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EFFECTS OF AIR PULSED STREAMER CORONA DISCHARGE ON SINGLE AND MIXED SPECIES BIOFILMS OF *S. AUREUS* AND *P. AERUGINOSA*

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The pulsed streamer corona discharge generated in ambient air was applied to single- and mixed-species bacterial biofilms of different growth stages. Both types of biofilms underwent significant losses of biofilm biomass, suppression of metabolic activity, and removal from polystyrene or glass substrates. Despite the strong immediate effect on bacterial viability, the regrowth of biofilms on the next day showed a inadequacy of the used plasma treatment. Also, hydrated form of biofilms was found more resistant than dry-surface one. The gaseous species including O_3 and NO_x combined with the etching of biofilms are probably the main factors responsible for overall antibiofilm eradication induced by the pulsed streamer corona discharge plasma.

1. Introduction

The existence of microbial biofilms in various aspects of the human community causes severe economic and health-related issues. Cells in biofilms demonstrate enhanced resistance to stressful environmental conditions than planktonic bacteria and require higher concentrations of disinfectants to inactivate on surfaces. Biofilms with harmful effects can be eradicated by physical and chemical methods [1]. Cold plasma represents one of the alternative methods for biofilm infection control [2]. It is believed the plasma treatment combine both physical (ion bombardment, electroporation, electrostatic disruption) and chemical (oxidative and nitrosative stress) mechanisms. Besides damage to DNA, proteins, and cell membrane as main targets of reactive oxygen and nitrogen species (RONS), the etching effect and interference of quorum sensing have been reported among antibiofilm mechanisms of plasma treatment [3]. There is a number of challenges to biofilm destruction by cold plasma, such as heterogeneity in biofilm extracellular polymeric substances, its thickness, O_2 content, nutrient gradients, microbial species, persister cells, and location of the biofilm. The adequate treatment of such coordinated communities of bacteria is crucial to avoid the appearance of more adaptive biofilms, persister cells, or resistance to RONS generated by plasma. Yet, knowledge of complex network of interactions between bacteria in mixed-species biofilms as well as plasma-biofilm inactivation pathways is limited. The objective of this work was to investigate the effects of the pulsed streamer corona discharge plasma on single- and mixed-species biofilms of clinically relevant bacterial species *S. aureus* and *P. aeruginosa*.

2. Experimental setup

The pulsed streamer corona discharge was used for the treatment of biofilms (Fig.1). The discharge was generated in ambient atmospheric air between the tip of the needle electrode and the surface of the grounded electrode. The biofilms on the polystyrene or glass substrate were directly placed between the electrodes and treated by the discharge. The discharge was driven by a high voltage (HV) pulse generator (*FID Technology* FPG 20-10NM10) and its electrical characteristics are monitored by a HV probe (*Tektronix* P6015A) and Rogowski type current probe (*Pearson Electronics* 2877) connected to a digitizing oscilloscope (*Tektronix* TBS 2000). The applied voltage was set at 10 kV at 1 kHz.

The effects of plasma treatment were evaluated against models of 24-h / 48-h old single– and mixed–species biofilms of Gram-positive *S. aureus* and Gram-negative *P. aeruginosa*. Bacteria viability, biofilm biomass, metabolic activity, regrowth of biofilms after treatment, and intracellular ROS accumulation were analyzed. Standard microbiological, spectroscopic, and fluorescence technics were used. Additionally, the gas-phase chemistry of the discharge was evaluated by emission and absorption spectroscopy.

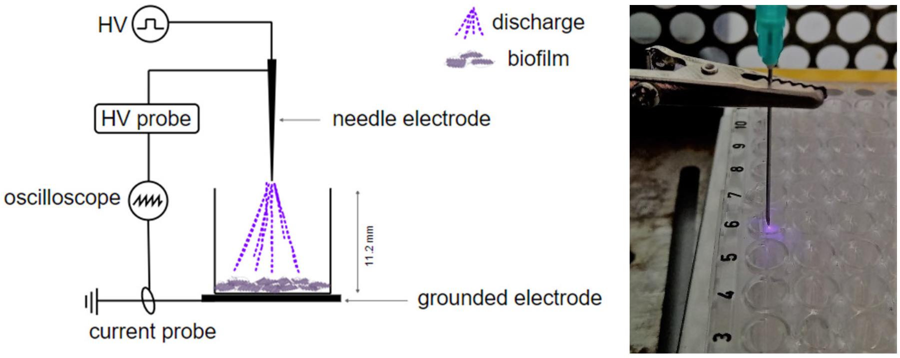

Fig. 1. Schematic diagram (left) and photograph (right) of the pulsed streamer corona discharge plasma.

3. Results and discussion

The pulsed streamer corona discharge plasma resulted in an effective biofilm inactivation (*P. aeruginosa* – complete inactivation, *S. aureus* ~ 4 log reduction). Both *S. aureus* and *P. aeruginosa* displayed higher resistance to plasma treatment in mixed-species biofilms compared to their single forms. A higher reduction in metabolic activity of bacteria was achieved for more mature 48-h biofilms. The fluorescence microscopy showed evident biofilm eradication from the glass substrate even at the shortest (30 s) treatment time. Interestingly, a significantly stronger inactivation was found for dry-surface biofilms compared with hydrated biofilms (Fig.2). Supposedly, plasma did not generate enough long-lived RONS in water responsible for bacterial lysis. Moreover, O_3 and NO_2 were dominant gaseous species generated by the discharge that seemingly contribute to the predominant etching mechanism of biofilm destruction.

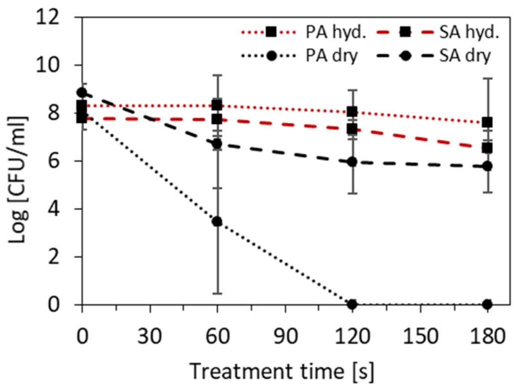

Fig. 2. Survival of 24-h old single species biofilms as a function of plasma treatment time.
(PA - *P. aeruginosa*, SA – *S. aureus*; hyd. – hydrated, dry – dry-surface biofilm).

4. Conclusion

Air streamer corona discharge has shown the ability to efficiently eradicate bacterial biofilms. The results showed the plasma did not lead to the complete inactivation of bacteria within biofilms since they regrew again the next day. A thorough fundamental understanding of cold plasma physicochemical properties and revealing of plasma-induced biological responses are required for the safe application of plasma in biomedicine.

5. Acknowledgments

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6. References

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