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Antimicrobial and Anti-Biofilm Activity of Non-Thermal Plasma

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Abstract : Microbial colonization of medical instruments, catheters, implants, etc. is a serious problem in the spread of nosocomial infections. Biofilms exhibit enormous resistance to environment. The resistance of biofilm populations to antibiotic or biocides often increases by two to three orders of magnitude in comparison with suspension populations. Subjects of interests are substances or physical processes that primarily cause the destruction of biofilm, while the released cells can be killed by existing antibiotics. In addition, agents that do not have a strong lethal effect do not cause such a significant selection pressure to further enhance resistance. Non-thermal plasma (NTP) is defined as neutral, ionized gas composed of particles (photons, electrons, positive and negative ions, free radicals and excited or non-excited molecules) which are in permanent interaction. In this work, the effect of NTP generated by the cometary corona with a metallic grid on the formation and stability of biofilm and metabolic activity of cells in biofilm was studied. NTP was applied on biofilm populations of Staphylococcus epidermidis DBM 3179, Pseudomonas aeruginosa DBM 3081, DBM 3777, ATCC 15442 and ATCC 10145, Escherichia coli DBM 3125 and Candida albicans DBM 2164 grown on solid media on Petri dishes and on the titanium alloy (Ti6Al4V) surface used for the production joint replacements. Erythromycin (for S. epidermidis), polymyxin B (for E. coli and P. aeruginosa), amphotericin B (for C. albicans) and ceftazidime (for P. aeruginosa) were used to study the combined effect of NTP and antibiotics. Biofilms were quantified by crystal violet assay. Metabolic activity of the cells in biofilm was measured using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) colorimetric test based on the reduction of MTT into formazan by the dehydrogenase system of living cells. Fluorescence microscopy was applied to visualize the biofilm on the surface of the titanium alloy; SYTO 13 was used as a fluorescence probe to stain cells in the biofilm. It has been shown that biofilm populations of all studied microorganisms are very sensitive to the type of used NTP. The inhibition zone of biofilm recorded after 60 minutes exposure to NTP exceeded 20 cm^2, except P. aeruginosa DBM 3777 and ATCC 10145, where it was about 9 cm^2. Also metabolic activity of cells in biofilm differed for individual microbial strains. High sensitivity to NTP was observed in S. epidermidis, in which the metabolic activity of biofilm decreased after 30 minutes of NTP exposure to 15% and after 60 minutes to 1%. Conversely, the metabolic activity of cells of C. albicans decreased to 53% after 30 minutes of NTP exposure. Nevertheless, this result can be considered very good. Suitable combinations of exposure time of NTP and the concentration of antibiotic achieved in most cases a remarkable synergic effect on the reduction of the metabolic activity of the cells of the biofilm. For example, in the case of P. aeruginosa DBM 3777, a combination of 30 minutes of NTP with 1 mg/l of ceftazidime resulted in a decrease metabolic activity below 4%.

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