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Effect of Non-Thermal Plasma, Chitosan and Polymyxin B on Quorum Sensing Activity and Biofilm of Pseudomonas aeruginosa

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Abstract : Increasing the resistance of pathogenic microorganisms to many antibiotics is a serious threat to the treatment of infectious diseases and cleaning medical instruments. It should be added that the resistance of microbial populations growing in biofilms is often up to 1000 times higher compared to planktonic cells. Biofilm formation in a number of microorganisms is largely influenced by the quorum sensing regulatory mechanism. Finding external factors such as natural substances or physical processes that can interfere effectively with quorum sensing signal molecules should reduce the ability of the cell population to form biofilm and increase the effectiveness of antibiotics. The present work is devoted to the effect of chitosan as a representative of natural substances with anti-biofilm activity and non- thermal plasma (NTP) alone or in combination with polymyxin B on biofilm formation of Pseudomonas aeruginosa. Particular attention was paid to the influence of these agents on the level of quorum sensing signal molecules (acyl-homoserine lactones) during planktonic and biofilm cultivations. Opportunistic pathogenic strains of Pseudomonas aeruginosa (DBM 3081, DBM 3777, ATCC 10145, ATCC 15442) were used as model microorganisms. Cultivations of planktonic and biofilm populations in 96-well microtiter plates on horizontal shaker were used for determination of antibiotic and anti-biofilm activity of chitosan and polymyxin B. Biofilm-growing cells on titanium alloy, which is used for preparation of joint replacement, were exposed to non-thermal plasma generated by cometary corona with a metallic grid for 15 and 30 minutes. Cultivation followed in fresh LB medium with or without chitosan or polymyxin B for next 24 h. 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. Activity of N-acyl homoserine lactones (AHLs) compounds involved in the regulation of biofilm formation was determined using Agrobacterium tumefaciens strain harboring a traG::lacZ/traR reporter gene responsive to AHLs. The experiments showed that both chitosan and non-thermal plasma reduce the AHLs level and thus the biofilm formation and stability. The effectiveness of both agents was somewhat strain dependent. During the eradication of P. aeruginosa DBM 3081 biofilm on titanium alloy induced by chitosan (45 mg / l) there was an 80% decrease in AHLs. Applying chitosan or NTP on the P. aeruginosa DBM 3777 biofilm did not cause a significant decrease in AHLs, however, in combination with both (chitosan 55 mg / l and NTP 30 min), resulted in a 70% decrease in AHLs. Combined application of NTP and polymyxin B allowed reduce antibiotic concentration to achieve the same level of AHLs inhibition in P. aeruginosa ATCC 15442. The results shown that non-thermal plasma and chitosan have considerable potential for the eradication of highly resistant P. aeruginosa biofilms, for example on medical instruments or joint implants.

Keywords : anti-biofilm activity, chitosan, non-thermal plasma, opportunistic pathogens
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