Mitigation of Biofilm Formation with an Elastomeric Barrier Membrane System

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Statement of Purpose: While barrier membranes can prevent alveolar bone resorption in dental extractions and periodontal defect grafts, infection and dehiscence of the overlying soft tissue are two frequent mechanisms of material failure. For defects in communication with the oral cavity, infection from biofilm-producing pathogens is particularly devastating, as implantable biomaterials can act as bacterial substrate. D-amino acids, such as D-tyrosine, have been found to mitigate biofilm production at concentrations as low as 50 µM [1]. To take advantage of this new class of antibacterials, we fabricated barrier membrane constructs (BMCs) from a biodegradable elastomer loaded with D-amino acids. This barrier membrane can be injected and photocrosslinked by dental curing light. By altering material crosslinking, the rate and dosage of delivered D-amino acid can be controlled.

The primary objective of this study was to develop a biodegradable barrier membrane system capable of tunable daily release of D-tyrosine at physiologically-relevant concentrations (at least 3 µM) over at least 4 weeks (the span of time in which oral mucosa is generally regenerated over defects), with the released D-tyrosine maintaining bioefficacy against *Staphylococcus aureus* (a common biofilm-producing pathogen). As substrate stiffness has been shown to affect local cell migration, adhesion, and differentiation [2], the secondary objective of this study was to produce BMCs with stiffness (as reflected by Young's modulus) closely approximating oral mucosa. BMCs that meet these design criteria represent a new, flexible platform for promoting soft tissue growth while specifically mitigating biofilm formation, without relying on traditional antibiotics.

Methods: Poly(glycerol sebacate) (PGS) was synthesized by the condensation of glycerol and sebacic acid (1:1 molar ratio). PGS was acrylated to produce poly(glycerol sebacate) acrylate (PGSA) by the addition of acryloyl chloride [3]. The degree of acrylation (low (L) and high (H)) was controlled by altering the ratio of acryloyl chloride to PGS. PGSA was mixed with the photoinitiator Irgacure 819 (1 wt%) and D-tyrosine at 0, 5, or 10 wt%. The mixture was injected into polytetrafluoroethylene molds and photocrosslinked for 80s with a commercially available dental blue light curing system to create BMCs (discs; 2 mm in height and 7 mm in diameter) or rectangular test specimens for mechanical testing (6 mm x 2.5 mm x 0.7 mm). Uniaxial tensile testing (EnduraTEC ELF 3200) was performed to measure Young's modulus and ultimate tensile strength (n = 6 per group). BMCs (n = 3 per group) were placed in 2 mL PBS (pH 7.4) and incubated at 37°C under mild agitation for 4 weeks. Supernatant was collected, replaced, and assayed by high performance liquid chromatography (Waters 2695) for released D-tyrosine concentration. *S. aureus* (ATCC 29213) was grown for 48 hours in a 1:1 mixture of the supernatant and Mueller Hinton Broth, and biofilm generation was measured using standard biomass crystal violet staining protocols.

Results: PGS was synthesized and acrylated to different degrees as confirmed by H^1-NMR. PGSA was photocrosslinked with and without the blending of D-tyrosine and presented Young's modulus within one order of magnitude of oral mucosa. All BMCs loaded with D-tyrosine achieved release of at least 9.06 µg per day (equivalent to 50 µM in 2 mL media) over 4 weeks. Increasing the degree of acrylation significantly decreased the rate of drug release, while increasing incorporation of D-tyrosine significantly increased cumulative drug release (Fig. 1).

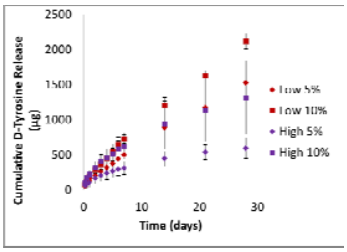

Figure 1. Cumulative release of D-tyrosine (Constructs that were not loaded showed no release)

By the end of 4 weeks, BMCs had degraded at least 20% by mass (no statistically significant trends) and increasing degree of acrylation significantly decreased swelling ratio, regardless of the amount of D-tyrosine incorporation. D-tyrosine released from the BMCs was bioactive and demonstrated efficacy in mitigating biofilms produced by *S. aureus* compared to no treatment and unloaded BMCs.

Conclusions: PGSA was blended with D-tyrosine and photocrosslinked to produce elastomeric barrier membrane constructs. These constructs achieved D-tyrosine release at appropriate dosage (over 9.06 µg daily) for a clinically relevant span of time (4 weeks). Release was controlled by altering the loading concentration and degree of construct acrylation. The released D-tyrosine remained bioactive and significantly mitigated biofilm formation by *S. aureus*. The constructs possessed a stiffness similar to oral mucosa. These injectable, biodegradable elastomers with tunable release are capable of both mitigating biofilm formation as well as providing a biomimetic substrate with mechanical properties similar to native tissue.

References:

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