Chitosan as an Antibacterial Coating: Tetracycline Release and Activity Against Staphylococci

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Statement of Purpose: Although success rates of dental implants are high, infectious complications can account for about 10 % of implant failures [1]. It has been hypothesized that the localized delivery of antibiotics, in conjunction with systemic prophylaxis, is beneficial in the treatment implant infection [2]. This strategy may be particularly useful to patients with periodontitis, and/or who smoke. Chitosan a natural bio-polymer has shown promise as a bioactive dental implant coating due to it's osteoconductive, and degradation properties [3]. While chitosan films are most often made using acetic acid solvents, other organic acid solvents can be used to modify the physiochemical properties of the coating material. The aim of this study was to determine the effect of different organic acid solvents on the release of an antibiotic from chitosan coatings on titanium.

Methods: Chitosan or tetracycline-loaded chitosan coatings were covalently bonded to commercially pure titanium coupons via silane-glutaraldehyde molecules[3]. Coatings were made by solution casting a 78% deacetylated chitosan (Agratech, MS), dissolved to 1 wt% solution in 1% acetic acid, lactic acid, or formic acid. The antibiotic-loaded coatings contained 20 wt% tetracycline. Controls did not contain tetracycline. Elution testing was performed to determine the amount of antibiotic released at 3, 6, 12, 24, 48, and 72 hrs, then every couple days up to day 28. Five replicate coated coupons were placed in 15 mL of phosphate buffered saline containing 500 mg/mL lysozyme, and agitated in a 37°C water bath. Solutions were sampled and renewed at each time point. Tetracycline concentrations were determined using UV-Vis spectroscopy at 320 nm. A bacterial zone of inhibition (ZOI) study was also conducted to assess the antibacterial activity of the coatings (n=5). A lawn of *staphylococcus epidermidis* was prepared on agar, and the coated coupons were placed on the agar with plain titanium and chitosan coatings used as negative controls. Comparisons between groups were performed using ANOVA and SNK tests.

Results / Discussion:

Drug elution from the films made from the three solvent acids in the first 24 hours was found to be significantly different ($p<10^{-5}$) (Fig. 1). Acetic acid coatings eluted the most tetracycline (131.8±21.1 µg/mL), followed by formic (64.9±26.1 µg/mL) and lactic acid coatings (27.0±6.25 µg/mL). Concentrations fell off most rapidly with lactic acid coatings, followed by formic and acetic acid. After 24 hours, the eluted concentration of drug was low (approx. < 1 µg/mL), and did not vary between coating types ($p>0.05$). Zones of inhibition for the three antibiotic coatings were significantly larger than controls, but not different from each other. The zones extended 15.6±1.1 mm showing that the concentrations were capable of inhibiting growth of bacteria in all cases. Titanium and chitosan coatings alone were unable to inhibit growth (Fig. 2).

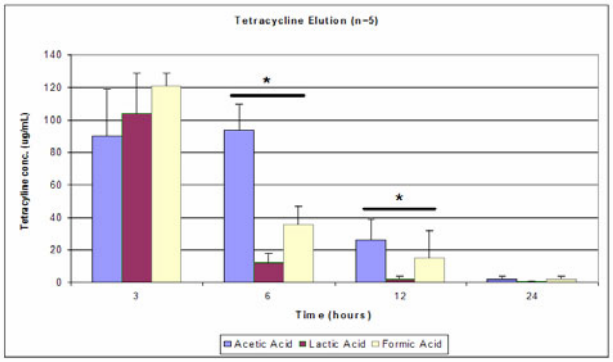

Figure 1 - Tetracycline Elution (µg/mL) from chitosan coatings on Ti made with different organic acid solvents (* indicates difference at the α=0.05 level)

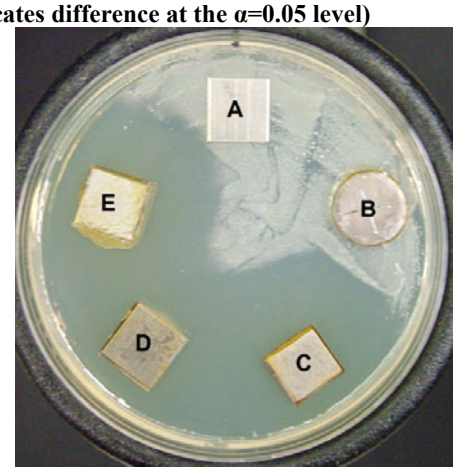

Figure 2 – Zones of inhibition produced against *staphylococcus epidermidis* a)Titanium, b) Chitosan Coated, c) Tetracycline (formic acid) Chitosan coating, d) Tetracycline (acetic acid) Chitosan Coating, e) Tetracycline (lactic acid) Chitosan Coating.

Conclusions: We have shown the ability of chitosan coatings to deliver tetracycline in a rapid manner. This burst delivery of tetracycline is capable of inhibiting growth of bacteria. The concentrations reached with these coatings are well above the minimum inhibitory concentration (MIC=4-12µg/mL) of tetracycline for the first 12 hours, before falling off to negligible levels. Delivery of tetracycline occurred most rapidly from coating made from lactic acid solvent, but the greatest amount of drug was eluted from acetic acid coatings. Future work includes testing of bond strengths, inhibition of biofilm formation, and cytocompatibility.

References:

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