Evaluation of Chitosan Sponges as a Localized Drug Delivery System

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Statement of Purpose: Complex open wounds obtained from traumatic injuries are ideal sites for infection. Conflicts abroad have left many military personnel with such wounds due to explosive devices. These devices cause massive tissue damage and leave the injured with extremity fractures in 82% of cases[1]. These wound sites are ideal sites for contamination followed by infection from any number of pathogenic bacteria. Methods of controlling and eradicating these infections are in need of newer, more effective clinical treatment options. Delivering antibiotics locally as an adjunctive treatment method to systemic dosing can reduce overall serum concentration of antibiotic while increasing the local concentration to bactericidal levels. Chitosan is a well-known, well-researched biocompatible polymer. Chitosan has been shown to be effective at providing a resorbable matrix to deliver therapeutic agents[2,4].

There is a need for a biocompatible, resorbable carrier for use in contaminated extremity injuries that can be custom loaded based on the suspected bacterial species in a wound. Non-restrictive loading could potentially reduce bacterial colonization by orders of magnitude and may be able to reduce infection rates and loss of functionality in limbs in compromised patients with contaminated wounds. The hypothesis of this study was that lyophilized chitosan sponges could serve as a carrier for antibiotics to act as an adjunctive therapy to standard irrigation and debridement for orthopaedic trauma and other musculoskeletal applications. The potential of chitosan sponges as a customizable drug delivery system was evaluated in the present work.

Methods: Sponge preparation: Chitosan solution was prepared by dissolving 5.0 grams (g) of chitosan into 500 milliters (ml) of 1% (v/v) acidic solvent. The chitosan used was 61 and 71% deacetylated (DDA) from Primex (Iceland). 25 ml of aqueous chitosan was cast into aluminum dishes and frozen for one hour at -80°C. The samples were then lyophilized for 48 hours and neutralized in sodium hydroxide, followed by washing in distilled water. Samples were re-frozen and lyophilized before being sterilized via low-dose gamma irradiation (25-32 kGy).

Elution tests: Samples were submerged into 15 ml amikacin solution (5 mg/ml) and allowed to hydrate for 2 minutes. Samples were then subjected to elution tests by submerging into 50 ml of 1x Phosphate Buffered Saline (PBS) and agitated in a 37°C incubator for the duration of the study. One ml aliquots were taken at 1,3,6,24,48, and 72 hours. Aliquots were tested for antibiotic concentration using a fluorescence polarization immunoassay technique (TDxFLx, Abbott Labs, Abbott Park, IL).

- **Activity tests:** Drug activity of the aliquots was tested using a turbidity assay. Samples were tested against *Pseudomonas aeruginosa.* Samples (200 µl) were added to 1.75 ml of Trypticase Soy Broth (TSB) and 50 µl of *P. aeruginosa* inoculum. Samples were incubated for 24 hours at 37°C. Absorbance measurements at 530 nm on a spectrophotometer (BioTek).

Results:

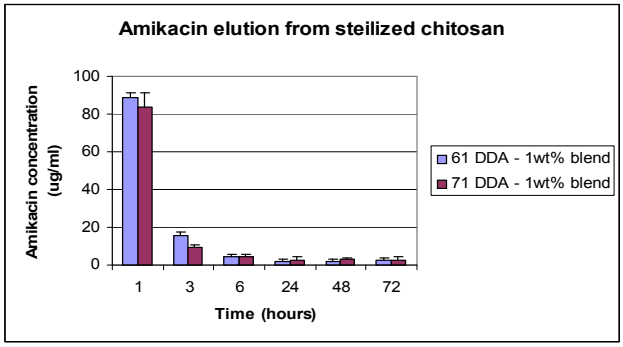

Fig. 1: Graph detailing the elution of amikacin from two different types of sterilized chitosan sponges. Sponges were soaked in 5 mg/ml amikacin solution before immersion in 50 ml of 1x PBS. (n=3)

Amikacin release at one hour was found to be 88.7±2.4 µg/ml and 83.7±7.3 µg/ml for the 61 and 71 DDA samples, respectively. Amikacin was evident at 72 hours as the 61 DDA samples released 2.8±1.3 µg/ml and the 71 DDA samples released 2.2±1.9 µg/ml (Fig 1). Bacterial growth inhibition of *P. aeruginosa* was found to be 98.7% after one hour and 93.1% after 72 hours.

Discussion: Chitosan is a well-studied biocompatible polymer that has been used in localized drug delivery applications. Chitosan is currently being used by the United States military as a haemostatic wound dressing material[3]. This study tested the ability of lyophilized chitosan sponges to be used as a delivery system for antibiotics. The ability to customize the antibiotic choice is potentially desirable for clinicians as they can tailor treatment regimens based on suspected bacterial species present. Chitosan sponges allow for larger uptake of antibiotic solution and higher release concentrations than chitosan films as evidenced by previous work done in our labortories[4]. The sponges are also customizable in terms of degradation as manufacturing alterations can change degradation rates. Current studies involve evaluation of this technology in animal models assessing degradation, local tissue response, and bacterial eradication from the wound site in addition to characterization as a local drug delivery system. The results presented in this study offer evidence that incorporation of antibiotics into chitosan can potentially provide a local drug delivery system that can be used in conjunction with irrigation and debridement therapies.

References:

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