Evaluation of acyl-modified chitosan membranes loaded with cis-2-decenoic acid and bupivacaine for infection prevention

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INTRODUCTION

Burn injuries cause complex wounds that can lead to multifaceted complications. Burn wounds are incredibly susceptible to infection, with approximately 51% of burn victim deaths resulting from wound infection[1]. With the formation of possible biofilms (bacterial community), these immunocompromised wounds can be clinically harder to treat. Chitosan nanofibrous membranes are well suited for tissue healing and drug delivery applications due to their increased surface area, high degree of biocompatibility, and ability to mimic the extracellular matrix. Previous work has demonstrated that acylation using short chain fatty acids protects chitosan nanofibers from swelling, resulting in hydrophobic characteristics that provides extended drug release of hydrophobic therapeutics,[2] such as anti-biofilm molecules and local anesthetics. In this study, we fabricated and acylated electrospun chitosan membranes (ESCM) and loaded with anti-biofilm agent cis-2-decenoic acid (C2DA) and the local anesthetic bupivacaine (BUP). We then investigated the antimicrobial activity of loaded ECSM against common burn pathogen A. baumannii.

METHODS

Figure 1. electrospinning box

Membranes were electrospun (Figure 1) using a high molecular weight chitosan (ChitoLytic) with a 86.5%-degree deacetylation. Chitosan was dissolved overnight at 5.5 (w/v) %, of 70% (v/v) trifluoroacetic acid & 30% (v/v) dichloromethane then centrifuged to remove any particulates. Membranes were spun to 15 cm diameters and ~ 0.7 mm thickness. 10 mm membranes were treated with pyridine and Decanoic anhydride (DA) at ratios of 1:1 (pyridine: anhydride).
Membranes were washed, frozen, and lyophilized before loading with therapeutics. Membranes were loaded with either 1.5 mg C2DA, 1.5 mg BUP, or a combination of both therapeutics (C2/BUP). Commercially available chitosan sponges and gauze were used as controls.

For antimicrobial studies (Figure 2), wells of a 48-well plate were inoculated with 0.5 mL tryptic soy broth containing 10^6 colony forming units (CFU) of *A. baumannii* incubated for at 37° C for 24h to allow for biofilm growth. At 24 and 48 h, membranes were removed from wells, rinsed twice with sterile PBS, and sonicated for 5 minutes at 40 to remove biofilm-associated bacteria.

Quantification of biofilm was determined using BacTiter-Glo® Microbial Cell Viability Assay (Promega). Supernatant from wells containing membranes and bacteria was removed and added to a new 96 well plate, then combined with BacTiter-Glo® to quantify the amount of planktonic bacterial growth after 24 and 48 hours**.** Significant differences were determined by ANOVA with Holm-Šídák post-hoc tests ($\alpha<0.05$). Membranes were fixed, dried, and kept for scanning electron microscopy analysis.

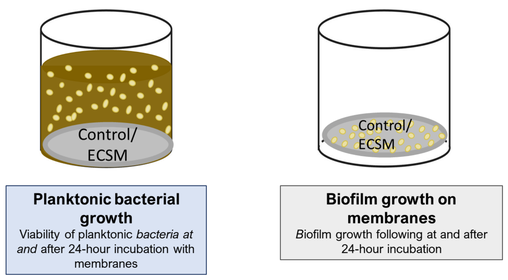

Figure 2.) Schematic of antimicrobial studies

RESULTS

Planktonic results showed that an increased sample size may be required to detect differences in planktonic growth, although most planktonic growth at 48 hours showed signs of decreased viability (Figure 3). All membrane groups decreased *A. baumannii* biofilm viability at 24 hours compared to sponge controls, although at 48 hours this effect was not detected.

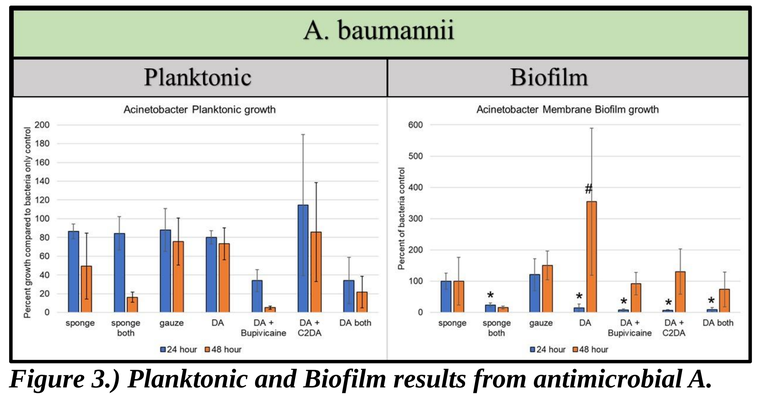

Figure 3.) Planktonic and Biofilm results from antimicrobial A. baumannii exposure for 24 and 48 hours. *Bars represent the mean and error bars represent the standard deviation. Significant differences are made with comparison to control(sponge) with # larger and * lesser than control.*

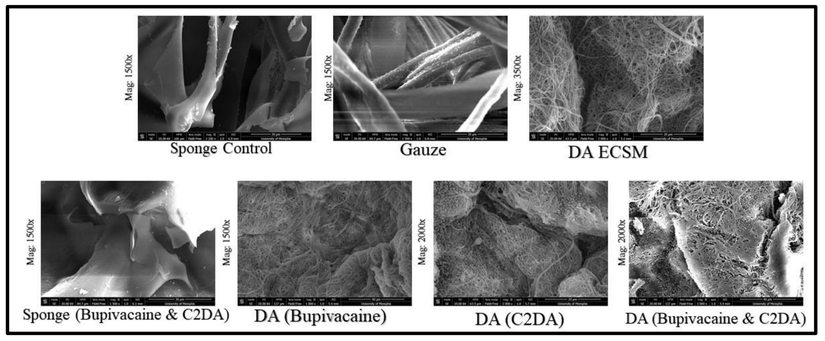

Figure 4.) SEM membranes exposed to A. baumannii for 24 hours

Loaded and unloaded membranes displays visible *A. baumannii* imbedded within the fibers as well as control groups (Figure 4 & 5). Control groups show a slight increase in frequency of visible bacteria compared to experimental groups.

Loaded and unloaded membranes show an increase in *A. baumannii* activity. Control and experimental groups show an increase in potential biofilm formation as depicted in Figure 3.

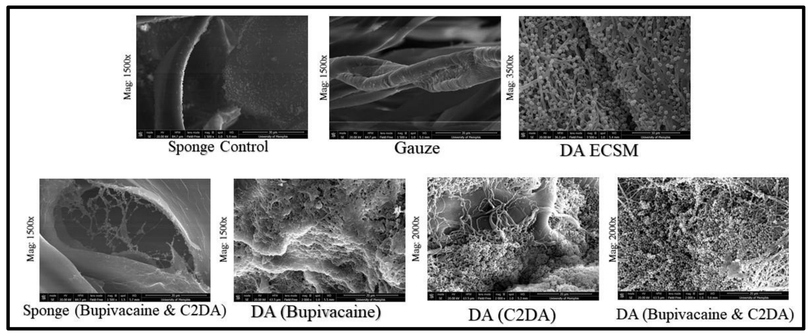

Figure 5.) SEM membranes exposed to A. baumannii for 48 hours

CONCLUSION

Results from the antibacterial activity from these membranes showed decreased biofilm viability at 24 hours then possible signs of decreased viability for planktonic bacteria after 24 hours. As expected, the membrane displays and addresses biofilm growth with antibacterial effects, but for A. baumannii perhaps the concentration needs to be increased or these therapeutics are not as efficient for inhibition of *A. baumannii*. Although the presence of C2DA facilitates the inhibition of biofilm for other common burn pathogens, planktonic *A. baumannii* did not appear to be as sensitive to C2DA as pathogens such as S. aureus and P. aeruginosa[3]. Future studies will entail revaluation of antimicrobial studies with varying concentrations, as well as 24- and 48-hour biofilm and planktonic study for *S. aureus* and *P. aeruginosa*.

REFERENCES

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ACKNOWLEDGEMENTS

Research is supported by the Military Burn Research Program (MBRP) under Award #: W81XWH-20-1-0430.
A special thanks to Ezzudin Abuhussein and Zoe Harrison for assistance with experimental methods.