

# CO-IMMOBILIZATION OF ANTIMICROBIAL LIPOPEPTIDE PALM AND DNASE I TO CREATE BI-FUNCTIONAL ANTIBACTERIAL COATINGS

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Bacterial colonization of indwelling devices is very often a prelude for biofilm formation and infection. Biofilm infections remain a clinical challenge with serious medical and economic consequences, due to their resistance to antimicrobials and to the host immune system. The development of novel approaches to fight biomaterial-associated infections (BAI) is, therefore, in great demand. Since bacterial adhesion to the surface of a biomaterial is the first step in biofilm formation, a number of surface modifications have been developed aiming to reduce the contact with approaching bacteria. These so-called anti-adhesive coatings are well known in the literature but none of them is able to completely prevent microbial adhesion. The next logical step to improve anti-adhesive coatings functionalities is to add moieties that prevent adhering bacteria from growing into a biofilm.

In this study, using a mussel-inspired coating strategy it was possible to introduce both anti-adhesive and antimicrobial functionalities on silicone material. The anti-adhesive moiety was assured by DNase I, an enzyme targeting an important component of biofilms matrix, and the antimicrobial component by the lipopeptide PALM-KGK-NH<sub>2</sub>. Silicone substrates were immersed in an alkaline solution of dopamine to form a thin layer of polydopamine and then transferred into a solution containing different proportions of the antimicrobial lipopeptide and the enzyme. Contact angle measurement and SEM analysis confirmed the immobilization of both compounds alone onto silicone. A fluorescamine assay indicated that the coating efficiency of peptide was about 65 % and it did not detach from the surface for up to 5 days. The mono-functional enzymatic coating was able to prevent *Staphylococcus aureus* adhesion while the coating functionalized with the antimicrobial lipopeptide was able to kill most of the adhered cells. Furthermore, cells adhered to these modified surfaces exhibited the same susceptibility pattern as cells adhered to unmodified surfaces, suggesting no development of resistance. The combination of both compounds resulted in a bi-functional coating able to prevent bacterial adhesion and kill the adherent ones. Similar results were obtained when co-adhesion of *S. aureus* and *Pseudomonas aeruginosa* was investigated. To better discriminate co-adhesion of both species on modified surfaces, PNA FISH (Fluorescence in situ hybridization using peptide nucleic acid probes) was further employed and results suggested that *P. aeruginosa* was the dominant organism with *S. aureus* adhering afterwards on *P. aeruginosa* agglomerates. A preliminary cytotoxicity assay on both mono-functional and bi-functional coatings has showed no toxicity towards mammalian cells. The overall results suggest that silicone functionalization with DNase I and the antimicrobial lipopeptide PALM holds great potential in the development of materials able to prevent BAI.

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