Blocking of *Pseudomonas aeruginosa* biofilm formation by a colistin coating

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Bacterial colonisation of indwelling devices followed by biofilm formation remains a serious threat in clinical field as it is commonly associated to persistent infections. Once adhered to a surface, bacteria embed themselves in a self-produced matrix mainly composed of extracellular polymeric substances which confers them protection against antimicrobial agents and the host immune system. Early bacterial adhesion is a crucial step in biomaterial associated infections pathogenesis, representing therefore a promising target for the development of biofilm preventive measures. Several strategies have been developed to prevent bacterial adhesion and biofilm formation on the surfaces of medical devices, based mainly on the use of anti-adhesive, antiseptic and antibiotic coatings. Although some of these coatings have been shown efficient in the prevention of biofilm formation, an important drawback associated to them is the development of microbial resistance that limits the usefulness of classical antimicrobials. A promising solution to overcome this problem may rely on the use of new alternatives, as antimicrobial peptides (AMPs) that are unlikely to induce any resistance because of their evolutionary path.

The aim of this work was to evaluate the potential role of colistin, a traditional AMP, as an antimicrobial coating for biomaterials. Based on the observation that the presence of colistin as a biofilm growth media complement was able to significantly impair *Pseudomonas aeruginosa* biofilm formation at concentrations below its MBC, polystyrene (PS) surfaces were coated with this AMP and its ability to prevent biofilm formation was assessed.

A *P. aeruginosa* reference strain (ATCC 10145) and a *P. aeruginosa* clinical isolate (U147016) were used as biofilm producers. PS surfaces were pre-coated with several concentrations of colistin and the biofilms formed on conditioned and clean surfaces were then characterized in terms of biomass (CV), respiratory activity (XTT) and number of viable cells (CFU). The susceptibility of biofilms formed on colistin-conditioned surfaces to ciprofloxacin (CIP) treatment was further investigated.

Results showed that the clinical isolate produces biofilms with more activity, less biomass and similar number of cells than the reference strain. Random deposition of colistin residues on the adhesion surfaces significantly reduced biofilm activity and mass accumulated in a dose-dependent manner for both strains. Regarding biofilm entrapped cells, the conditioning film proved to be less efficient, causing significant reductions for the highest concentrations tested. Concerning the combined application of colistin surface conditioning and biofilm treatment with CIP, it was observed that biofilms formed on colistin-conditioned surfaces were more susceptible to CIP treatment in terms of biofilm-entrapped cells. The presence of colistin during biofilm formation may have interfered in the transition from reversible to irreversible interactions during the early steps of bacterial adhesion to PS, disturbing and delaying the mature biofilm development.

Biomaterial associated infections remains a major drawback to the long-term use of medical devices. This study demonstrates the potential of the AMP colistin as an excellent candidate for biomaterials coating limiting biofilm formation on their surfaces.

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