AWARD NUMBER: W81XWH-20-1-0430

TITLE: Acylated Electrospun Biopolymer Membranes for Burn Wound Coverage, Infection Prevention, and Pain Relief

PRINCIPAL INVESTIGATOR: Dr. Jessica Jennings

CONTRACTING ORGANIZATION: University of Memphis
315 Administration Building, Memphis, TN 38152

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5b. GRANT NUMBER

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6. AUTHOR(S)
Jessica Amber Jennings: jjnnings@memphis.edu
Zoe Harrison: zlhrrson@memphis.edu
Emily C. Montgomery: cclman22@memphis.edu

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5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Memphis (Herff College of Engineering)
315 Administration Building
Memphis, TN 38152

8. PERFORMING ORGANIZATION REPORT NUMBER

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14. ABSTRACT
We have developed novel biopolymer membranes with advantageous features (physical coverage, infection prevention, pain relief) for immediate care of burn wounds and during prolonged field care. Electrospun chitosan membranes (ESCM) serve to address burn wound coverage in several ways, including 1) acting as a barrier to microbial contamination, 2) releasing local anesthetics in a controlled manner that reduce pain and modify the inflammatory response, and 3) releasing natural antimicrobial fatty acids that prevent biofilm contamination. To assess these ESCM, bulk fabrication of ESCM was performed as well as loading treatments (Bupivacaine and/or C2DA) to evaluate elution and antimicrobial properties. Elution results displayed similarities between the single and dual release of C2DA, where controls (sponge & gauze) did not elute therapeutics past 9 hours. Experimental groups (hexanoic, octanoic, and decanoic acylated) eluted therapeutics throughout the study. Antimicrobial results displayed antimicrobial properties of treated membranes against planktonic and biofilm microorganisms.

15. SUBJECT TERMS
biofilm; anesthetic; bupivacaine; electrospinning; chitosan; biomaterial; local drug delivery; wound dressing; infection; Staphylococcus; Pseudomonas; animal model; burn wound; antimicrobial; elution; SEM; FTIR; biopolymer

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRDC
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2. INTRODUCTION

Burn wounds sustained during combat can become infected and cause significant pain. These traumatic burn injuries can be subject to biofilm infection, which are often antibiotic-resistant and difficult to treat. Dressings may be used as wraps over multiple types of soft tissue wounds, including burns, and have advantages in military wound care in that they are able to remain in place with minimal maintenance during the evacuation process. While proven effective in reducing microbial colonization, current standard of care creams and dressings have drawbacks in that they may delay the wound healing process and they do not address the management of pain.
The purpose of this study is to develop electrospun chitosan membranes (ESCM) loaded with local anesthetics (LA) and the antimicrobial agent cis-2-decenoic acid (C2DA) to serve as burn wound dressings that prevent pain and infection. There are several milestones to demonstrate successful application of loaded ESCM for burn wound treatment in a prehospital setting, including: selecting the most effective LA based on antimicrobial activity, manufacturing and characterizing ESCM, evaluating release of both therapeutics, and determining anti-biofilm properties of loaded ESCM. To ensure these wound dressings do not delay the wound healing process, ESCM will be tested for cytocompatibility with fibroblasts and keratinocytes, collagen and cytokine production will be determined, and monocyte to macrophage differentiation in the presence of loaded ESCM will be investigated. The overall effectiveness of loaded ESCM to prevent burn wound infection in an established contaminated comb scald wound rat model will also be determined. These milestones and the current progress are outlined and described in this report.

3. KEYWORDS

biofilm; anesthetic; bupivacaine; electrospinning; chitosan; biomaterial; local drug delivery; wound dressing; infection; Staphylococcus; Pseudomonas; Acinetobacter; animal model; burn wound; antimicrobial; elution; SEM; FTIR;

4. ACCOMPLISHMENTS

What were the major goals of the project? (Goals to be accomplished and status.)

Specific Aim 1: Evaluate antimicrobial effects of LA, C2DA, and combinations released from ESCM (months 1-12)

- STATUS: completed Y2Q7, completed Major Task 1. Completed Major Task 2, subtask 1 complete, subtask 2 complete, subtask 3 complete. Subtask 4 complete.

Specific Aim 2: Evaluate dermal and inflammatory cell responses to LA, C2DA, and combinations released from ESCM (months 8-20)

- STATUS: Major Task 3: subtask 1 complete. Subtask 2 completed. Subtask 3 begun, 50% complete, Major Task 4 not started.

Specific Aim 3: Compare ESCM with and without LAs and C2DA to commercially available casualty care standards in an in vivo contaminated rat comb scald wound model (months 12-36)

- STATUS: Major task 5, subtask 1 is complete. IACUC has been submitted and approved. Subtasks 2 underway with animal study in progress. Subtasks 3 and 4 yet to start.

What was accomplished under these goals? (Detailed progress and results.)

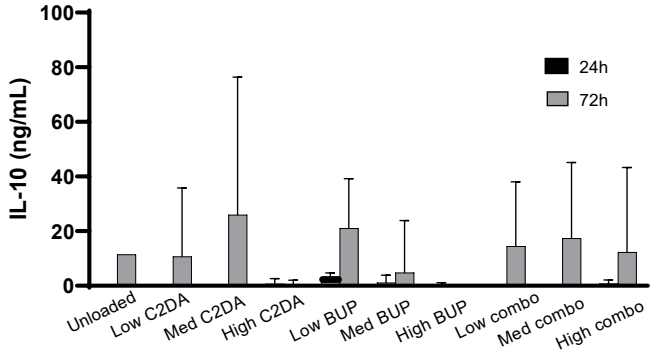


Figure 5. IL-10 production by NHEK in contact with DA-ESCM for 24 or 72 hours (n = 4). Individual data points are shown as bars representing mean and error bars representing standard deviation. No significant differences were determined between groups..

Specific Aim 1: Evaluate antimicrobial effects of LA, C2DA, and combinations released from ESCM (months 1-12)

5. Major Task 1: Investigation of antimicrobial activity of LA therapeutics

Completed. Reported in Q1 and Q7 reports.

6. Major Task 2: Manufacturing and Characterization

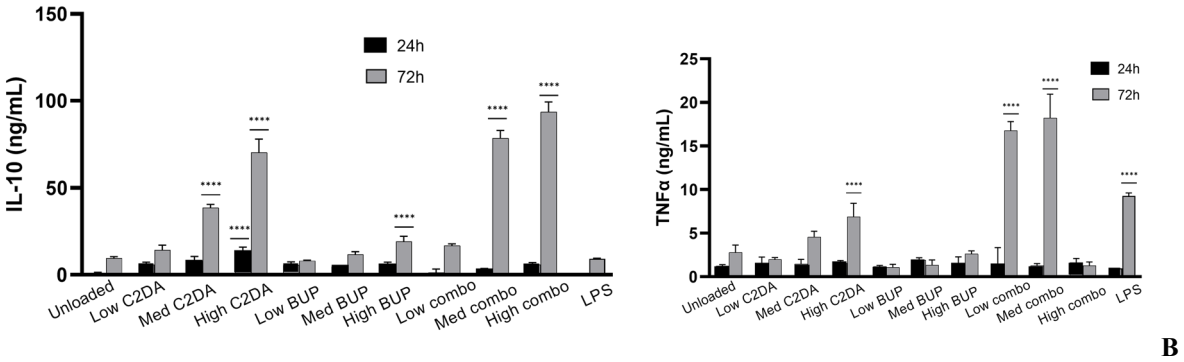
Subtask 1: Fabrication
Completed. Reported in Q1.

Subtask 2: Characterization
Completed. Reported in Q1, Q2, Q6, and Q7 reports.

Subtask 3: Release
Subtask 4: Antimicrobial studies
Zone of Inhibition, biofilm formation, and planktonic cell viability studies were reported in Q3-7 reports.

Specific Aim 2: Evaluate dermal and inflammatory cell responses to LA, C2DA, and combinations released from ESCM (months 8-20)
Studies to investigate dermal and inflammatory responses to ESCM are underway. Cytocompatibility with NHDF and NHEK cells has previously been reported, with viability improved when C2DA and BUP are delivered together instead of alone. RAW264.7 rat monocytes were studied in this quarter and had similar results, with simultaneous delivery of both molecules increasing viability over delivering either molecule alone.

Collagen production of fibroblasts cultured with the loaded membranes was reported in Q7. Further studies have been performed to evaluate cytokine production of NHEK and RAW264.7 cells using ELISAs. The production of cytokines IL-10 and IL-12 were evaluated at 24 and 72 hours in NHEK cell culture. No cytokine production was detectable at 24 hours. After 72 hours, IL-10 was seen in all groups that delivered both C2DA and BUP (Figure 5), however, it was not statistically different than controls with only membranes in culture. No IL-12 response was seen in any of the NHEK groups. RAW264.7 cells showed increased IL-10 production after 72 hours in all groups that had high concentrations of C2DA, with minimal production at 24 hours. The RAW264.7 cells also showed increases in TNF-alpha after 72 hours in simultaneous delivery groups delivering low to medium concentrations of the therapeutic molecules (Figure 6).



A **B**

Figure 6. Production of (A) IL-10 and (B) TNF-α by monocytes in contact with DA-ESCM for 24 or 72 hours ($n = 4$). Individual data points are shown as bars representing mean and error bars representing standard deviation. Concentrations were normalized based on viability for each sample. **** indicates significantly higher cytokine production compared to unloaded control, detected using one-way ANOVA with Tukey's post hoc tests ($p < 0.0001$).

Specific Aim 3: Compare ESCM with and without LAs and C2DA to commercially available casualty care standards in an in vivo contaminated rat comb scald wound model (months 12-36).

Not completed. We have obtained approvals from UM IACUC and ACURO. Cytocompatible concentrations of C2DA and bupivacaine have now been obtained for use in the animal model. Animal studies are underway following planning meetings with animal care staff and Environmental Health and Safety staff to define requirements of working with biosafety level 2 animal model in the animal care facility. Work has begun with all treatment groups at the 3-day time points.

What opportunities for training and professional development has the project provided?

This project has a variety of synthesis and analysis methods needed to evaluate the appropriate samples. Due to this, our staff/students, even if not funded through this grant, have been given the opportunity to learn and operate many of the equipment and studies. Our undergraduate students have been a key example of this, as they have been trained and tasked to assist with studies to reduce analysis time and to learn for future analysis. The following are a list of undergraduates and interns that assisted in the ongoing work:
Madelyn Wicker, Hanna Jones, Brenna Ballard, Payton Freeman, Tibirni Yusuf
This also applies to graduate students, as they have been tasked with training undergrads by producing SOPs and leading a majority of the studies. The following list of graduate assistants and their professional development activities stemming from this project:
Emily Montgomery
Yogita Dintakurthi — Cell culture experience aiding in her dissertation and for future applications.
Jermiah Tate
Additionally, some of our students have been able to present research related to this project on campus at the Student Research forum this year, and in return gained experience with presenting scientific research at conferences. The following student presented with their presentation titles and conference:
Student Research Forum:
Yogita Dintakurthi – Cytocompatibility of Electrospun Chitosan Membranes Treated with Decanoic Anhydride and Loaded with Biofilm Inhibitors and Bupivacaine.

How were the results disseminated to communities of interest?

The following are the ways the PI and collaborators have shared information regarding this project with the scientific community and the general community:
Poster presentation at MHSRS, September 12-15, 2022.
Poster presentation at Society for Biomaterials Annual Meeting, April 19-22, 2023.
Poster presentation at Nashville Biomaterials Day at Vanderbilt University.
Article accepted in Journal of Applied Microbiology.
Article under review at MDPI Pharmaceuticals.

Recent progress and potential translational activities have been included in the University of Memphis Research Foundation Board meetings, Additionally, see publications and/or presentations.

Plans for the next reporting period to accomplish the goals

We plan to finish subtask 3 of Major Task 3, and finish Major Task 5.
Subtask 3 of Major Task 3 consists of cytokine production studies using a MAGPIX panel measuring multiple targets. Major Task 4, subtask 1 will follow with a similar method looking at cytokine production of monocytes.

Major Task 5, subtask 2 is performing the animal scald model. The animal model is underway.

7. IMPACT

What was the impact on the development of the principal discipline(s) of the project?

The development of a biopolymer modification strategy to tailor drug release over time impacts the field of biomedical engineering and drug delivery. Our understanding of material properties and how they may be applied could lead to the development of other functional materials for antimicrobial or other therapeutic delivery. Chitosan is a biodegradable, antimicrobial, and versatile biopolymer. In this project we electrospin chitosan solution into a fibrous membrane, one that has an increased surface area, woven to be breathable, and capable of loading therapeutics and other agents. Biomaterial fabrication strategies such as electrospinning may be useful in burn wound dressings, orthopaedic wraps, or tissue

engineering scaffolds. Our evaluation of the effect of materials and released therapeutics on dermal and inflammatory cells shapes our understanding of the potential benefits and risks of these novel dressings as we move toward translating them into the clinic and battlefield use.
A key impact of this project is the development of clinical tools to protect the patient from infection and promote healing. Our understanding of antimicrobial material characteristics has been expanded to evaluate both the planktonic and biofilm formation characteristics they inhibit or promote. As we further investigate the interactions between biofilm inhibitors and anesthetic molecules, we can design materials and loading strategies to heal and protect burn wounds, as well as other combat or non-combat-related injuries. Our investigation of multiple different strains of pathogenic bacteria impacts the field of microbiology in adding knowledge of the effects of locally delivered therapeutics on bacterial viability and biofilm formation. Similarities or differences in response can guide development of therapeutics and may impact clinical guidelines for infection prevention and treatment. Our observation of altered exopolymeric substance production in response to these materials could lead to new understanding of the biofilm response for these microorganisms.

What was the impact on other disciplines?

With the advancement in evaluating therapeutics and biomaterials, related to the anti-bacterial/anti-biofilm affects they possess, knowledge gained in this project may apply to other engineering materials that require antimicrobial properties. For instance, electrospun chitosan modified with antimicrobials may be used in water filtration media in civil engineering.

What was the impact on technology transfer?

Discussions and negotiations have been in progress with Chitolytic to acquire a license to further develop this into a commercially available bandage. A meeting with the CEO of this small business will occur in August next quarter. We are also connecting with investigators at Tripler Air Force Base regarding potential collaborations moving forward.

What was the impact on society beyond science and technology?

By introducing a biomaterial to address wound healing we are supporting improved patient care through the use of biomaterials in medical treatments. As we move closer to our goal, these therapeutics could help a wide range of people that have been burned and in need of antimicrobial/pain relief. This could impact society by improving overall patient outcomes and reducing the costs of burn therapies to patient and society.

8. CHANGES/PROBLEMS

Changes in approach and reasons for change

NMR was proposed to study the degree of substitution of carboxylic acids on the surface of the chitosan membranes. This equipment is no longer available, and XPS was used to determine elemental ratios on the surface so that a degree of substitution could be calculated as reported in Q7.

The animal study approach has changed to use only male rats. Originally both male and female animals were planned. However, in collaborating on a separately funded burn model, we observed that skin of female rats is thinner than the male rats and would require a different burn time than for males to prevent an injury that was too severe. In addition, the smaller size of female rats posed a challenge and would have resulted in a large increase in percentage of the body burned compared to the male animals. To continue using both male and female rats, the male rats would need to be younger, and the female rats would need to be much older, resulting in a large age difference in the animals. To continue with a consistent model and decrease the experimental variables, we have chosen to continue with only male rats, which are larger at a younger age and have thicker skin.

Actual or anticipated problems or delays and actions or plans to resolve them

THP-1 cells were received but are exhibiting issues proliferating. Literature search and amendment of THP1 cell proliferation protocol is underway.

Changes that had a significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects

Not applicable.

Significant changes in use or care of vertebrate animals

TOTAL PROTOCOL(S): 1
PROTOCOL (X of Y total):
IACUC Protocol Number: **0865**

ACURO Protocol Number: MB190046.e001
Protocol PI: Jessica Jennings, PhD
Protocol Site: University of Memphis
Protocol Title: Acylated Electrospun Biopolymer Membranes for Burn Wound Coverage, Infection Prevention, and Pain Relief
Number of Animals Approved for Use: 120
IACUC Initial APPROVAL DATE: 1/21/2021 (expires 01/21/2024)
ACURO initial APPROVAL DATE: 4/21/2021
RENEWAL APPROVAL DATES: Due 4/21/2024
AMENDMENTS:
- **Updating protocol to longer burn time, using only male rats and to add investigators and new students**

adverse events or unanticipated problems:
- **None.**

Significant changes in use of biohazards and/or select agents

Not applicable.

9. PRODUCTS

Journal publications

1. Wells, CM, Harrison, ZL, Coleman EC, Jennings, JA, Antimicrobial and anti-biofilm efficacy of local anesthetics combined with cis 2 decenoic acid against Staphylococcus aureus, Pseudomonas aeruginosa, and Acinetobacter baumannii. Frontiers in Cellular and Infection Microbiology.
    a. Original manuscript
    b. Under Revision
    c. Directly related to SOW, specific aim 1
    d. DoD funding acknowledged

Books or other non-periodical, one-time publications

Nothing to report

Other publications, conference papers, and presentations

1. Harrison, Z., J. Bush, J. Bumgardner, T. Fujiwara, D. Baker, and J. Jennings. Simultaneous Delivery of Diffusible Signaling Factor and Anesthetic Improves Cytocompatibility for Burn Wound Treatment. in Military Health System Research Symposium. 2022. Orlando, FL
    a. Poster
    b. Presented
    c. Directly related to SOW, specific aim 1
    d. DoD funding acknowledged

2. Montgomery, E., Z. Harrison, and J. Jennings. Decanoic anhydride-modified chitosan membranes loaded with bupivacaine and cis-2-decenoic acid affect cytokine expression of keratinocytes. in Materials Science and Technology Annual Meeting. 2022. Pittsburgh, PA
    a. Talk
    b. Presented
    c. Directly related to SOW, specific aim 1
    d. DoD funding acknowledged

3. Yusuf, T., Z. Harrison, E. Montgomery, and J. Jennings. Cytocompatibility of Electrospun Chitosan Membranes Treated with Decanoic Anhydride and loaded with biofilm inhibitors and bupivacaine in Vanderbilt Biomaterials Day. 2022. Nashville, TN.
    a. Poster
    b. Presented
    c. Directly related to SOW, specific aim 1
    d. DoD funding acknowledged

Website(s) or other Internet site(s)

Nothing to report

Technologies or techniques

Nothing to report

Inventions, patent applications, and/or licenses

Nothing to report

Other Products

Nothing to report

10. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Jessica Amber Jennings
Project Role: PI
Researcher Identifier: https://orcid.org/0000-0002-2760-6948
Nearest person month worked: 0.75
Contribution to Project: Project management and supervision of graduate assistants

Name: Daniel Baker
Project Role: co-I
Researcher Identifier:
Nearest person month worked: 0.3
Contribution to Project: Synthesis of C2DA and input on detection methods

Name: Tomoko Fujiwara
Project Role: co-I
Researcher Identifier: https://orcid.org/0000-0002-3329-0361
Nearest person month worked: 0.3
Contribution to Project: Analysis of FTIR and input on fabrication methods

Name: Joel Bumgardner
Project Role: co-I
Researcher Identifier:
Nearest person month worked: 0.25
Contribution to Project: Input on electrospinning and modification of chitosan, analysis of release results

Name: Landon Choi
Project Role: MS graduate assistant (funded from this grant)
Researcher Identifier:
Nearest person month worked: 1.5
Contribution to Project: Release Studies, electrospinning of chitosan, performing acylation treatments, and SEM

Name: Zoe Harrison
Project Role: PhD graduate assistant (funded by departmental funds)

Researcher Identifier: https://orcid.org/0000-0002-5276-450X
Nearest person month worked: 1.5
Contribution to Project: Release studies and analysis, SEM imaging

Name: Rabeta Yeasmin
Project Role: PhD graduate assistant (funded on another grant, Project course work)
Researcher Identifier:
Nearest person month worked: 1
Contribution to Project: Antimicrobial studies of modified membranes

Name: Ezzuddin Abuhussein
Project Role: PhD graduate assistant (funded on another grant, Project course work)
Researcher Identifier:
Nearest person month worked: 0.5
Contribution to Project: HPLC and analysis

Name: Brian C. Hoffman
Project Role: PhD graduate assistant (Chemistry)
Researcher Identifier:
Nearest person month worked: 0.5
Contribution to Project: synthesis of C2DA

Name: Emily C. Montgomery
Project Role: MS graduate assistant
Research Identifier: https://orcid.org/0000-0003-1389-0586
Nearest person month worked: 1.2
Contribution to Project: Release and antimicrobial studies

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Nothing to report

11. SPECIAL REPORTING REQUIREMENTS

QUAD CHART
Convert this report to a PDF file and append updated quarterly Quad Chart in PDF as an appendix.

12. APPENDICES

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.