Tobramycin and Vancomycin Release from Local Delivery Devices

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Statement of Purpose: Effectively treating infections resulting from bacteria strains present clinical challenges that are ongoing. These infections are commonly associated with orthopedic implants. Inhibiting the bacteria once formed has proven to be an arduous and costly excursion. Two of the more common strains are methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*. Antibiotics have shown success against one or the other but in combination no antibiotic has found success. The combination of vancomycin and tobramycin is being investigated to perceive if they are able to develop synergism against a colony consisting of both strains. The results of this study will directly impact a preclinical model proposed to utilize a local deliver device to counter the aforementioned strains *in vivo*.

Methods: Antibiotic-loaded sterile sponges (polyethylene glycol (PEG)/chitosan, acetic/lactic acid/chitosan, Bionova Medical (commercial) –Memphis, TN) were made by soaking the sponges for 1 minute in solution. Sponges were sectioned to average volumes of 703.88 mm^3, 1601.01 mm^3 and 2499.25 mm^3 respectively. The tobramycin and vancomycin concentration of the soaking solution was 5 mg/mL in sterile 1x PBS. Antibiotic-loaded polymethylmethacrylate (PMMA) bone cement (Simplex P with tobramycin, Kalamazoo, MI) beads were fabricated by adding 2.6 g of tobramycin and 1.0 g of vancomycin. Bone cement beads were fabricated in aseptic environment in accordance with clinical preparation to average volumes of 781.95 mm^3. All samples were submerged in sterile 40 mL 1x PBS and placed in 37 °C incubator. Eluates were sampled at designated time points and medium refreshed every 2 days for 10 days. Concentration of vancomycin in eluates was measured by absorbance at 470 nm with a BioTek Synergy H1 plate reader (BioTek, Winooski, VT, USA). Tobramycin concentration in eluates was measured by reacting 10-fold diluted samples with a mixture of acetylacetone and formaldehyde in a buffer solution of boric acid, acetic acid, and phosphoric acid at pH 2.7 and boiling for 20 minutes [1]. Fluorescence of the reacted product was measured at 471 nm (410 nm excitation) with a BioTek Synergy plate reader.

Results: The Bionova sponge displayed the highest burst release of both antibiotics initially. Concentration of vancomycin in eluates from the Bionova sponge showed steady release at approximately 20 µg/mL throughout the time of study (Figure 1). Tobramycin concentration in eluates declined after the initial burst in all sample groups except for the PMMA beads (Figure 2). The Bionova sponge displayed the ability to release at latter stages a concentration in eluates that may exceed the MIC. Sponges were all tested for their degradability by submersion in 35 mL of 1% antimycotic/antibiotic and 1 mg/mL lysozyme in 1x PBS. The results for all tested sponges were not significantly different with an approximate 10% weight loss over 10 days. PMMA beads have been investigated to demonstrate minimal degradability and thus were not further investigated during study.

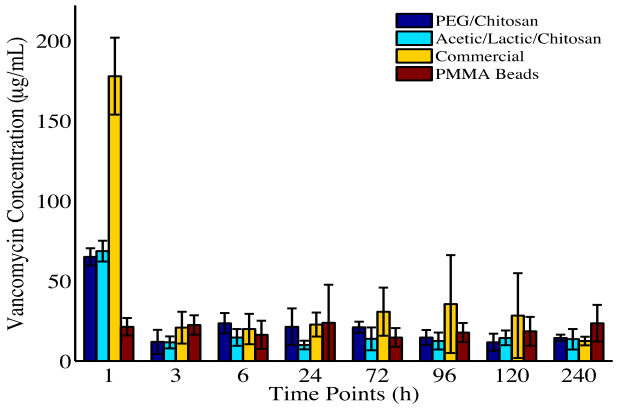

Figure 1. Concentration of vancomycin in eluates over the course of 10 days.

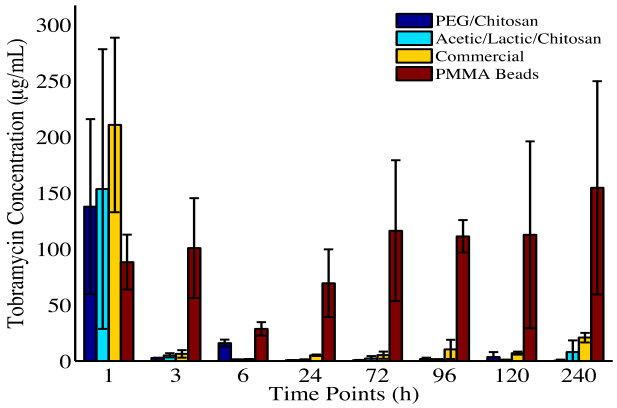

Figure 2. Concentration of tobramycin in eluates over the course of 10 days.

Conclusions: The release profile for the Bionova sponge shows promise in its ability to elute concentrations above MICs for vancomycin consistently over a period of time. The sponge also demonstrates the ability to release a concentration of tobramycin above the MIC at latter stages. The initial burst may lead to an inhibitory effect that is sustainable until the latter release of tobramycin. Further studies are needed to verify and validate the trends seen in this study. Equally loading all sample participants with an equivalent concentration of antibiotics should be explored to discern if the tobramycin concentration in eluates from PMMA beads are a result of the higher initial concentration or a resultant of properties delegated to PMMA.

References:

1. Jennings, J.A., et al., *Novel Antibiotic-loaded Point-of-care Implant Coating Inhibits Biofilm*. 2014, University of Memphis: Clinical Orthopaedics and Related Research.