In vitro evaluation of chitosan films as a localized drug delivery system

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Introduction
Complex open wounds obtained from various injuries are ideal sites for infection. Conflicts abroad have left many military personnel with complex open wounds due to explosive devices. These devices cause massive tissue damage and leave the injured with extremity fractures in 82% of cases1. These complex open 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. The ideal local drug delivery system is one that will deliver therapeutic agents while degrading. Chitosan is a well-known, well-researched biocompatible polymer. Chitosan has been shown to be effective at providing a resorbable matrix to deliver therapeutic agents2. Chitosan has also been studied as a wound dressing material.

There is a need for a biocompatible, resorbable carrier for use in contaminated extremity injuries that can be specifically loaded based on the suspected bacterial species in a wound. This operative 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 films 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 films as a customizable drug delivery system was evaluated in the presented work.

Materials and Methods

• Film preparation: Chitosan films were prepared by dissolving 98.5 grams (g) of chitosan into 1.5 milliliters (ml) of 1% (v/v) acetic acid solvent. The chitosan used was 80% deacetylated (DDA) from AgraTech (Goose Creek, SC). The chitosan solution was cast into glass Petri dishes and placed into a convection oven at 37°C for 24 hours. After drying, the films were removed and neutralized in sodium hydroxide and washed in distilled water. Films were then frozen at -80°C and lyophilized for 24 hours. Sterilization of the films was performed after lyophilization by using low-dose gamma irradiation (25-32 kGy). After lyophilization, films were submerged into 10 ml of amikacin-loaded solution (10 mg/ml) and vancomycin-loaded solution (10 mg/ml). Films were allowed to re-hydrate for 2 minutes.

• Elution tests: Films were subjected to elution tests by submerging 5/8 inch disks into 50 ml of 1x Phosphate Buffered Saline (PBS), kept 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. Two different strains of bacteria were used in this study. Vancomycin samples were tested against Staphylococcus aureus and amikacin samples were tested against both Staphylococcus aureus and Pseudomonas aeruginosa. 200 µl of each aliquot was added to 1.8 ml of Mueller-Hinton II broth combined with 20 µl of S. aureus inoculum. Amikacin samples (200 µl) were also 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 were taken and recorded after incubation at a wavelength of 530 nm (A530) on a spectrophotometer. Blanks were used to zero the spectrophotometer (1.8 ml of MHB broth and 200 µl of PBS) and a percent inhibition determination was made when comparing eluate absorbance measurements to positive control absorbance measurements.

Results

Significant elution of both amikacin and vancomycin occurred after the first hour in solution. Vancomycin release was determined to be 50.6 ± 4.5 µg/ml at hour 1. The 48 hour release of amikacin was found to be 4.72 ± 0.48 µg/ml dropping to 0.60 ± 0.48 µg/ml at 72 hours (fig. 1).

Discussion
Chitosan is a well-studied biocompatible polymer that has been used in localized drug delivery applications. The study presented here tested the ability of lyophilized chitosan to be used as a carrier for antibiotics. The ability to customize the antibiotic choice is potentially desirable for clinicians as they can tailor dosing regimens based on suspected bacterial species present. The films are also customizable in terms of degradation. Manufacturing alterations can change the degradation rates. These films can become a hydrogel or remain in film form based on clinician preference for the particular application. Chitosan is currently being used by the United States military as a haemostatic wound dressing material3. 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. Future studies will involve evaluation of this technology in three separate animal models assessing degradation, local tissue response, and bacterial eradication from the wound site.

References

Acknowledgements
USAMRAA – W81XWH-08-1-0312 (grant support)

Table 1: Concentration values of released drug from chitosan during the elution testing (72 hours). Values given are concentrations in µg/ml±standard deviation (n=4).

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>1 hr</th>
<th>3 hr</th>
<th>6 hr</th>
<th>24 hr</th>
<th>48 hr</th>
<th>72 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin</td>
<td>83.5±17.3</td>
<td>19.4±7.9</td>
<td>0.6±0.9</td>
<td>2.5±0.67</td>
<td>9.7±1.9</td>
<td>6.1±0.4</td>
</tr>
<tr>
<td>Amikacin</td>
<td>50.6±4.5</td>
<td>2.2±1.1</td>
<td>3.2±1.1</td>
<td>3.9±0.6</td>
<td>4.7±2.5</td>
<td>0.6±0.5</td>
</tr>
</tbody>
</table>

Activity studies performed on the eluates proved that the antibiotics being released were active in inhibiting bacterial growth. The samples containing vancomycin inhibited 98.1% of S. aureus. Samples containing amikacin inhibited 93.1% of P. aeruginosa and 98.7% of S. aureus. (n=4)

Fig. 1: Release profile of Vancomycin and Amikacin from chitosan films through 72 hours. Concentrations are presented as µg/ml (n=4).