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TITLE: Validation of a Multi-Use Scalable, Long-Acting Nanoparticle Anesthetic Tilapia Dressing for Complex Burn Trauma

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14. ABSTRACT

Objective: The proposal goal is to identify the novel topical long-acting lidocaine nanoparticle anesthetic Dressing (NPD) optimal safe dosing strategy by examining its pharmacokinetic safety profile and efficacy. Specific Aim 1 further characterizes and develops the physicochemical properties of NPD, with and without collagen targeting ligand and determine the cold chain logistics, optimal dosing and administration for application of the NPD to a burn wound. Specific Aim 2 evaluates the efficacy and safety of the NPD applied to an acute burn wound by evaluating pain and pharmacokinetics in a pig burn model after application of the NPD to optimize dosing. Study Design: In Aim 1 we will synthesize and characterize the NPD, with and without collagen targeting ligand and then determine the cold chain logistics, optimal dosing and administration strategies for application of NPD to burn wounds. Aim 2 will evaluate pain in a 10% pig burn model treated with different doses of the NPD to assess pharmacokinetics and assure efficacy for pain, wound colonization, and healing.

15. SUBJECT TERMS None listed.

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1. Introduction

Each year >1 million people in the United States are treated for burn injury. 45,000 are hospitalized and 4,500 die. In wartime, thermal injuries account for 5-20% of military casualties, primarily soldiers in their early 20s with an average burn size of 15%. Combined burn/trauma injuries constitute 23-55% of wartime injuries, with many of the injured having soft tissue injuries. In Iraq and Afghanistan, the most severe injury in 33% of the U.S. military personnel was open soft tissue wounds. (1) Chronic pain and the associated risk of opioid addiction are common following these injuries. (2-4) Burns are expected to be prevalent in future multi-domain operations (Dense Urban Environment Analysis Report). Hence, improving care of burn, trauma, and accompanying major soft tissue injury is a military imperative. The overarching objective of this project is to improve outcomes and maintain warfighter capabilities after burn, combined burn/trauma, or major soft tissue injury, particularly in the prolonged field care situation. Burns combined with major trauma and major soft tissue injury often remove the soldier from the fight due to pain and wound care needs. A dressing that can be applied in the field to mitigate pain, control infection, and accelerate wound healing would vastly improve burn injured warfighter capabilities as well as conserve valuable medical supplies, including narcotics, which are likely to be in short supply in prolonged field care situations. We propose to employ a multiple-use scalable wound-care solution that addresses delivery of therapeutics, prevention of infection, and promotion of healing in major burn or soft tissue injury in an operational setting. Our proposal focuses on the validation of a novel topical long-acting nanoparticle anesthetic dressing, efficacious for 5-7 days, that can be applied in the field to decrease pain, facilitate wound healing, and prevent infection in the soldier with burn, burn/polytrauma, or extensive soft-tissue injury. This dressing can enable a soldier to remain functional in the field, conserve dressing and narcotic medication resources, and promote wound healing, enabling earlier return to duty. This study is unique in that it leverages technology to address pain, infection and wound healing simultaneously at the time of injury as in the hospital setting. Alignment with Focus Areas. This application addresses FY21 MBRP IDA Focus Area for development of therapeutic interventions for burn injury in the polytrauma patient with concomitant pathology to improve service member outcomes by addressing gaps in injured combatant wound care, pain control, and infection prevention at the point of injury. Our proposal develops a multi-use scalable wound-care solution applied at the point of injury to treat pain, decrease infection, and promote healing for both burns and complex

traumatic injuries (blast, penetrating, complex tissue injury). Our proposed long-acting lyophilized nanoparticle anesthetic tilapia dressing (NPD) can be reconstituted at all care levels and applied to any wound to decrease pain, improve wound healing, and mitigate infection for 5-7 days. This versatility gives further impetus for its development and makes it ideal for mass casualty events, in which traditional wound care systems are overwhelmed. The product could transform battlefield and civilian wound care by bringing sustained effective wound and non-narcotic pain treatment to the point of injury, optimizing field care and soldier battle-readiness after injury. Reasoning behind the work: Lidocaine is a commonly used readily available highly effective topical analgesic that also inhibits bacterial growth, including Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Streptococcus. (5-7) Although lidocaine is commonly used in small areas, such as a cut, to control pain, topical application of lidocaine to burns and large wounds is limited due to erratic absorption, short duration of therapeutic efficacy, and systemic toxicity (seizures, cardiac dysfunction). Local anesthetic toxicity can occur following topical application, direct wound infiltration, or nerve block administration. (8-12) A liposomal local anesthetic formulation can provide longer effective analgesia duration, but its use is limited to infiltration or nerve block injection thus requiring expertise, equipment and sterile conditions, (13-15) ,) and has associated toxicity risks despite the slower drug release. Pain control using nerve block catheters has been shown to safely improve pain control and reduce opioid needs but requires expertise, supplies and a facility that allows administration in a sterile manner. (16-18) Further, implanted catheters can become infected, and infusion of continuous analgesia presents a risk of local anesthetic toxicity (19-20) Nanotechnology enables the development of a product that can simultaneously target and control lidocaine release, making it feasible as a long term (multiple day) primary wound treatment. Nanotechnology has been used successfully in the development of cancer therapeutics, and we propose to extend this to anesthetic drug delivery. Dr. Kit Lam, CoPI on this study, is a pioneer in nanotechnology drug delivery in cancer patients. He has formulated a topical nanoparticle lidocaine anesthetic that controls lidocaine release and a second one that can be easily modified to bind to collagen (details in specific aim 1). Biologic dressings have been used for >20 years in burn and wound care to optimize the wound environment, provide growth factors, decrease bacterial invasion, prevent water loss, and protect the wound. (21) A recently introduced biological, tilapia skin, has been developed and utilized by Dr. Peyton (CoPI in this proposal), to treat burned animals from the California wildfires, with improved healing and decreased pain. Similarly, tilapia skin has been used

selectively in burned humans. (22-23) Animal studies suggest that tilapia skin improves wound healing by accelerating keratinocyte proliferation. (24-25) Traditional biologic dressings are difficult to transport, expensive, and have limited shelf life. However, recent introduction of lyophilization of biologics has enhanced their usability in austere conditions. Dr. Peyton has created a lyophilized version of tilapia skin (details in specific aim 2). We will utilize this lyophilized tilapia skin as a wound treatment modality. To establish efficacy of the novel nanoparticle anesthetic dressing, we will evaluate the pharmacokinetics of the nanoformulation at different concentrations and wound sizes and evaluate the efficacy of the nanoparticle anesthetic in relieving pain. This will form the foundation for future comparative studies of the nanoparticle anesthetic alone, the biologic (tilapia skin) alone, and the combination of the two to each other and to traditional topical burn treatment to optimize wound healing, minimize infection, and improve pain control. This combination of analgesic, antibacterial, and wound healing optimizer may immediately improve warfighter capabilities after injury (pain control), conserve medical resources (dressings and narcotics) and return the injured warfighter to duty more rapidly (accelerate wound healing, prevent infection). II. Hypotheses: 1. Adding a collagen targeting ligand to the surface of the topical long-acting nanoparticle anesthetic will improve delivery, adherence, and retention of the nanocarrier, thus allowing sustained release of the anesthetic at the wound. 2. The novel topical long-acting nanoparticle anesthetic can be formulated such that it is compatible with cold chain logistics. 3. Pharmacokinetic profiling of the NPD allows identification of optimal safe and effective dosing to relieve pain, improve wound healing, and decrease bacterial wound colonization. III. Specific aims: Aim 1: Further development and physicochemical characterization of a novel topical long-acting nanoparticle anesthetic (NPD). 1.a. Synthesize and further characterize a NPD, with and without collagen targeting ligand. 1.b. Determine the cold chain logistics for the NPD for a burn wound. Aim 2: Evaluate the safety and efficacy of the NPD applied to an acute burn wound. 2.a. Evaluate the pharmacokinetics of the NPD in a porcine burn model. 2.b. Determine the optimal dosing of the NPD based on wound size, NPD concentration, and pain relief.

2. Keywords

Burn injury, tilapia, nanoparticle anesthetic dressing, pharmacokinetics, pain

3. Accomplishments

Specific Aim 1: Further development and physicochemical characterization of a novel topical longacting nanoparticle anesthetic. Duration to achieve this aim: 12 months

Major tasks in Aim 1
1.a. Prepare and characterize nano-particle anesthetic. Timeline: 1-6 months
Progress to date: nanoparticle lidocaine anesthetic in preparation by Dr. Lam.
1.b. determine the cold chain logistics, optimal dosing and administration strategies for application of the long-acting nanoparticle anesthetic to a burn wound. Timeline: 6-12 months

Milestone goal: Synthesis of anesethetic nanoformlation of lidocaine by polymers optimized; enough nanoformlations for aim 2 studies. Completion target month 16.

Specific Aim 2: Evaluate the pharmacokinetics and dosing of the nanoanesthetic. Duration for this aim: 6-36 months

Major Task Aim 2
2.a. Evaluate the pharmacokinetics of the NPD in a porcine burn model using 12 pigs.
Progress to date: IACUC and ACURO approval obtained.
2.b. Determine the optimal dosing of the NPD based on wound size, NPD concentration, and pain relief (using same 12 pigs as in 2.a.)

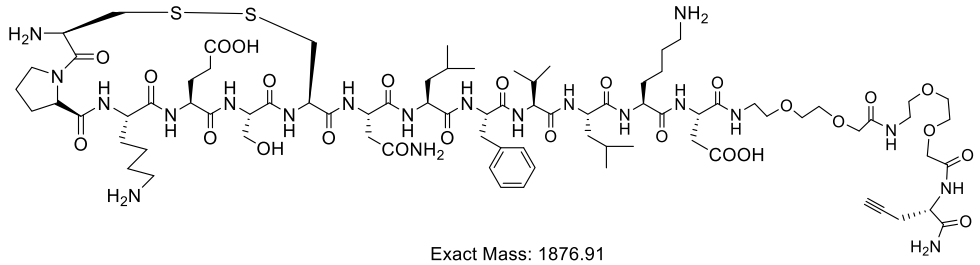
Milestones goals:
Local IACUC/ACURO Approval
IACUC/ACURO Approval
Pig burn model acclimation and optimization
Experiments to determine pharmacokinetics of nanoanesthetic
Efficacy studies completed

Major Activities

1.a. Preparation of nano-particle anesthetic. Timeline: 1-6 months

Progress to date:

1. In the past 3 months of the project, the Lam lab has successfully prepared the collagen binding peptide CPKESCNLFVLKD with an alkyne tethered to the carboxyl terminus via two short hydrophilic AeeA linkers to form CPKESCNLFVLKD-AeeA-AeeA-Pra (**Figure 1a**). We then purified it by reverse phase HPLC and its identy was confirmed by Orbitrap-ESI mass spectrometry (**Figure 1b and c**).



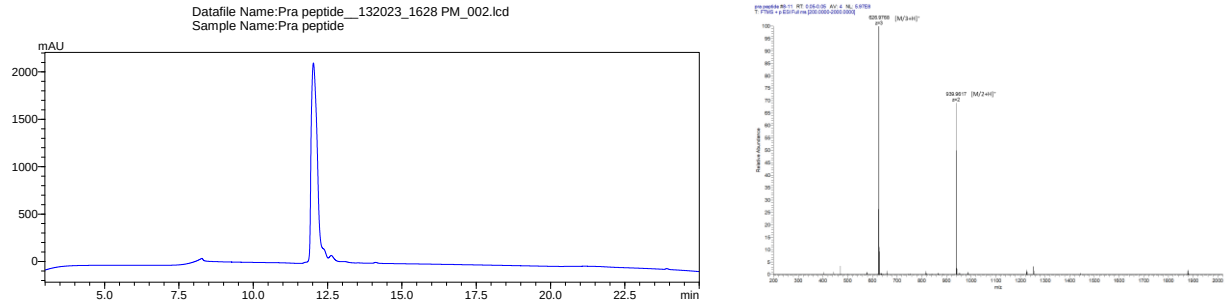
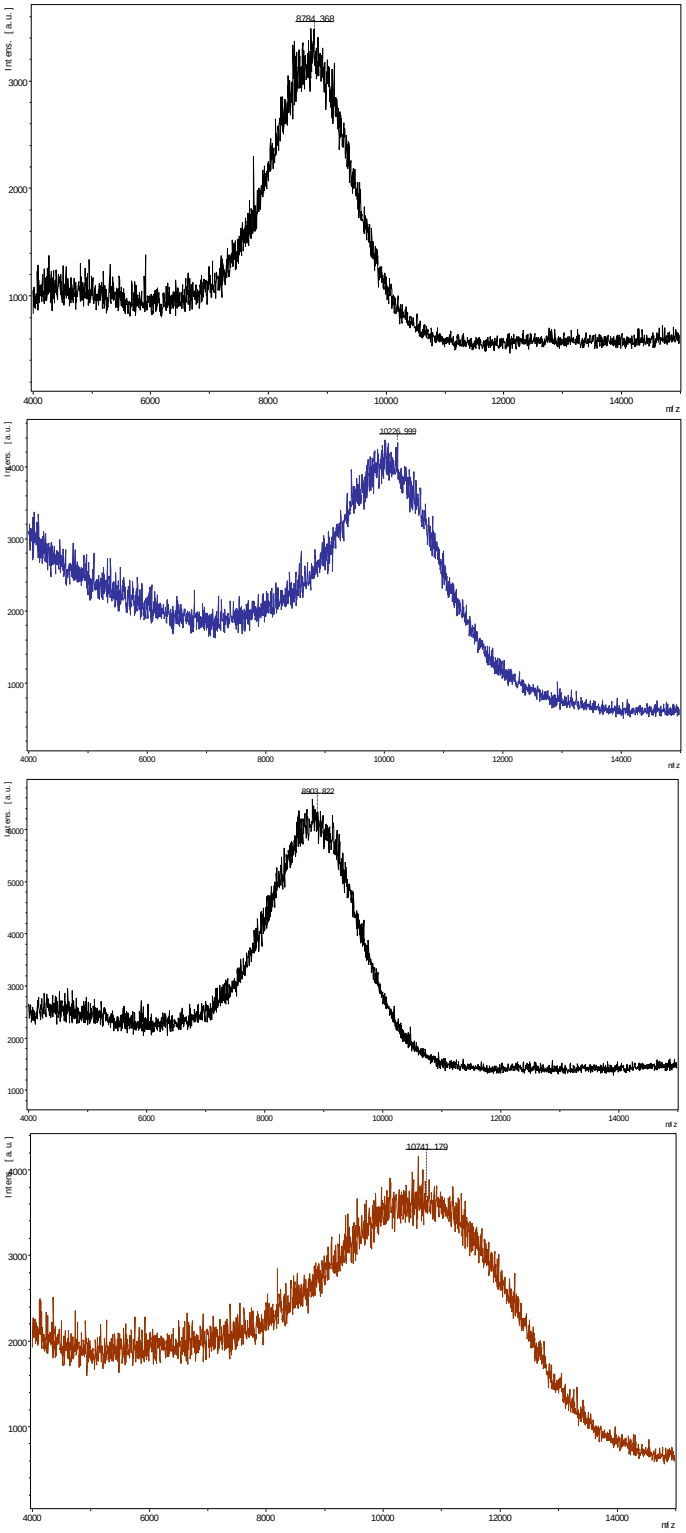


Figure 1 a). Chemical structure of the collagen binding peptide tethered to an alkyne (CPKESCNLFVLKD-AeeA-AeeA-Pra), b). HPLC profile (214nm) of the purified peptide, and c). Orbitrap-ESI analysis of the peptide confirmed the identify of the compound.

2. We have also prepared necessary telodendrimers for the initial phase of the project. This include the successful conjugation of CPKESCNLFVLKD-AeeA-AeeA-Pra to N_3-telodnerimder. Mass spectrometry analysis of these products confirmed their identity (**Figure 2**).



N_3-PEG^{5k}-CA_8
Calculated molecular weight: 9029.72
Found molecular weight 8784.368.

CPKESCNLFVLKD-PEG^{5k}-CA_8
Calculated molecular weight: 10906.63,
found molecular weight 10227.00, 10600.017.

PEG^{5k}-CA_8
Calculated molecular weight: 9018.89,
found molecular weight 8903.722

PEG^{5k}-Cys_4-L_8-CA_8
Calculated molecular weight: 11271.94,
found molecular weight 10741.179.

Figure 2. MALDI-MS analysis of the four telodendrimers prepared for the initial phase of the project.

3. Lidocaine Nanodformulation*.*

We prepared and characterized a total of three lidocaine nanoformulations, with and without collagen-binding ligands: *a. PEG^{5k}-CA_8; b. 1:1, PEG^{5k}-CA_8: PEG^{5k}-Cys_4-L_8-CA_8; c. 1:1:1, Ligand-PEG^{5k}-CA_8: PEG^{5k}-CA_8: PEG^{5k}-Cys_4-L_8-CA_8.*

We also performed some cold chain experiments by evaluating the stability of the nanoformulation, as lyophilized powder, over time (up to 231 days) at room temperature. The stability analysis encompassed the utilization of Dynamic Light Scattering (DLS, **Figure 3**) and High-Performance Liquid

Chromatography (HPLC, **Figure 4**) methods.

General procedure for preparing nanoparticles: 20 mg of telodendrimers was dissolved in chloroform/methanol (10:1, v/v, 2mL), vortexed to make sure it was dissolved well. Five mg of lidocaine was then dissolved in chloroform (1mL) and combined with the telodendrimer solution. The solvents were removed under vacuum gradually until a thin film was formed. Two mL of DI water was introduced, and it was vortexed for 20 min until the solution became homogeneous. A 0.22 μm filter was used to remove any unloaded lidocaine (precipitate) and to sterilize the solution of nanolidocaine. The clear nanolidocaine formulation was then lyophilized to dryness for room temperature storage.

3.1 Assessing Prepared Nanoparticle Stability via DLS

We mixed 10 μL of nanoparticles with 990 μL MiniQ water, and the mixture was filtrated through a 0.22 μm filter before DLS analysis by Zetasizer nano ZS. Samples were collected at various time points for particle size distribution analysis using DLS and drug concentration assessment using HPLC. The results are shown below (**Figure 3**).

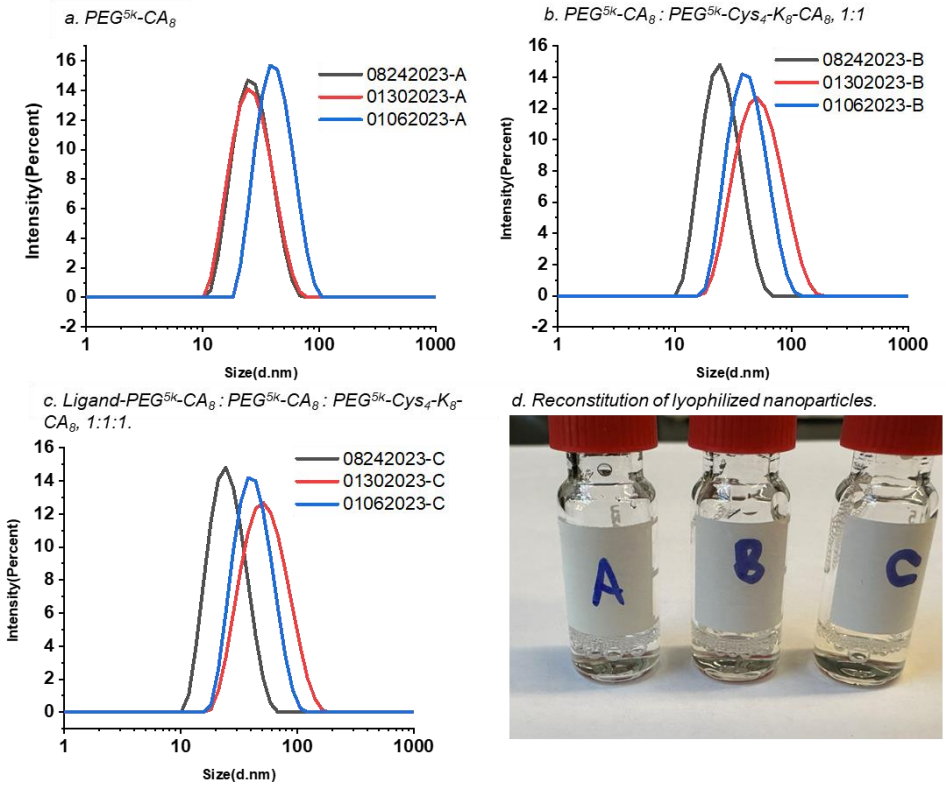
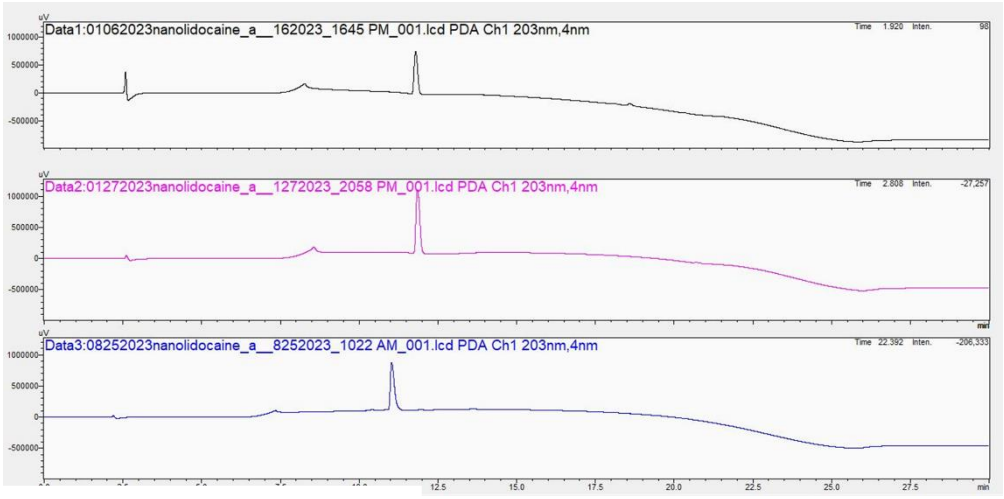


Figure 3a-c. Nanoformulations (A), (B) and (C) collected at various time points post-preparation were analyzed for nanoparticle size distribution at specific intervals: 0, 24, and 231 days, respectively. **d)** Following the lyophilization of the prepared nanoparticles and subsequent storage at room temperature for 231 days, the reconstitution of nanoformulation (A) (B) and (C) in MiniQ water resulted in a visibly clear solution.

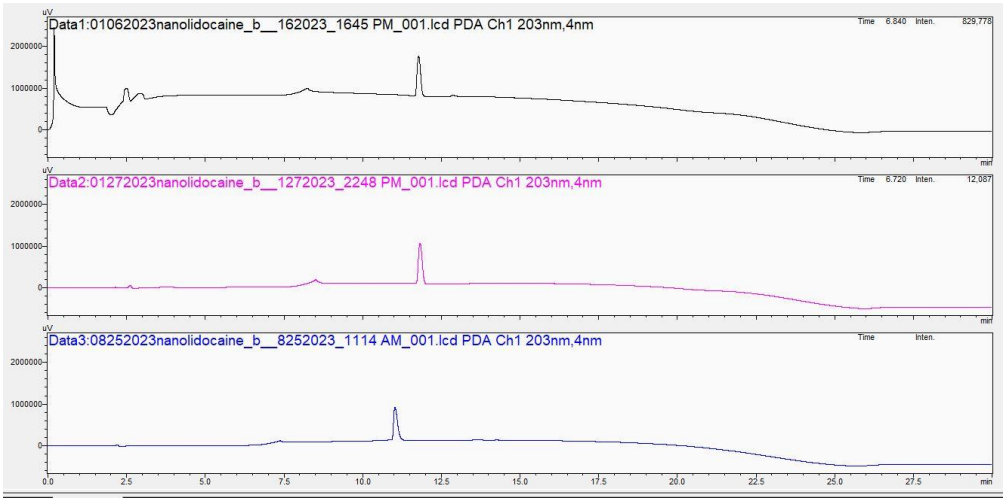
3.2 HPLC Evaluation of Prepared Nanoparticle Stability;

We mixed 20 μL of nanoparticles (nanoformulation A, B or C) and mixed it with 180 μL of methanol and then injected the sample into HPLC to analyze the content of lidocaine, and the experiment was repeated twice more. From the HPLC spectrum, the UV absorption area at retention time11.0-11.8 minutes was recorded.

A. PEG^{5k}-CA$_8$



B. PEG^{5k}-CA$_8$: PEG^{5k}-Cys$_4$-K$_8$-CA$_8$, 1:1



C. Ligand-PEG^{5k}-CA$_8$: PEG^{5k}-CA$_8$:PEG^{5k}-Cys$_4$-K$_8$-CA$_8$, 1:1:1.

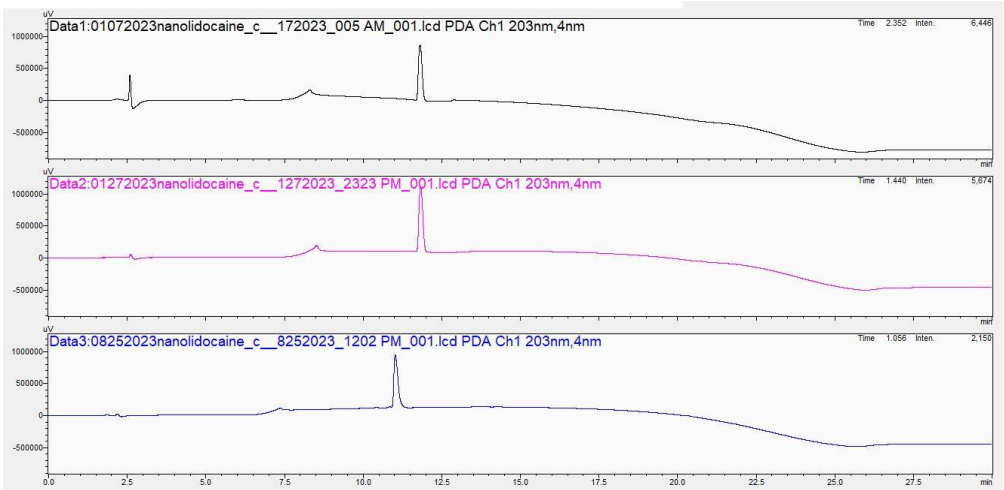


Figure 4. Samples A, B and C collected at various time points post-preparation were analyzed by HPLC at specific intervals: 0, 20, and 231 days.

3.3 Lidocaine Loading Capacity within Telodendrimer;

10 mg of telodendrimers was dissolved in chloroform / methanol (10:1, v/v, 2mL), vortexed to make sure it was dissolved. Lidocaine (2, 4, 6, 8 or 10 mg) was dissolved in chloroform (1mL) and combined with the telodendrimer solution. The solvents were removed under vacuum gradually until a thin film was formed. Two mL of DI water was introduced, and it was vortexed for 20 min until the solution became homogeneous. A 0.22 μm filter was used to remove any unloaded lidocaine (precipitate) and

to sterilize the solution. HPLC was used to make a standard curve ($Y = 1.83887\times10^7 X + 2.64149\times10^6$; $R^2 = 0.9951734$, $R = 0.9975838$), and subsequently it was used to analyze the concentration of Loaded Lidocaine (**Figure 5**).

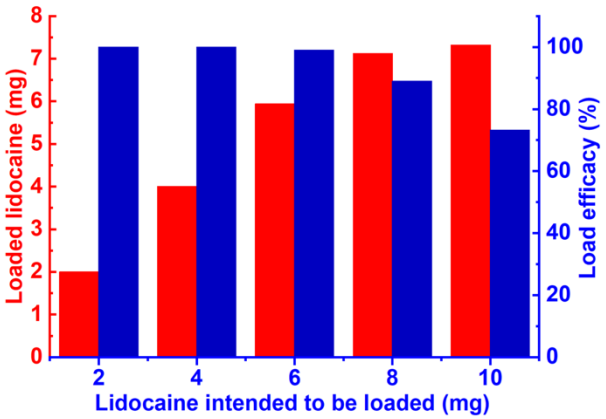


Figure 5. Lidocaine loading capacity by the telodendrimer. The red histogram depicts the quantity of loaded lidocaine, while the blue histogram illustrates the loading efficiency.

Goal for nanoparticle anesthetic creation on schedule

Specific Aim 2: Evaluate the pharmacokinetics and dosing of the nanoanesthetic
Major Task Aim 2
a. Prepare animal protocol to local IACUC for approval:
IACUC protocol number 22926 approved 9/15/22 by the University of California Davis IACUC
b. Prepare animal protocol to ACURO for approval
ACURO protocol MB210052.e001 approved as of 10/28/22

IACUC and ACURO goals met ahead of schedule

Describe the Regulatory Protocol and Activity Status (if applicable).
Describe the Protocol and Activity Status for sections a-c, as applicable, using the format described for each section. If there is nothing significant to report during this reporting period, state "Nothing to Report."

(a) ***Human Use Regulatory Protocols: NA***

(c) Animal Use Regulatory Protocols
TOTAL PROTOCOL(S):
State the total number of animal use protocols required to complete this project (e.g., 2 animal use research protocols will be required to complete the Statement of Work.). If not applicable, write "No animal use research will be performed to complete the Statement of Work."

1 animal protocol will be required to complete the statement of work.

PROTOCOL(S):

Identifier and Protocol Title: Proposal Number MB210052, Award Number W81XWH-22-1-0575 ntitled, "Validation of a Multi-Use Scalable, Long-Acting Nanoparticle Anesthetic Tilapia Dressing for Complex Burn Trauma"
IACUC protocol number 22926 approved 9/15/22 by the University of California Davis IACUC
ACURO protocol approved as of 10/28/22
Protocol 1 of 1 total):
Protocol MB210052.e001:
Title: Validation of a Multi-Use Scalable, Long-Acting Nanoparticle Anesthetic Tilapia Dressing for Complex Burn Trauma
Target required for statistical significance: 12
Target approved for statistical significance: 12

Submitted to and Approved by:

Provide bullet point list of protocol development, submission, amendments, and approvals (include IACUC in addition to ACURO).
Initial IACUC submission: 7/7/22
First IACUC revision: 7/11/22
Second IACUC review comments: 8/2/22; resubmission that day
Third IACUC review comments: 8/5/22; resubmission that day
Fourth IACUC review comments: 8/9/22; resubmission 8/31/22
IACUC protocol number 22926 approved 9/15/22 by the University of California Davis IACUC
Initial ACURO protocol submitted: 9/18/22
ACURO protocol approved as of 10/28/22

4. Impact

The project is still in progress and hence has not had a direct impact on clinical care.

5. Changes/Problems

Supply chain issues were encountered in the development of the nanoparticle anesthetic dressing; however, we were able to synthesize and characterize the NPD. Our next challenge is to load the nanoparticle anesthetic into the tilapia dressing.

6. Products

None to date, but the nanoparticle formulation with lidocaine has been created

7. Participants & Other Collaborating Organizations

Participants include Dr. Tina Palmieri, PI, burn surgeon and researcher at UC Davis Health; Jamie Peyton, DVM, from the University of California Davis Veterinary School; Dr. Kit Lam, Professor of Biochemistry at UC Davis; and Dr. Richard Applegate from UC Davis Health Department of Anesthesiology. Dr. Applegate left UC Davis in July and has thus not received any funding since his departure.

8. Special Reporting Requirements

None

9. Appendices

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