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Antibiotic-loaded chitosan film for infection prevention: A preliminary *in vitro* characterization

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Abstract

The growing infection rate by methicillin-resistant *Staphylococcus aureus*, especially with bone fracture fixation implants, is a major concern in extremity musculoskeletal wound treatment. This preliminary investigation evaluates the ability of chitosan film to be loaded with daptomycin and vancomycin in the operating room, *in situ* loading, and applied to musculoskeletal fixation devices to lessen or prevent infection. Films with 61, 71, and 80% degrees of deacetylation (DDA) made using lactic or acetic acid solvents were analyzed for their antibiotic uptake, elution, and activity along with film swelling ratio, ultimate tensile strength, Young's modulus, adhesive strength, and degradation. Chitosan films after 1 min of rehydration were able in a simulated, clinical setting to maintain mechanical integrity and adhesive strength to be applied to bone fracture fixation devices or implant surfaces. The film percent degradation increased with DDA increasing from 61 to 80%, but film degradation rate decreased in the presence of antibiotics. Eighty percent DDA chitosan films were optimal for absorbing and eluting antibiotics. Antibiotics eluted by the films were active against *Staphylococcus aureus*. These findings indicate that an 80% DDA chitosan film is potentially advantageous as a clinically adjunctive treatment in musculoskeletal injuries to lessen or prevent infections.

Keywords

antibiotics; drug delivery/release; infection; bone fracture devices; chitosan

INTRODUCTION

The primary aims of treatments for complex extremity trauma are bone fracture stabilization, soft tissue repair, and infection prevention. Complex extremity injuries are associated with compromised vasculature and high rates of bacterial contamination, that is, ~65% in the severe case of open fractures are contaminated.^{1,2} Three hundred sixteen (316)

liters stainless steel (SS) and 6–4 titanium (Ti) alloy bone fracture fixation devices are prone to bacterial contamination and biofilm formation, increasing the risk for wound infection by 5%.^{3,4} With the recent advent of community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) in the United States, infected musculoskeletal trauma patients are faced with greater lengths of hospitalization, higher morbidity, and increased healthcare costs.⁵

Using screening techniques, MRSA's presence within a musculoskeletal wound can be determined prior to treatment.^{6,7} The use of last resort antibiotics such as vancomycin is necessary for the treatment of MRSA contaminated musculoskeletal wounds.^{8,9} However, recently vancomycin-resistant *Staphylococcus aureus* infections have been reported, resulting in the increased use of daptomycin for MRSA treatment.⁸ Daptomycin is a semisynthetic antibiotic with concentration dependent activity against MRSA that uses a method of action distinct from β -lactam antibiotics.^{10,11} Vancomycin and daptomycin can be administered intravenously for musculoskeletal infections but both antibiotics have reduced efficacy due to the distance from the injection to wound site, the biochemical environment the drug must traverse, and inhibition of the antibiotic arrival by the compromised vasculature at the wound site.¹² These factors require an increased level of systemic antibiotics to fight the infection.¹

Because of the problems facing systemic therapy for complex extremity trauma, antibiotic-impregnated local delivery vehicles have been used as an adjunct to systemic antibiotic therapy.¹³ This technique offers lengthened drug release time, high local concentrations of active antibiotic and negligible serum antibiotic levels.¹³ High local antibiotic concentrations facilitate delivery to avascular areas of complex musculoskeletal wounds by diffusion.¹² With local antibiotic delivery, antibiotic potency is maximized and the risk of systemic toxicity is minimized, producing an optimal local delivery system.¹⁴ Biodegradable materials, in local drug delivery devices that biodegrade through natural hydrolytic or enzymatic mechanisms, enhance drug delivery and avoid secondary surgical procedures to remove the foreign material after antibiotic elution.²

The biomaterial chitosan is biodegradable,¹⁵ antibacterial,¹⁶ and allows for drug storage and delivery.¹⁷ Chitosan is a linear polysaccharide composed of randomly distributed β -(1-4)-2-amino-2-D-glucosamine (deacetylated) and β -(1-4)-2-acetamido-2-D-glucosamine (acetylated) units, with the number of deacetylated units reported as chitosan's degree of deacetylation (DDA). Lysozyme, an active enzyme in humans,¹⁸ degrades chitosan to saccharide and glucosamine byproducts that are incorporated into proteoglycans or metabolized in the Krebs cycle.^{15,19,20} Chitosan is a cationic weak base soluble in dilute acid solutions and exhibits bioadhesive properties to negatively charged surfaces.^{21,22} Dehydrated chitosan films have the ability to rapidly rehydrate and absorb drugs.²³ DDA, acid solvent, antibiotic uptake and elution can affect the chitosan film properties. Manipulations of the film's properties during manufacture could theoretically optimize an adhesive, local drug delivery implant device for manual application to fracture fixation devices and implants to lessen or prevent infection.^{17,23}

Chitosan films have previously been researched preloaded with antibiotics and have shown success in *in vivo* trials.^{24–26} In a recent article, McLaren states that currently there is no

polymer that can be hand mixed with antibiotics in the operating room, or *in situ* loaded, for clinical use.¹⁴ The ability of chitosan film to be *in situ* loaded, coupled with a physician's knowledge of contaminating bacterial species allows the chitosan film to be customized to fit the patient's therapeutic needs. To address this research void, we undertook this preliminary investigation to evaluate *in vitro* performance of a chitosan film loaded with antibiotics *in situ*. The potential of chitosan film to prevent musculoskeletal infections caused by bacteria such as *Staphylococcus aureus* (*S. aureus*) was determined by evaluating antibiotic uptake, elution and activity. These tests determined if the device could deliver antibiotics above the minimum inhibitory concentration (MIC) within the first 72 h after implantation, effectively inhibiting the bacteria's growth. The potential of chitosan film to maintain mechanical properties enabling it to be applied by hand to an implant surface was determined by film tensile strength, elasticity, and adhesive strength testing. These tests were used as screening tools to evaluate which chitosan film variations had the most beneficial mechanical properties, where the higher values from each test would indicate better surgical manipulation and handling ability. Additionally, the film's degradation properties were ascertained to determine how quickly the film could clear the wound, where the film with the fastest degradation rate would be considered the most beneficial.

MATERIALS AND METHODS

Film preparation

Using three chitosan DDAs and two acid solvents, six chitosan test groups were evaluated. To indicate the film variations, the following labeling system was used: daptomycin was abbreviated as D, vancomycin as V, and neither as N; the numbers 61, 71, and 80 was used to indicate the % of DDA; and the acid solvents—lactic acid and acetic acid— were abbreviated LA and HAc, respectively.

Primex ChitoClear (Iceland) chitosan powder at 61, 71, and 80% DDA with 124, 1480, and 332 cP intrinsic viscosities, respectively, was used to create the films. A 1.5% (w/v) chitosan solution was prepared by dissolving the different DDA chitosan powders in either 1% (v/v) acetic or lactic acid solution, under constant stirring for 24 h. To remove insolubilities from the chitosan solution, it was filtered through an 180 µm nylon screen and allowed to degas at 20°C. The solution was placed in a flat-bottomed glass dish at ~0.8 mL/cm² and the solvent was allowed to evaporate in a convection oven at 38°C for 24 h. This produced a dried film which was neutralized by dipping the film in 2M sodium hydroxide for approximately 1 s, followed by pouring 2 L of distilled/deionized water over the film for rinsing. The neutralized films were dried on a large-pore sized nylon screen in a convection oven at 38°C for 12 h.

Antibiotic quantification

High-pressure liquid chromatography (HPLC) was used to quantify the uptake and elution of the antibiotics vancomycin from MP Biomedicals and daptomycin from Cubist Pharmaceuticals (Lexington, MA). The Varian HPLC system was comprised of ProStar 240 Solvent Delivery, ProStar 410 Autosampler, and ProStar 325 UV-vis Detector modules. Module control and data processing were performed using Varian's Galaxie

Chromatography Data System. Both HPLC separation methods were modified from previous research.^{26–28} For daptomycin quantification, the mobile phase consisted of a HPLC grade acetonitrile and water (62:38, v/v) solution including 40 mM ammonium dihydrogen phosphate brought to a pH of 3.25 using phosphoric acid. Separation was accomplished using a Varian Microsorb-MV C₈ column, 150 mm length and 4.6 mm inner diameter with a flow rate of 1 mL/min. Daptomycin was detected at 232 nm with a retention time of 13.8 min. For vancomycin quantification, the mobile phase consisted of a HPLC grade acetonitrile and water (92:8, v/v) solution, including 50 mM ammonium dihydrogen phosphate brought to a pH of 4 using phosphoric acid. Separation was accomplished using a Varian Microsorb-MV C₁₈ column, 150 mm length and 4.6 mm inner diameter with a flow rate of 1 mL/min. Vancomycin was detected at 208 nm with a retention time of 24.4 min. Both methods were performed in a temperature range of $23.3 \pm 1.1^\circ\text{C}$.

Film and antibiotic characterization

Antibiotic uptake—To determine the film's antibiotic uptake characteristics, a 1 mg/mL vancomycin or daptomycin phosphate-buffered saline (PBS) solution was created. Using six replications, films of known weights were submerged in 50 mL of the antibiotic solution for 1 min, where 1 min is representative of effective operating room usage. This method of antibacterial loading is defined as *in situ* loading, as opposed to preloading, where antibiotics would be incorporated in the chitosan solution during film creation. After 1 min, the film was removed and a sample of the remaining antibiotic solution was used in HPLC to determine its concentration. Antibiotic uptake was normalized by film weight and determined using the following relations:

$$\text{Antibiotic uptake} = \frac{[(\text{Initial antibiotic solution concentration} - \text{Final antibiotic solution concentration}) \times \text{Antibiotic solution volume}](\text{mg})}{\text{Chitosan film weight}(\text{mg})}$$

Swelling ratio—Simultaneous to the antibiotic uptake procedure, the swelling ratio of the chitosan films were determined after 1 min submergence in the presence and absence of daptomycin and vancomycin solutions. To quantify swelling ratio, the initial volume of chitosan films were determined using electronic digital calipers accurate to 0.03 mm within a range of 0 to 150 mm. The final volume was determined immediately after the antibiotic uptake procedure was performed. This data allowed the swelling ratio after 1 min to be determined using the following relationship:

$$\text{Swelling ratio (\%)} = (\text{Final film volume} - \text{Initial film volume}) / (\text{Initial film volume}) \times 100$$

Ultimate tensile strength, Young's modulus—Using six replications, film variations were punched out into ASTM E8 tensile testing specimens with an initial gauge length of 25 mm and 175 mm² area. Dehydrated film thickness was 0.29 ± 0.6 mm. Using an Instron 33R, model 4465 (Norwood, MA) Universal Testing Machine with a 50 N load cell automated by Instron's Bluehill 2 (v2.13) software, the ultimate tensile strength (UTS) and Young's modulus of dehydrated films were determined. Because of the necessity of controlling the test specimen's precise dimensions, it was necessary to perform this test

using dehydrated chitosan film samples. The test specimen was securely placed in the hydraulic grips and tested in tension at a rate of 1 mm/min with data recorded at 200 ms intervals.

Adhesive strength—To determine adhesive strength, six specimen replications from all chitosan film variations were cut into minimal 38 × 38 mm squares. Films were then submerged in 50 mL of PBS solution for 1 min, to simulate the *in situ* loading procedure and were then positioned between cylindrical fixtures with a diameter of 35.1 mm to facilitate the adhesion test. The adhesion testing was modeled from ASTM D5179-02 in order to be performed in-house. Both Instron Universal Testing Machine hydraulic grips were made to hold either 316L SS (ASTM F138) or Ti (Ti-6Al-4V, ASTM 136) alloy fixtures (Figure 1). The mechanical fixture surfaces which faced each other were smoothed by superfinishing to a roughness, R_a , value of 0.025 μm . The superfinishing on the testing cylinders were used to provide comparison testing, not to replicate typical implant surfaces. The rehydrated chitosan film was sandwiched between the two mechanical fixtures with an automatic compression preload of 15 N. Immediately after reaching the pre-load force, the movable crossheads were reversed at 50 mm/min with data recorded at 30-ms intervals. Film thickness varied at 0.18 ± 0.8 mm and the software gave data output in maximal force (N) which was converted into adhesive strength (kPa).

Antibiotic elution—The number of film variations was reduced by half for the remaining procedures by excluding variations exhibiting unfavorable, negligible antibiotic uptake characteristics. Lactic acid films with the three variations of DDAs for daptomycin elution, and acetic acid films with the three variations of DDAs for vancomycin elution yielded six total variations for testing. The elution experiment was performed in triplicate by submerging the films in 50 mL of PBS immediately following *in situ* antibiotic loading at 3 mg/mL of antibiotic. The samples were then incubated at 37°C with 0.5 mL samples taken at 1, 3, 6, 12, 24, 48, and 72 h. This elution procedure excluded PBS solution refreshment at each time point and the antibiotic concentrations of eluant samples were determined using HPLC resulting in an elution profile for each variation of film/antibiotic combination.

Antibiotic activity—Antibiotic activity against *S. aureus* (Cowan I strain) was determined by utilizing the remaining antibiotic elution samples (0.4 mL), in triplicate, in a turbidity assay. In this turbidity study, solution clarity after sufficient bacterial incubation with antibiotic eluates indicated bacterial inhibition due to antibiotic activity. In triplicate, 200 μL of vancomycin and daptomycin eluates were individually added to the inoculum containing 1.75 mL of tryptic soy broth (TSB) and 25 μL of *S. aureus* in 5 mL polystyrene test tubes. Blanks containing neither *S. aureus* nor eluate samples, positive controls containing *S. aureus* without antibiotic eluates, and negative controls containing both *S. aureus* and high concentration antibiotic standards were mixed and incubated at 37°C along with the eluate samples. After 24 h of incubation, the tubes were vortexed and the absorbance at 530 nm was recorded using a spectrophotometer.

Chitosan degradation—A modified procedure from Tomihata and Ikada was used to quantify the antibiotic effect on chitosan degradation.²⁹ *In situ* loaded and nonloaded

chitosan film groups—the same groups used in the antibiotic elution and activity experiments with additional nonloaded groups yielded a total of 12 experimental groups to be tested with five replicates each—were subjected to degradation testing. The weight of clean 90 mm diameter Petri dishes and dehydrated chitosan films were established. Films marked for *in situ* loading were submerged in 50 mL of a standard antibiotic solution and then all films were submerged in 25 mL of 100 µg/mL 2 × crystallized, chicken egg white lysozyme (MP Biomedicals), PBS solution. The samples were allowed to sit for 20 h at 37°C in a convection incubator. After 20 h, the lysozyme solution was removed and the films dehydrated using the same method with the convection oven. The lysozyme/PBS solution was replaced and data gathering was performed every 20 h of degradation for 100 h. The new film weights were measured, which enabled the percent of the film that remained to be determined using the following relationship:

$$\text{Percent remaining (\%)} = \frac{(\text{Petri dish and film weight at } \times \text{ hours} - \text{Petri dish weight})(\text{mg})}{(\text{Petri dish and initial film weight} - \text{Petri dish weight})(\text{mg})} \times 100$$

Statistical analysis

All data are presented as mean ± standard deviation and analyzed initially by one-way ANOVA; if statistically significant differences were found then each pair of variations were compared using the Student *t*-test. Two-way ANOVA was used to identify differences between DDA and acid solvent independent variables. Analysis was performed using JMP 7.0.1 (Cary, ND). Statistical occurred when $p < 0.05$ as indicated in the text.

RESULTS

The uptake of daptomycin and vancomycin indicated the amount of antibiotic a dehydrated film could absorb in 1 min in terms of milligrams of antibiotic per grams of chitosan (Figure 2). Film variations V80HAc, D61LA, D71LA, and D80LA were all statistically similar ($p = 0.7769$) and absorbed significantly more antibiotic than other variations ($p < 0.0001$). When absorbing D, LA films had a significantly higher absorption than HAc films ($p < 0.0001$). When absorbing V, HAc films had a significantly higher absorption than LA films ($p < 0.0001$). LA films absorbed daptomycin at an average of 270.80 ± 125.08 mg/g which was significantly higher ($p < 0.0001$) than its average exclusion of vancomycin at 0 mg/g. Conversely, HAc films absorbed vancomycin at an average of 118.39 ± 101.93 mg/g which was significantly higher ($p < 0.0001$) than its average exclusion of daptomycin at -46.43 ± 77.56 mg/g. There was an upward trend of increasing antibiotic absorption as the DDA of the films increased. Antibiotic exclusion from the chitosan film during *in situ* loading was indicated by a negative uptake value.

The swelling ratio indicated the increase in volume of the 1 min rehydrated films in percentage of volume increased (Figure 3). After 1 min in PBS alone, results indicated that non-loaded films exhibited an ~100% swelling or doubling in film volume. Analysis indicated that D80LA and V80HAc variations' swelling ratios were similar ($p = 0.8381$) and significantly higher than every other variation ($p < 0.0001$), except for V71HAc ($p = 0.1703$).

and 0.0852, respectively). Generally, when the films were in an antibiotic environment that favored increased uptake, the films had a higher swelling ratio.

The UTS and the Young's modulus of the dry and dehydrated films are shown in Figure 4. The 71HAc and 80HAc groups exhibited UTS values of 55.47 ± 11 and 49.89 ± 3.12 MPa, which were statistically similar ($p = 0.2131$). These films also exhibited UTS that were significantly higher than the other test groups ($p < 0.0001$). Similarly, Young's moduli for the 71HAc and 80HAc were statistically similar (810.99 ± 207.64 , 762.53 ± 49.62 , $p = 0.06597$) and significantly greater than other test groups ($p < 0.0001$). No differences were detected in UTS or elastic modulus values of the other test groups.

Adhesion testing indicated the adhesion strength, or the maximum tensile load per area in kPa (Figure 5). The alloy/film variations Ti71LA, SS61LA, and SS80LA were statistically similar and had significantly higher adhesive strength than every other alloy/film combination ($p = 0.0310$). Generally, HAc film variations had lower adhesive strength than LA variations. In HAc films, there was an upward trend in adhesive strength as DDA increases. Subsequent studies have indicated that *in situ* antibiotic loaded chitosan films are not significantly different from nonloaded chitosan films using the current method.

Two-way ANOVA was performed to determine if there was any interaction among DDA and acid solvent independent variables. For antibiotic uptake, swelling ratio, UTS, Young's modulus, and adhesive strength there was no statistical interaction between DDA and acid solvent ($p = 0.0674$).

As discussed in the materials and methods section, the remaining experiments were performed with the exclusion of acetic acid film variations when daptomycin was included and lactic acid film variations when vancomycin was included. The exclusion was based on the unfavorable antibiotic uptake data for specific variations. Antibiotic elution results indicated the concentration of antibiotic present in solution per chitosan film sample weight over a period of time given in (mg/mL)/g (Figure 6). For daptomycin [Figure 6(A)], 80LA variations eluted consistently significantly higher quantities of daptomycin over the 72 hr period. This increased elution could be correlated to the increased antibiotic uptake (Figure 2). In vancomycin elution [Figure 6(B)], 80HAc variations had a higher average elution rate. The 72 h elution's approximate antibiotic release for each individual film variations remained in the same range, except for V71HAc and D71LA. The V71HAc and D71LA elution trends represented a short but relatively extended release in comparison to their maximum eluted concentration, although this difference was not significant.

Antibiotic activity was determined using turbidity assays, indicated by the percent inhibition of *S. aureus* growth (Figure 7). Overall, the eluates from the films did inhibit *S. aureus* at all time points. The following four variations did show more variability at their respective time points: V80HAc at 12 h; V71HAc, D61LA, and D71LA at 24 h. A potential source of some variability for V80HAc and V71HAc at 12 and 24 h, respectively, may have been caused by a precipitous interaction between the eluates and a component in the TSB media [Figure 7(B)]. Ultimately, the 48 and 72 h eluate samples for both vancomycin and daptomycin were found to be active in inhibiting the growth of *S. aureus*.

The degradation study with lysozyme indicated the chitosan film weight that remained after a period of time in percentage of original film weight (Figure 8). Film variations with 61% DDA degraded to a lesser extent and with no significant difference between antibiotic loaded and non-loaded variations. Variations with higher DDAs degraded more than those with a lower DDA. When antibiotic loading had an effect, the effect was a decrease in the film degradation amount. After ~60 h, the degradation rate slowed considerably as each individual variation's percentages were statistically similar after that point.

DISCUSSION

Local drug delivery from degradable biomaterials continues to be a vital research field. This preliminary research proposes the use of biodegradable chitosan film to be loaded with antibiotics *in situ*, as opposed to during manufacture, and elute the antibiotics in the wound site to lessen or prevent infection.

Antibiotic uptake is a critical property for a device with *in situ* loading. An abundance of literature concerning the loading of drug delivery devices,^{30–34} and chitosan films specifically,^{21–23,35} with antibiotics can be found. It has been suggested that a local drug delivery polymer which can be loaded with antibiotics during surgery would be ideal for its ability to be tailored to the patients needs with respect to the antibiotic choice and concentration.¹⁴ However, no previous studies have evaluated chitosan's *in situ* antibiotic loading properties. In this preliminary study, the chitosan films absorb antibiotics differently depending on both the type of acid and DDA, with best results occurring when 80LA absorbs daptomycin and 80HAc absorbs V. Similarly, no previous studies were found for an accurate comparison of swelling ratio. Independent experimentation using the same chitosan film variations indicated that maximum swelling ratio occurred after approximately 15 min and increased film volume by as much as 850%. However, the swelling ratio in this study was investigated after 1 min of film rehydration to simulate the use of the device in a surgical environment, for example, with *in situ* loading. Other studies which determine chitosan film's swelling ratio investigated the film's maximum rehydration at 4–24 h which is not feasible for operating room use.^{23,24,29} This preliminary study investigated film swelling ratio after 1 min of rehydration, a feasible time for use in the operating room.

The ability of chitosan film to absorb antibiotics is equally as important as its ability to maintain its mechanical properties as a wrap on bone fracture fixation devices during and after surgery. Several other studies report UTS for chitosan films to be in the same range as those found in this study, such as 20–60 MPa.^{23,36–38} The difficulty in any direct UTS comparison is due to variations in chitosan film material source and creation methods, such as the use of different chitosan DDAs, molecular weights, acid solvents, solution concentrations, as well as the possible inclusion of additional compounds or crosslinking materials.^{36,38} For example, Arvanitoyannis et al. reported the Young's modulus of a highly concentrated, ~45% by weight, chitosan film to be in the range of 1000–2000 MPa, with a UTS exceeding 100 MPa,³⁹ where as films in our study were created at a lower chitosan concentration, 1.5% (w/v), had a UTS that exceeded 50 MPa, and a max Young's modulus of 800 MPa. Conditions that are generally accepted in previous chitosan film mechanical studies are (1) UTS increases as molecular weight increases, (2) HAc solvent have a higher

UTS that those of LA, and (3) Young's modulus is directly proportional to its UTS.^{23,36–39} This first generalization is potentially confirmed through the chitosan source's corresponding intrinsic viscosities; the second and third generalizations are confirmed through our data. Other material properties which were not investigated in this study, such as crystallinity, may impact the mechanical studies.¹⁵

An additional material property aiding in chitosan film's use as an adjunctive antibiotic wrap on a musculoskeletal device is chitosan film's adhesion. Preliminary experimentation determined that *in situ* antibiotic loading did not have a statistically significant effect on adhesion strength when compared to rehydration alone, and therefore was not included in the results. This property has not been as extensively studied in previous publications. A study by Khan measured bioadhesive strength of a chitosan film to a chicken pouch membrane using a texture analyzer.³⁷ This study reported a peak detachment force of 0.71 ± 0.02 N (1.2 kPa adhesive strength) for an 84% DDA, 1% (w/v) chitosan film using 1% (w/v) LA and 0.47 ± 0.03 N (0.80 kPa adhesive strength) for an 84% DDA, 1% (w/v) chitosan film using 2% (w/v) HAc. While Khan's bioadhesive study depicted an approximate level of film adhesion to soft tissues, our study provides a likely result of chitosan film's adhesion to musculoskeletal implant Ti and SS alloys. Khan's study and this study do agree that that films made from LA solvent provide a higher adhesive strength than HAc solvent films. Our study showed an approximate 900% increase in adhesion from Khan's study.

Antibiotic elution has been commonly investigated in many local drug delivery devices, including chitosan devices.^{19,21,25,33} These previous studies investigated pre-loaded chitosan with various drugs. In a previous study performed by Noel et al.,²⁵ a similar 80% DDA chitosan film variation had an extended release of preloaded daptomycin over 72 h, that is 10.17 ± 3.83 $\mu\text{g/mL}$ at 1 h increasing to 28.72 ± 6.80 $\mu\text{g/mL}$ at 72 h. This study's results differ from the elution results found by *in situ* loading and revealed that approximately 87% of the total daptomycin was eluted after 1 h. Results of the current study did indicate that eluted antibiotic activity against *S. aureus* was unaffected by the film *in situ* loading and antibiotic elution. Additionally, it was shown through activity testing that *in situ* loading with 3 mg/mL and elution into 50mL of solution successfully delivered antibiotics at levels above the MIC for both vancomycin and daptomycin, resulting in the inhibition of *S. aureus* growth throughout the 72 h experiment's duration. These results are similar and are supported by previous antibiotic preloaded film studies.²⁵

In order to produce an optimal local drug delivery device, chitosan film's degradability was also investigated. Tomihata and other studies found that as chitosan's DDA increases, its degradation rate decreases.^{19,22,29,40} The findings of this preliminary study were not similar to the established relationship between DDA and degradation. Other film properties mechanical properties, such as chitosan's molecular weight and crystallinity, could provide an explanation for this difference in results.¹⁵ Additionally, our preliminary study indicated that there was a significant decrease, as high as ~70%, in degradation when films were *in situ* loaded. These relations between chitosan film's physical properties and drug interaction have been reported with antibiotics by another group of researchers.^{21,23,25}

Chitosan film and molecular behavior studies by Nunthanid et al. have concluded that drug incorporation into chitosan films interacts with the chitosan molecules at amino functional groups.²³ Specifically, they found that the drug–polymer interaction in preloaded films resulted in sustained release of an acidic or negatively charged drug, whereas no drug–polymer interaction was observed in the release of a basic drug from the film.⁹ These drug–polymer interactions could explain the antibiotic uptake and release, and the degradation effects evident in this study. However, daptomycin and vancomycin in this study reacted differently based on the acid solvent used in the creation of the chitosan film; therefore, molecular polarities may not be the sole cause of the different results in film–drug interaction. To determine what effect the acid solvent and antibiotic have on chitosan film’s behavior, additional investigations will be required. These additional studies could provide vital information for a chitosan film variation with the efficacious elution of daptomycin, vancomycin, and other antibiotics.

SUMMARY AND CONCLUSION

This preliminary *in vitro* research determined that a novel *in situ* loaded, chitosan film has potential as an adjunctive device for musculoskeletal extremity wound treatment where bacterial contamination is likely and orthopaedic fixation devices and implants are used. The superior ability of 80% DDA chitosan films to absorb and deliver antibiotics at levels above the MIC and the LA film property of increased mechanical and adhesive capabilities shows chitosan film’s promising potential for traumatic injury infection prevention. One limitation of this study was the focus on 1 or 3 mg/mL *in situ* antibiotic loading of chitosan films for 1 min. The antibiotic activity against *S. aureus* was successfully shown to be unaffected by the film variations, loading, and elution processes in this study through its near complete inhibition of growth of *S. aureus*. This local chitosan drug delivery device at 80% DDA of this source holds the additional benefit of increased biodegradability, which eliminates the need for its removal through a secondary surgery. Additional studies are needed to ensure biocompatibility, confirm a sterilization procedure and establish a method for use in fracture device stabilization before *in vivo* evaluation commences. Further investigations into the interactions between the chitosan matrix, the antibiotic structure, and the acid solvent would yield information toward optimizing the film for a wider range of antibiotics and extended elution period. This preliminary data presents *in vitro* evaluations of chitosan film with *in situ* loaded antibiotics for adjunctive treatment of contaminated complex trauma and surgical sites.

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FIGURE 1.

Cylindrical adhesive testing fixtures positioned in the grips of the universal tensile testing machine with hydrated chitosan film in position between the adhesive fixtures.

FIGURE 2.

Antibiotic uptake of daptomycin and vancomycin by *in situ* loading of chitosan films. $n = 6$ measurements for all groups. Daptomycin with lactic acid and vancomycin with acetic acid film variations had the highest antibiotic uptake values. The results are represented as the average $\pm$ standard deviation (*, $p = 0.7769$; * vs. all others, **, †, ††, $p < 0.0001$).

FIGURE 3.

Swelling ratio of the chitosan film variations after 1 min rehydration by *in situ* loading. $n = 6$ measurements for all groups. Daptomycin with lactic acid and vancomycin with acetic acid film variations had a higher swelling ratio. The results are represented as the average $\pm$ standard deviation (*, $p = 0.8381$; * vs. all others except **, $p < 0.0001$).

FIGURE 4.

$n = 6$ measurements for all groups. A: The ultimate tensile strength of dehydrated chitosan films reported in stress, (*, $p = 0.02131$; * vs. all others, $p < 0.0001$). B: Young's modulus, or elasticity, of dehydrated chitosan films reported in the ratio of stress to strain. Variations of 71 and 80% DDA with acetic acid have the highest ultimate tensile strength and Young's modulus. Results are shown as the average $\pm$ standard deviation (**, $p = 0.00597$; ** vs. all others, $p < 0.0001$).

FIGURE 5.

The adhesive strength of chitosan films rehydrated for 1 min to titanium and stainless steel alloy substrates as adhesive strength, represented as the average $\pm$ standard deviation (* vs. all others, $p = 0.0310$). 61 and 80% DDA, lactic acid film variations on SS, and 71% DDA, lactic acid films on Ti variations have the highest adhesive strength. $n = 6$ measurements for all groups.

FIGURE 6.

Elution of (A) daptomycin and (B) vancomycin from *in situ* loaded chitosan films represented as the average $\pm$ standard deviation. Both antibiotic release profiles for 71% DDA films showed a bell-shaped release. $n = 3$ measurements for all groups.

FIGURE 7.

Antibiotic activity of (A) daptomycin and (B) vancomycin elution samples. Activity is indicated by the percent inhibition of *S aureus* growth and represented as the average $\pm$ standard deviation. All variation samples over 72 h had nearly complete inhibition. $n = 3$ measurements for all groups.

FIGURE 8.

The lysozyme-mediated degradation of chitosan films without antibiotics and *in situ* loaded with (A) daptomycin and (B) vancomycin represented as the average $\pm$ standard deviation (*, $p = 0.0243$; **, $p = 0.0300$; †, $p = 0.0133$; ††, $p < 0.0001$). At 80 h and on, 71 and 80% DDA films showed a significant decrease in degradation rate. $n = 5$ measurements for all groups.