Antibiofilm effect of argon plasma jet

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Abstract: Exposure to non-thermal plasma is an alternative method for treating superficial wounds and skin infections. When designing plasma sources for these medical applications, a key is to have an understanding of the interactions between plasma-borne species and microbial biofilms. The goal is to investigate the effect of ultra violet (UV) radiation and reactive oxygen species on these interactions. In this work a double barrier discharge (DBD) argon plasma jet is used to treat biofilms (formed by *Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans*) under different conditions including source-to-sample distance and treatment time. The bacterial biofilms were found to be more susceptible to plasma exposure than *C. albicans* biofilms. In the former group, survival rates were lower for the Gram-positive *S. aureus* than for the Gram-negative *P. aeruginosa* biofilms.

Keywords: plasma jet, sterilization, biofilm

1. Introduction

The inactivation of harmful microorganism can be achieved by physical or chemical means. Most microorganisms present in clinical and industrial environment are growing in a biofilm, i.e. attached to a surface and embedded in a self-produced extra-cellular matrix. Biofilm cells are intrinsically more resistant to antimicrobial agents such as antibiotics and disinfectants, compared to their planktonic counterparts [1]. There are different proposed mechanisms for biofilm resistance to antimicrobial agents. The antimicrobial molecule must defuse into the matrix biofilm in order to inactivate the covered cells. The matrix may act as a barrier influencing the rate of transport through the biofilm and by reacting with antimicrobials. In addition, the resistance of biofilms to antimicrobials could be a result of slower growing of biofilm cells compared to planktonic cells [1].

Resistance of biofilms to antimicrobials led to development of new techniques for killing of biofilms. Among them non-equilibrium atmospheric pressure plasma showed great promise. Plasma exposure causes killing of bacteria by one of four known factors or by synergistic combination of them. These factors are heat, UV radiation, charged particles and reactive neutral species. The extent of the influence of each factor depends on the plasma operating parameters such as structure of the plasma device, power, gas mixture, and flow rate. Cold atmospheric pressure plasmas are of particular interest for treatment of temperature sensitive material and living organism. In this type of plasma, the neutrals and ions remains relatively cold while electron temperature is typically a few electron volt. Although plasma does not cause any thermal effects, the electron temperature is sufficient to drive chemical reactions for modifying DNA, proteins and cell membrane. Additionally, cold atmospheric pressure plasma can be used without complication and cost of a vacuum chamber. In this paper, first an overview of the operating parameters of the plasma device which has been used to inactivate bacteria is presented. Then the preparation of the samples, treatment and studied parameters are discussed. Finally, the prospect of using cold atmospheric pressure plasma jet to inactivate microorganisms is outlined.

2. DBD argon plasma jet

The plasma device consists of a quartz capillary with inside and outside diameters of 1.3 and 3.0 mm, respectively. It is partially surrounded by a cylinder ground electrode with height of 10 mm. The high voltage electrode, a tungsten rod of 0.5 mm diameter, is placed inside the capillary. The distance between the ground electrode and tip of high voltage electrode is 40 mm and the ground electrode is placed 20 mm away from the edge of the capillary. The electric field is in the same direction as the gas flow which indicates electron energy is higher than cross-field source (jet comparison).

Plasma can be generated in argon and helium with a small amount of reactive gases such as oxygen. We used argon which requires about 43% less input energy and consumed merely 11% of the gas volume required in a helium plasma jet for the same sterilization efficiency. Therefore, it is more cost-efficient to use argon plasma. Moreover, since, the gas temperature of the argon plasma jet is relatively low, the living tissue would be protected from thermal damage. The electron density in argon plasma jet is 2-2.5 times more than in helium plasma which can produce more excited metastable species such as $O^*(^1D)$ and radicals such as $O(^3P)$. These species are very important in destruction of microorganism [2].

The gas flow was controlled by mass flow controllers model MKSPR20. Plasma jet is generated on sinusoidal wave voltage at fixed frequency of 50 kHz. The applied voltage peak to peak value varies between 8.0 to 10.0 kV. The plasma jet temperature is between 60 and 40 $^{\circ}$C dependent on the distance from the capillary nozzle.

3. Preparation of the samples

Some opportunistic bacteria can be found frequently in wound infections. Among them are *S. aureus* and *P. aeruginosa* which are often multidrug resistant. These two microorganisms are representative for Gram-positive and Gram-negative bacteria, respectively. *C. albicans* is used as a sample for eukaryotic cells (yeast).

To prepare the biofilm sample an overnight liquid culture has been diluted to contain approximately 10^8 CFU/ml (bacteria) or 10^7 CFU/ml (yeast). TSB is used for *S. aureus* and *P. aeruginosa* and SAB for *C. albicans*. For each biofilm experiment, a round-bottomed polystyrene 96-well microtiter plate (TPP, Trasadingen, Switzerland) is filled with 100 µl of these dilutions. Following 4 h of adhesion, the supernatant (containing non-adhered cells) was removed from each well and plates were rinsed using 100 µl physiological saline (PS). Subsequently, 100 µl of fresh medium was added to each well and the plates were further incubated for 24 h at 37 $^{\circ}$C. Before plasma treatment, the growth media is removed and biofilms are washed with PS. After exposure to plasma, a resazurine assay is used to quantify the bacteria [3]. Fluorescence (λ_{ex} :560 nm) was measured after 30-60 min of incubation at 37 $^{\circ}$C.

4. Result and discussion

In separate experiments, the contributions of main inactivation factors of cold atmospheric pressure plasma are studied. Temperature at the place of sampled was about 40 $^{\circ}$C which is low enough to use the plasma source in medical application.

a) UV

Although various kinds of UV lamps are used to sterilize surfaces, it is known [4] that UV radiation in the 200-300 nm wavelength range cause serious damage to cells. Radiation emitted from the plasma was measured using the Ocean Optics spectrometer (model S2000) at a distance of 1.5 cm from the plasma. It contains molecular bands of OH-radical and lines of excited argon atoms between 700 and 900 nm. There is no

detectable emission in the UV range between 200 and 400 nm.

b) Applied voltage

The sterilization effect of argon plasma has been studied with different applied voltages. While the applied voltage has been increased from 8.0 kV to 10.0 kV, power remains almost constant around 1 W. The result for *S. aureus, P. aeruginosa* and *C. albicans* shows that at a fixed distance and gas flow, the sterilization effect does not change significantly with with different voltages (figure 1).

c) Gas flow rate

Effect of gas flow rate on inactivation of biofilm is studied. The experiment has been done for *S. aureus* at d=8 mm and V=8 kV with flow rate changing from 0.5 to 2 slm. At a fixed distance and for a constant applied voltage, changing flow rate does not have a significant effect on sterilization. Also, at 2 slm blowing of the bacteria by gas flow has been seen.

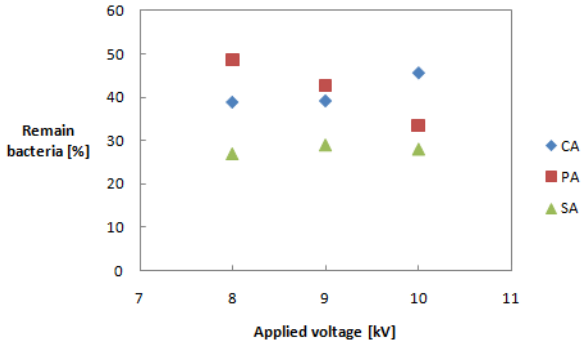

Figure 1 Sterilization effect of argon plasma jet with different applied voltage: gas flow rate =0.5 slm, distance= 10 mm, exposure time= 1 min.

d) Distance

The result for 1 min exposure to plasma generated by applied voltage of 8 kV and flow rate of 0.5 slm at different distance is presented in figure 2. The closest possible distance, which does not damage the sample is, 0.5 cm. if the distance is more than 2 cm from the edge of capillary, the observed effect is almost negligible. Therefore, it is important to have the sample as close as possible to the plasma. The sterilization effect is inversely proportional to the distance between the plasma and sample.

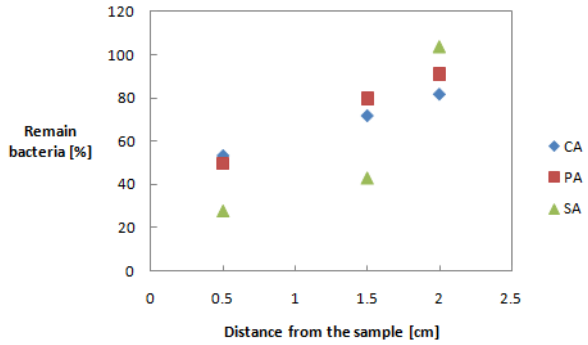

Figure 2 Sterilization effect of argon plasma jet at different distance from the sample: gas flow rate =0.5 slm, applied voltage= 8 kV, exposure time= 1 min.

e) Exposure time

The sterilization effect is more pronounced when the exposure time is increased. However, a long exposure time is not possible at short distance because it may cause damage to the sample plate. Therefore, an optimization of distance and exposure time was needed. Figure 3 shows the effect of exposing *S. aureus* to plasma at 10 mm from the edge of capillary and for different time. The antibiofilm effect was proportional to the exposure time. For shorter distance, the exposure time should be shorter which results in lower killing effect.

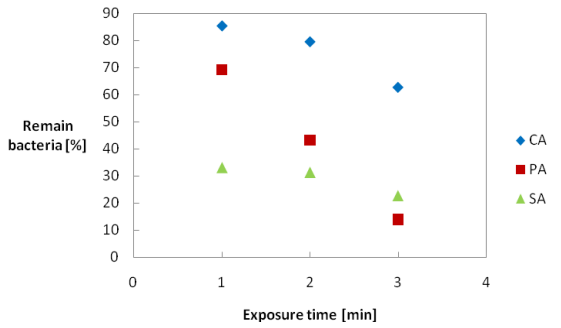

Figure 3 Sterilization effect of argon plasma jet for different exposure time: gas flow rate =0.5 slm, applied voltage= 8 kV, distance from the sample= 10 mm.

5. Conclusion

In this paper, some of the effective parameters of cold atmospheric pressure plasma on microorganism have been discussed. By exposing biofilms to plasma, about 90% inhibition is observed. The results are promising and point to the possibility of success in the use of cold plasma for inactivation of biofilm cells. Exposure time and distance to the samples are important factors to be optimized. The inactivation was not due to UV or heat, as these factors are not present at the place of samples. To optimize the condition, it is important to find out which plasma species are responsible for inactivation mechanism. Charged particles are suggested to play an important role in the rupture of the outer membrane of bacterial cells. Mendis *et al*. [5] and Larousi *et al*. [6] showed that the electrostatic force accumulated on the outer membrane could overcome the tensile strength of the membrane and cause it to rupture. Cell membranes are susceptible to attacks by hydroxyl radical (OH). Proteins present in the membrane are susceptible to oxidation by atomic oxygen or metastable oxygen molecules. It can alter the passage of macromolecules in and out of cells which is controlled by proteins.

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