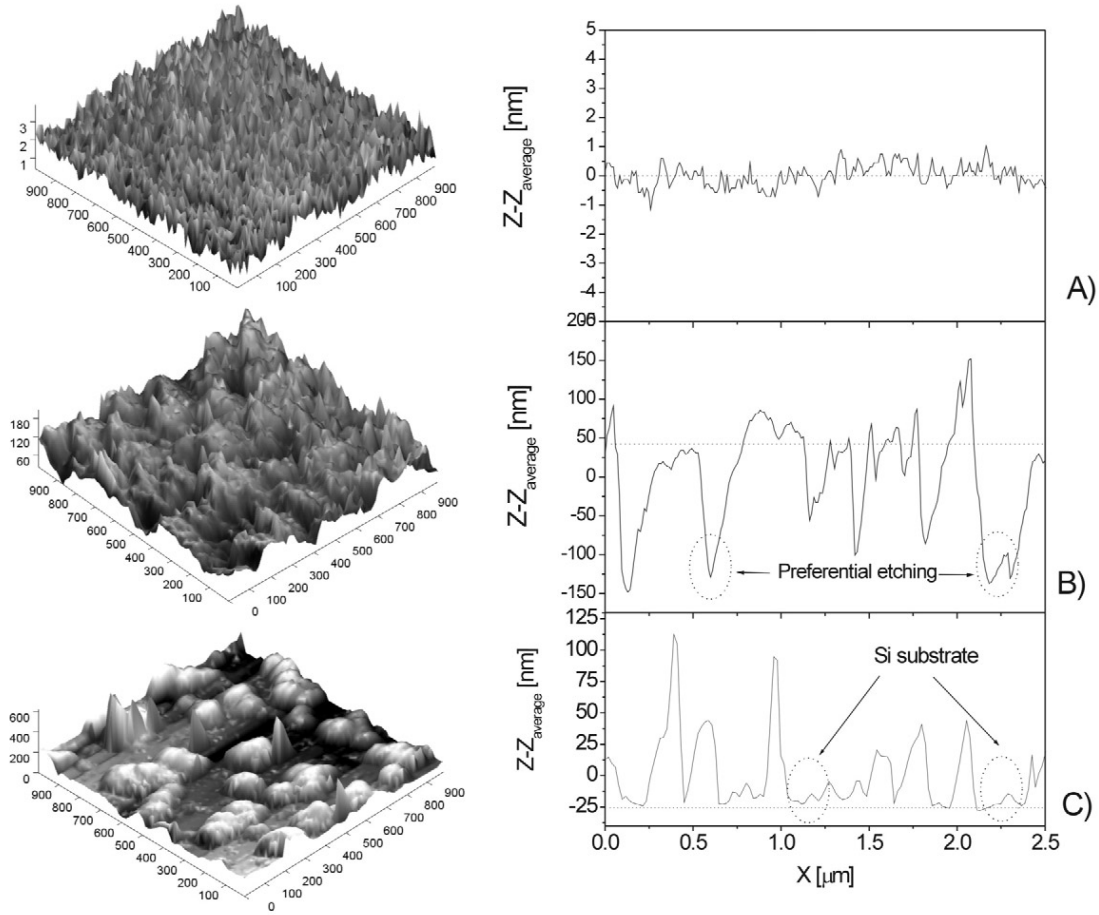

Fig. 9 AFM image of the surface (1 × 1 μm) of a BSA film at different times of O_2 plasma treatment (left panel) and corresponding measured roughness. (A) untreated; (B) 2 min; (C) 8 min (right panel).

ferences. After subsequent treatment, the apparent roughness decreases, due to the fact that the BSA film has been totally removed locally, letting the Si substrate appear underneath. However, even after 8 min of plasma treatment, localized islands of protein residues are still detectable.

The stylus profilometry and AFM results described above show that there is a residue on the surface that the plasma treatment does not succeed in removing (see Figs. 8 and 9). In order to understand this fact, TOF/SIMS analysis of the surface was done after the end of the experiment. Spectral images of the BSA deposits are presented in Fig. 10. It can be seen that after 8 min of treatment, the organic part of the BSA (as measured by the lysine peak $C_5H_{10}N+$, normalized atomic mass 84.085 [38]) has disappeared. On the other hand, the signals corresponding to the inorganic compounds (originating either from the native structure of protein or from the preparation of the protein solution) increase, showing the progressive enrichment of the surface in nonvolatile compounds, which in turn explains the reduction of the removal rate for long treatment duration.

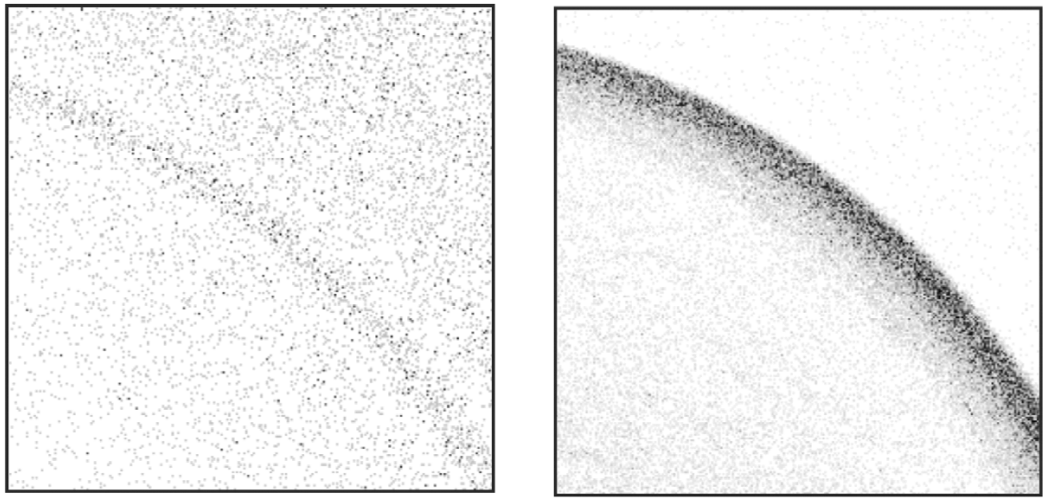

Fig. 10 TOF/SIMS maps of protein lysine peak, m/z = 84.085 A) and calcium peaks (m/z = 39.96) B) on the BSA sample treated for 8 min.

CONCLUSIONS

In this contribution, the potential of low-pressure, plasma-based sterilization and decontamination has been discussed with emphasis on the role of physical removal of the residuals of biological origin. According to the results presented, it can be concluded that bacterial spores as well as two different kinds of biomolecules exhibiting high resistance to traditional decontamination methods—proteins and bacterial endotoxins—can be largely removed from the treated surface by means of low-pressure ICP discharges sustained in pure oxygen. Nevertheless, complete removal of deposited materials was not achieved, even after substantial prolongation of the treatment time. This is caused predominantly by the build-up of inorganic nonvolatile compounds, forming highly resistant deposits that need to be washed or removed by plasma discharges sustained in different gas mixtures (currently tested hydrogen-containing ones). In spite of this phenomenon, whose relevance for the safety of medical instruments is a subject of ongoing investigations, the results presented are promising mainly in regard to the requirements posed recently by the medical services.

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