Sterilisation and decontamination by means of O_2:H_2 low pressure microwave discharge

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Ensuring sterility is a crucial requirement in different fields, which is in particular true in the medical praxis or pharmaceutical industry. However, it appeared that current sterilisation and decontamination techniques are either not capable to remove or inactivate at all various potentially harmful micro-organisms or substances of biological origin or only with inducing major damage to the sterilised substrate or tissue itself. From this point of view non-equilibrium discharges are considered to be promising alternative to commonly used techniques. The main aim of present contribution is to demonstrate feasibility and advantages of O_2:H_2 low pressure microwave plasma not only for destruction of bacterial spores, but also for deactivation of bacterial endotoxins or removal of protein films, e.g. substances difficult to remove by other techniques.

1. Introduction

Application of non-equilibrium discharges for sterilisation of various instruments and devices currently appeared to be promising alternative to commonly used sterilisation techniques. This is mainly due to their high efficiency at relatively low costs, possibility to maintain low temperature operation necessary for treatment of heat sensitive instruments frequently used in the medical praxis and pharmacological industry, and non-toxic operation reducing both environmental and occupational impacts. These key advantages naturally triggered extensive investigations of plasma interaction with biological samples pointing on the demonstration of the feasibility of plasma based sterilisation.

Regarding these studies it has been already demonstrated by many groups that non-equilibrium discharges are capable to reduce significantly number of bacteria and bacterial spores, i.e. the most resistant kind of living organisms, employing both low pressures plasmas (e.g. [1,2,3,4,5,6,7]) and discharges sustained at atmospheric pressure (e.g. [8,9,10,11,12,13]). The sterilisation process is in this case relatively well described and understood. It has been found that in usual configuration of the low-pressure discharges the overall sterilisation efficiency is driven predominantly by UV radiation capable to induce irreversible alternations of spores DNA and by the etching of spores, which is important to uncover spores shielded from the direct view of UV radiation. The later process has been furthermore identified to determine the total sterilisation rate in common situation of stacked bacterial spores [14] and therefore it implies necessity to employ discharges having high etching efficiency.

Moreover, it has been pointed out by several authors (e.g. [15]) that in many cases the sterility itself is not sufficient since certain fragments of death bacteria could be still pathogenic, which is primarily true in the case of bacterial endotoxins originating from the outer walls of bacteria or spores and which are capable to invoke immune reaction of a human body with severe consequences (e.g. fever, sepsis, multiple organ failure).

Another family of potentially harmful biomolecules represent proteins, which can be transmissible agents of serious neurodegenerative diseases or to invoke allergic reactions; typical examples are prions responsible for Creutzfeldt-Jakob Disease in humans.

A common point to endotoxins and proteins is that they are extremely resistant to commonly used sterilisation methods. Furthermore the sterilisation methods based on the maximisation of the UV emission are not efficient to remove or de-activate them. The possible options for their de-contamination thus lies either on the modification of their chemical structure leading to the decrease of their bioactivity, or in their complete removal from surfaces. The second point, in analogy to the case of bacterial spores, requires a high etching rate.

The high etching rates of biomaterials are usually achieved in mixtures providing high amount of atomic oxygen, since O atoms can easily volatilise hydrocarbons. The main intention of current contribution is to demonstrate that this effect can be markedly enhanced by presence of hydrogen.

2. Experimental

2.1. Plasma treatment

Sterilisation and decontamination tests were carried out using the plasma reactor (developed and manufactured in-house) identical to the one used in our previous studies [14, 16] and schematically depicted in figure 1. It consists of a stainless steel cylindrical vacuum chamber (200 mm in diameter and 380 mm in length) equipped with several diagnostics windows and one port for the sample introduction. The processing chamber is connected to the gas inlet system, which is composed of MKS mass flow controllers (*F*) attached to the gas lines and it is evacuated by a primary pump and a roots blower.

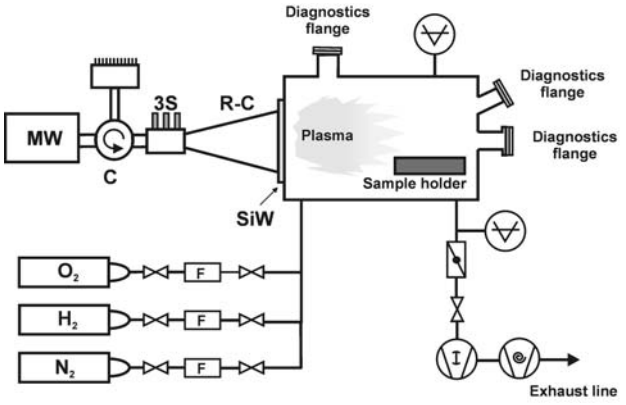

Figure 1. Schematics of plasma reactor

The plasma is sustained by microwaves with excitation frequency of 2.45 GHz. The microwave circuit includes the microwave supply (*MW*), a circulator (*C*) protecting the power supply from the reflected power, a three-stub impedance matching system (*3S*), and a rectangular-circular wave-guide transition (*R-C*). Microwaves are introduced in to the plasma chamber through a silica window (*SiW*) placed at the extremity of a circular 100 mm wave-guide.

The results presented were obtained at pressure 16 Pa, applied MW power 1000 W, and at total gas flow 100 sccm.

2.2. Bacterial spores

Spores of *Geobacillus Stearothermophilus* deposited on the stainless steel disks produced by Raven Biological Laboratories, INC. with declared spore's population 2.5×10^6 were employed as the bacterial indicators. After the plasma treatment, a thin gold layer (~ 10 nm) was deposited on the samples for SEM examination (LEO 435VP) in order to evaluate the degree of spores' destruction induced by the plasma discharge.

2.3. Bacterial endotoxins

For the depyrogenation experiments, lipopolysaccharides (LPS) derived from *E.coli* were used. To create a controlled surface contamination by pyrogens, 24-well-plates (Becton Dickinson S.p.A., Buccinasco–MI, Italy) were incubated with 100 µl of LPS diluted to a final amount in a range of 10-0.1 ng/ml. The plates were dried overnight in a common flow-hood, exposed to the plasma discharge and afterwards processed with the whole blood incubation [17] the next day in order to estimate remaining biological activity of the deposit. All accessories and solvents were used in pyrogen-free quality.

2.4. Protein films

The efficiency of plasma treatment in terms of proteins removal has been studied on BSA (Bovine Serum Albumin, Sigma Aldrich) deposited on one side polished Si wafers. The drops of 0.5% water solution of BSA has been deposited on Si surface by means of sterile syringes and allowed to dry at ambient temperature in a common flow hood. Such prepared samples have been afterwards treated by plasma and examined by means of different diagnostics methods, namely profilometry (Aplha-step), imaging ellipsometry (EP^3, Nanofilm Surface Analysis GmbH), AFM (Solver P47H from NT-MDT Co.) and XPS (AXIS ULTRA Spectrometer, KRATOS Analytical) in order to evaluate both their morphological changes and changes in their chemical composition.

3. Results

3.1. Destruction of bacterial spores

In order to evaluate effect of hydrogen addition to oxygen discharge for etching of bacterial spores, *Geobacillus Stearothermophilus* spores were treated in O_2, O_2/N_2 and O_2/H_2 discharges under otherwise the same experimental conditions. As can be seen in figure 2, the most potent mixture within the tested ones in terms of spores etching is O_2/H_2. According to the statistical analysis of SEM images of spores before and after treatment the spores mean size reduction after 1 min of plasma in this mixture reaches 55%, whereas other tested discharges leads for the same treatment time to reduction lower than 5%.

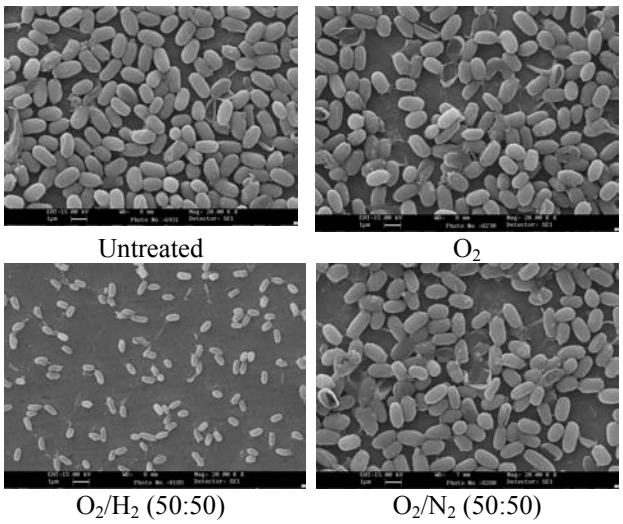

Fig 2. Scanning electron microscope images of untreated and plasma treated bacterial spores (Applied MW power 1000 W, treatment time 1 min, pressure 16 Pa, total gas flow 100 sccm).

3.2. LPS deactivation

Regarding the deactivation of lipopolysaccharides it was found that all tested discharges are capable to induce significant decrease of their bioactivity expressed in terms of amount of cytokines release when brought in to contact to human whole blood. However, as can be seen in figure 3 the efficiency of the treatment is almost one order of magnitude higher in the case of O_2/H_2 mixture compared to the O_2 or O_2/N_2 ones, which similarly to the case of bacterial spores etching shows clear advantages of this particular mixture.

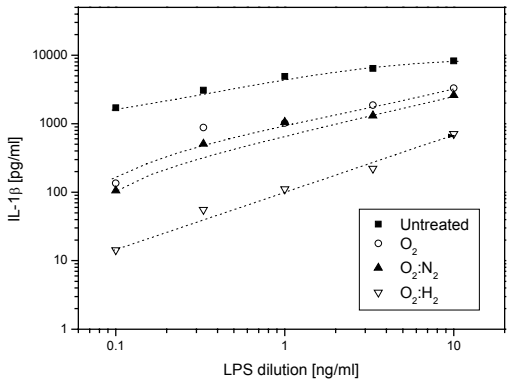

Fig. 3. Comparison of the plasma treatment efficiency LPS in different discharges measured by the human whole blood incubation and following IL-1β ELISA. (Applied MW power 1000W, treatment time 1 min, pressure 16 Pa, total gas flow 100 sccm).

3.3. Removal of protein films

Regarding the BSA deposit before plasma treatment it has been found that the chosen protocol of samples preparation results in highly spatially inhomogeneous organisation of BSA on Si wafer. As can be seen in figure 4, there can be distinguished two regions – the border of the drop with a high amount of protein and the central part, were only small portion of BSA has been deposited. According to the profilometric measurements the thickness of these two parts is different by more than one order of magnitude (typical height of the border is μm, whereas the central part is not higher than ~ 50 nm).

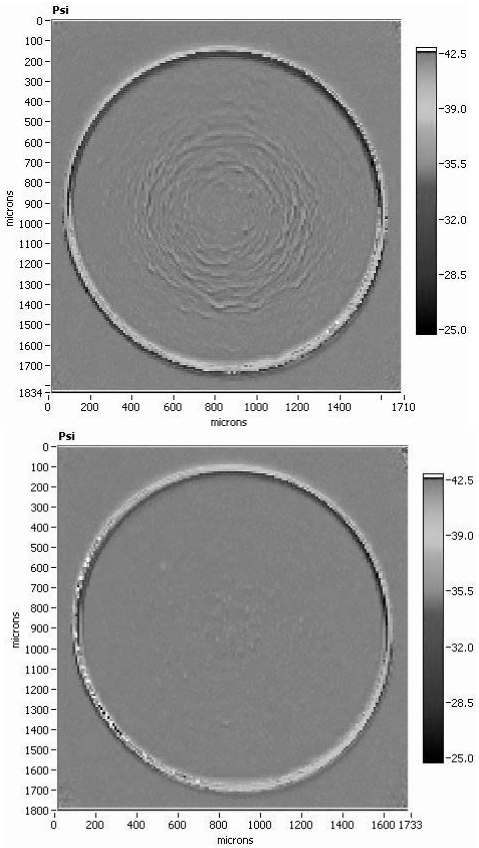

Fig. 4. Elliposmetric image of BSA before and after plasma treatment in O_2/H_2 50:50 discharge.

After a plasma treatment the BSA samples are markedly modified; from the central part of the drop is BSA almost completely removed (figure 4) and the thickness of the border is significantly decreased, which is accompanied by the increase of its roughness and alternations of chemical composition indicating strong oxidation observed by XPS. However the rate of BSA removal is again most pronounced in the O_2/H_2 mixture as can be seen in figure 5 showing temporal decrease of height of the drop border observed after the plasma treatment.

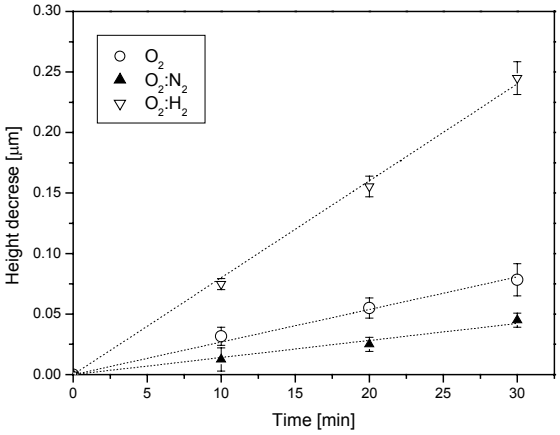

Fig. 5. Decrease of the BSA thickness after a plasma treatment (16 Pa, 100 sccm, and applied MW power 1000W)

4. Conclusions

According to the results presented, it can be concluded that the low-pressure microwave discharges sustained either in pure O_2 or in its binary mixtures with N_2 or H_2 are capable to destroy bacterial spores as well as to remove or substantially modify/deactivate model biological substances. Furthermore, it has been demonstrated that application of oxygen-hydrogen mixture accelerates process efficiency compared to the other discharges and thus represents interesting option for both sterilisation and decontamination.

5. Acknowledgements

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6. References

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