Colistin surface conditioning impairs *Pseudomonas aeruginosa* biofilm formation and enhances ciprofloxacin antimicrobial activity

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Bacterial biofilms on medical devices (MD) are commonly associated with persistent infections. Biofilm formation is central to pathogenesis due to the ability of the biofilm-entrapped bacteria to evade the host immune responses and the increased antimicrobial resistance phenotype often shown by sessile microorganisms. In order to control the infections related with MD, antimicrobial peptide conditioning of MD surfaces can be an attractive answer. Colistin (COL), an important cationic antimicrobial peptide (AMP) produced by *Bacillus polymyxa* strains, was used to coat polystyrene (PS) surfaces. This work aims at characterizing the antimicrobial effect of COL surface coating to impair *Pseudomonas aeruginosa* adhesion and subsequent biofilm formation. The response of *P. aeruginosa* 24 h biofilms treatment with Ciprofloxacin (CIP) in non-conditoned and COL-conditioned surfaces was also assessed

P. aeruginosa from collection (ATCC 10145) (PAC) and *P. aeruginosa* isolated (PAI) from a medical device (endoscope) were used as biofilm producers. PS surfaces were pre-conditioned with 64 mg/L of COL during 30 min. Biofilms were then developed in unconditioned and COL-conditioned surfaces, being after treated with Ciprofloxacin (CIP) at 0.75 mg/L. Biofilms were phenotypically characterized in terms of biomass, respiratory activity and cell number.

Results showed that, in general, the MD isolate produces biofilms with more mass and activity but less number of cells than the reference strain, being the action of COL conditioning or CIP treatment similar for both strains. The surface conditioning with COL was very efficient, as it impaired significantly biofilm formation in terms of mass and activity, allowing the adhesion of just 3-log of cells. The CIP treatment of biofilms developed in unconditioned and COL-conditioned surfaces, promoted reduction of biofilm mass, activity and 2-log of number of biofilm cells. Concerning the combined application of COL surface conditioning and biofilm treatment with CIP it was observed an increase in CIP efficacy in biofilm sanitation, especially regarding biofilm-entrapped cell reduction. In fact, the combination of conditioning/treatment promoted an accentuated reduction of the biofilm mass and activity and caused a reduction of 4-log of biofilm-entrapped cells.

This study demonstrates the potential use of COL surface conditioning since this surface treatment impairs biofilm formation, probably interfering in the transition from irreversible attached cells to mature biofilms. Moreover, and as consequence of the reduced amount of biofilms attached to COL-conditioned surfaces, adhered cells or thin biofilms become more exposed to the subsequent action of CIP. This study highlights a promising use of COL as MD coating and a synergistic effect between COL surface conditioning and CIP antimicrobial activity.

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