Plasma effects on chronic infection models

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Résumé

Chronic infections, such as chronic ulcer and wound infections, lung and bronchoalveolar infections or infections of the urogenital tract represent the major concern for the modern therapy of infectious diseases. Causative agents of chronic infection are often resistant to the standard antimicrobial treatments. The resistance might be due to wide spreading of strains with multiple resistance to antibiotics, but as well due to changes in general metabolism that take place in bacteria during prolonged persistence in the human body. There are two major ways for bacteria extended survival, which are forming of biofilms and intracellular parasitism. Physical treatments present an alternative approach when effectiveness of chemical agents is weak due to natural pathogen or biofilm resistance. This study investigated the bactericidal effect of nonthermal plasma against bacteria in biofilms and inside eukaryotic cells.

The argon plasma source MicroPlaSter β (Tetsuji, 2008) was used that produced plasma by argon ionization with superhigh frequency electromagnetic field. To grow biofilms, Gram-negative bacteria *B. cenocepacia* and *P. aeruginosa* were used. A coverglass of 1.5 cm^2 in area was placed vertically into the flask where bacteria were cultured to allow a biofilm to form on its surface. The glass was removed after 72h, carefully washed with PBS and the biofilms were treated. Plasma or non-ionized argon treated *P. aeruginosa* biofilms were labeled with Live/Dead® cell viability kit (Invitrogen) providing a 2-color fluorescence assay of bacterial cells based of membrane integrity.

Gram-negative bacteria *Chlamydia trachomatis*, which are obligate intracellular pathogens were used as model intracellular microoganisms. *Chlamydia* were grown in the McCoy cells. A bactericidal effect of nonthermal plasma was studied at major stages of the chlamydial lifecycle including infectious extracellular elementary bodies (EBs) in water suspension; EBs attached to the cell surface during the process internalization into the cells; actively multiplying intracellular bacteria (reticulate bodies, RBs) that form in 24 h after infection; intracellular EBs formed in infected cells in 48 h after infection of the McCoy cells.

Biofilms rather than isolated bacterial cells represent a major form of bacterial persistence on the surface of both medical equipment and chronic wounds. In general, bacteria in biofilms are less sensitive to antimicrobial treatments (Davey, 2000). Dead bacteria prevailed in the plasma-treated biofilms while the argon-treated biofilms included more living than dead bacteria. Moreover, plasma-treated biofilms had higher concentrations of living bacteria at deeper layers compared to control, non-ionized gas treated biofilms. Presence of dead in bacteria in argon-treated biofilms was not related to an effect of non-ionized argon gas. Quantitative assessment of biofilm treatment reveal the bactericidal effect with only 0.005% to 2% of survivals.

Both extracellular and intracellular *Chlamydia* forms were highly sensitive to plasma treatment. About 0.01 % bacteria survived treatment. The noticeable effect of plasma treatments on intracellular bacteria raised the question concerning susceptibility of host eukaryotic cells to argon plasma. In 24 h after 2 minutes treatment, about 35 % drop in the amount of viable epithelial McCoy cells was observed in regarding to control cells. There was no difference in viability between infected and non-infected cells.

Thus, plasma might be effective in elimination of pathogenic bacteria in biofilms and within cells, which are main bacterial localizations during chronic infection process.

References

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