Preliminary Results for the Addition of Fe_3O_4 Nanoparticle Impregnated Chitosan Microspheres to the Chitosan Sponge for Stimuli Responsive Antibiotic Delivery

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Statement of Purpose: Wound infections are becoming more difficult to treat with the introduction of increasingly antibiotic resistant bacteria. Local antibiotic delivery offers many advantages over systemic antibiotic delivery[1]. Chitosan is a biocompatible, biodegradable polymer that offers many advantages for drug delivery [2, 3]. Previous research has demonstrated that the chitosan sponge can be an effective, local antibiotic delivery system to combat and prevent bacterial infections[4].The development of a patient-specific stimuli-responsive local delivery system may prove useful to tailor release of antibiotics for optimal therapeutic efficacy. Our goal in this study is to obtain preliminary antibiotic elution data for the addition of iron oxide nanoparticle impregnated chitosan microspheres to the chitosan sponge for a stimuli-responsive antibiotic delivery vehicle.

Methods: The manufacturing of the components for this preliminary study were done as follows: Iron oxide nanoparticles were made following procedure in Hu et. al. [5],the water in oil emulsion procedure from Jain et. al. was used to make chitosan microspheres containing 40% w/v iron oxide nanoparticles [6], and chitosan sponges were made following the lyophilization procedure found in Noel et. al. [4]. Amikacin at 5mg/ml was added during the manufacturing process to half of the chitosan microspheres. These microspheres were kept separate from the non-amikacin loaded microspheres. All microspheres were then suspended in separate 5mg/ml amikacin solutions. Chitosan sponges were placed in these solutions containing amikacin and microspheres, frozen, and lyophilized. Then, 20ml of PBS was added to each sponge and samples of eluates were taken at time points of 1, 3, 6, 24, 28, and 72 hours. PBS was refreshed at each time point. To evaluate stimulus, chitosan microspheres containing 50% w/v iron oxide nanoparticles were loaded with dye during manufacturing [6]. These microspheres were divided into 4 different stimulus groups: magnetic field at 72 kHz from a Mini Ductor 2, 10 V_{pp} 10% duty cycle bipolar electric pulse using SAW resonators, ultra-sonication using a standard laboratory ultra-sonic cleaner, and a control group with no stimulus.

Results: During the 72 hour elution study, no statistical difference was found in the amikacin elution from the sponges containing microspheres loaded with amikacin and sponges containing microspheres not loaded with amikacin (Figure 1). All concentrations of amikacin were found to be above the MIC for *Pseudomonas aruginosa* which has been reported between 2 ug/ml and 16 ug/ml [4, 7].The stimulus results for the dye loaded microbeads show a visible dye release with respect to the control and stimulus amount (Table 1).

Table 1: Results of stimuli on dye loaded microspheres

Stimulus	Time	Dye Release
Electric Pulse	2min	Inconclusive
Magnetic Field	1.5min	None
Sonication	20hr / 1hr	High / Low
Control - None	N/A	None

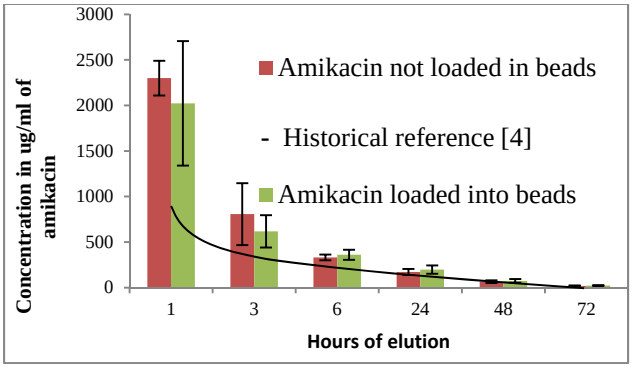

Figure 1: Graph of amikacin release over 72 hours from chitosan sponges.

Conclusions: Results show that there is no statistical difference in the elution of amikacin from this chitosan sponge-microbead composite regardless of loading technique without a stimulus. Previous research has shown the same elution profile from just a chitosan sponge loaded with 5mg/ml amikacin [4]. This suggests that the beads loaded with amikacin did not release amikacin which coincides with our control data for dye release with no stimulus. The effect of a sonication stimulus has shown noticeable release of dye which is promising. Based on the results of dye release from sonication stimulus, loading amikacin during manufacturing is a preferred loading method. Further studies need to be completed to assess the elution profile with a stimulus level/amount in order to pursue a stimuli-responsive antibiotic delivery system.

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