Long-term stability and effectiveness of antimicrobial coatings incorporating PSP-derived peptides

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Introduction

Infections are the most prevalent cause of failure for dental implants and orthopedic prostheses. The antimicrobial-peptide GL13K, derived from the Parotid Secretory Protein (PSP), showed bactericidal and bacteriostatic properties against putative pathogens associated with oral infections. We fabricated biofunctionalized Ti-surfaces by anchoring the antimicrobial GL13K-peptide using a simple and reliable chemical route. We already demonstrated that this novel coatings display bactericidal effect against *P. ginginvalis* for sustained periods of time; and retain good cytocompatibility. Here, we further studied the long-term mechanical and chemical stability of the antimicrobial coatings as infection of dental implants mostly occur after several months, sometimes years, after implantation. We also investigated its efficacy against other bacteria species.

Materials and methods

Coatings: Ti discs were ultrasonically-cleaned, activated by etching in NaOH (eTi), silanized with 3-chloropropyltriethoxysilane (eTi/CPTES), and coated with GL13K --NH_3-GKIIKLKASLKLL-C(O)NH_2— in Na_2CO_3 (eTi/CPTES/GL13K). Coatings of physisorbed peptides on etched (eTi/physi/GL13K), plain (pTi/physi/GL13K) surfaces and with two non-antimicrobial peptides, GK7 --NH_3-GQIINLK-C(O)NH_2-- (eTi/CPTES/GK7) and a scrambled version of GL13K (eTi/CPTES/Scram- GL13K) were also tested as controls. *Surface characterization*: Samples in each step of the fabrication process were characterized by advancing water contact angle (WCA), X-Ray photoelectron spectroscopy (XPS) and fluorescence labeling (FL). For the latter, fluorescent 5-FAM cadaverine was conjugated to GL13K and control peptides. After dialysis and purification, the fluorescent peptides were immobilized on silanized surfaces following the above protocol. Mechanical and thermochemical stability of the coatings were assessed by ultrasonication in distilled-water for 3 hours and immersion in PBS for up to 28 days at 37°C, respectively. Monitoring of the release of the FL-peptides as well as peptide retention on the surfaces over time were quantified using a fluoremeter and visualized using fluorescence microscopy. *Antimicrobial test*: *S. gordonii* were cultured on tested surfaces overnight. The antimicrobial effect of the coatings were characterized by ATP-bioluminescence and CFU. Ti/physi/Chitosan surfaces were tested as positive control.

Result and discussion

Ti discs were successfully biofunctionalized with the antimicrobial-peptide GL13K. The peptide coatings produced a hydrophobic surface with WCA above 125°. Presence of intense Nitrogen peaks in XPS spectra proved the presence of GL13K and full coverage of the titanium surface was further assessed by visualizing homogenous and intense fluorescent signal (FigA). The signal was significantly more intense for peptide coatings on silanized surfaces than on etched surfaces. The coatings were mechanically and thermochemically stable as WCA and N1s values did not significantly change after 3h sonication and 7 days in PBS, respectively. Furthermore, the profile of the release of peptides from the Ti surface over 28 days showed that GL13K peptides were robustly and continously retained on both etched and silanized surfaces (FigB). The robust peptide retention on eTi surfaces is due to the strong electrostatic attraction between etched surfaces and the highly-positively charged GL13K peptides. In contrast, GL13K physisorbed on plain Ti surfaces was vastly and mostly released during the first 3 hours of immersion in PBS. eTi/CPTES/GL13K surfaces had a significant antimicrobial effect against *S. gordonii* compared to the control surfaces ($p<0.05$, ANOVA+Tukey test). Cellular ATP and CFU assays showed similar antibacterial effect of GL13K coated surfaces as the chitosan coated positive control.

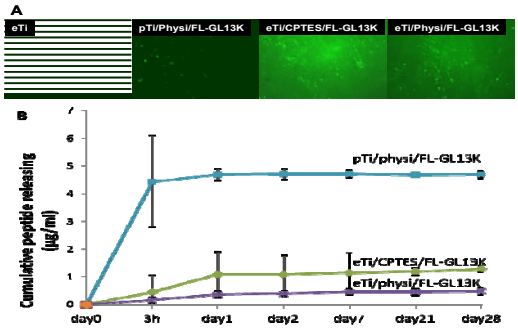

Fig. A-Images of coated surfaces with fluorescence-labeled peptides after 28 days in PBS at 37°C. B-Cumulative release of fluorescence-peptides in PBS solution during a 28 days period.

Conclusion

We have demonstrated a simple method to produce GL13K coatings with excellent mechanical and thermochemical stability over extended periods of time. These coatings have the potential to hinder detachment during surgical placement or under *in vivo* fluid flow forces while mantaining effective antimicrobial properties. We are using this same method to fabricate multi-functional coatings by co-anchoring peptides with different bioactivities.

The GL13K-coatings have shown notable antibacterial effect against various oral bacterial species while retaining appropriate cytocompatibility and thus, they are promising candidates for further studies to assess their *in vivo* efficacy against dental implant surface infections.