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**TITLE:** Novel Cyclic Lipopeptides for Treating Complicated Wound Infections

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**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

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Approved for Public Release; Distribution Unlimited

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**14. ABSTRACT**

The purpose for this grant is to develop a new class of antibacterial agents, cyclic lipopeptides, derived from the fusaricidin/LI-F family of naturally occurring antifungal antibiotics for the prevention and treatment of complicated combat-related or trauma-induced wound infections caused by multidrug-resistant (MDR) pathogens and biofilm formation. Three aims are planned: (1) To optimize/synthesize lead cyclic lipopeptides and assess their antimicrobial/antibiofilm activity and toxicity *in vitro*; (2) to develop and optimize a cyclic lipopeptide delivery system based on anionic graft copolymer nanoparticles for topical application; and (3) to characterize/optimize, evaluate dosing, efficacy, and toxicity/safety of the combined cyclic lipopeptide/polymer nanocomplexes in several porcine models for infection prevention, biofilm elimination, and wound healing. The modification of the amino acid sequences of lead depsipeptides will disrupt the balance between the charge and hydrophobicity leading to a better separation of antibacterial activity and nonselective toxicity. In vitro and in vivo studies will be performed to study antimicrobial and wound healing efficacy.

**15. SUBJECT TERMS**
Antimicrobial peptides, *Pseudomonas aeruginosa,* Burn Wounds, Healing

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## 1. Introduction

We propose to develop a new class of antibacterial agents, cyclic lipopeptides, derived from the fusaricidin/LI-F family of naturally occurring antifungal antibiotics for the prevention and treatment of complicated combat-related or trauma-induced wound infections caused by multidrug-resistant (MDR) pathogens and biofilm formation. Three aims are planned: (1) To optimize/synthesize lead cyclic lipopeptides and assess their antimicrobial/antibiofilm activity and toxicity *in vitro*; (2) to develop and optimize a cyclic lipopeptide delivery system based on anionic graft copolymer nanoparticles for topical application; and (3) to characterize/optimize, evaluate dosing, efficacy, and toxicity/safety of the combined cyclic lipopeptide/polymer nanocomplexes in several porcine models for infection prevention, biofilm elimination, and wound healing. The study hypothesis is that modification of the amino acid sequences of lead depsipeptides will disrupt the balance between the charge and hydrophobicity leading to a better separation of antibacterial activity and nonselective toxicity. Both *in vitro* and *in vivo* studies will be performed to evaluate the efficacy of the topical formulations.

## 2. KEYWORDS:

Antimicrobial peptides, Infection, Biofilms, *Pseudomonas aeruginosa,* Wounds, Healing

## 3. ACCOMPLISHMENTS:

### a) Major goals and objectives

**Specific Aim 1: Optimize lead cyclic lipopeptides using systematic and rational synthetic chemistry approach.**
**Major Task 1**: Optimize amino acid sequence of lead cyclic lipopeptides.
*Subtask 1*: Obtaine the University of Miami Institutional Animal Care and Use Committee (IACUC) and ACURO approval for the use of porcine model in the proposed in vivo experiments.
*Subtask 2:* Synthesize unusual amino acid building blocks.
*Subtask 3:* Synthesize cyclic lipopeptides with different amino acid sequences.
*Subtask 4:* Assess synthesized peptides in vitro antimicrobial/antibiofilm activities and toxicity.
**Major Task 2:** Optimize the lipidic tail of the lead cyclic lipopeptides.
*Subtask 1:* Synthesize cyclic lipopeptide with optimized lipidic tail length and intercharge distance.
*Subtask 2:* Assess synthesized peptides in vitro antimicrobial/antibiofilm activities and toxicity.
**Major Task 3:** Large scale synthesis and purification of selected cyclic lipopeptides.
*Subtask 1:* Synthesize and purify large gram quantities of selected cyclic lipopeptides required for research outlined in Aims 2 and 3.

**Specific Aim 2: Develop and optimize cyclic lipopeptide delivery system for topical application.**
**Major Task 4:** Synthesize graft copolymers and their cyclic lipopeptide nanocomplexes.
*Subtask 1:* Validate synthesis protocol for graft copolymers from PMAA and PPAA backbones and polyetheramines at 1g/batch scale.
*Subtask 2:* Prepare cyclic lipopeptide/polymer nanocomplexes with various peptide/polymer charge ratios and peptide concentrations.
**Major Task 5:** Characterize properties of cyclic lipopeptide/polymer complexes.
*Subtask 1:* Assess efficacy of nanocomplex binding and release kinetics for cyclic lipopeptide using ultrafiltration, dialysis and RPHPLC.
*Subtask 2:* Determine stability of cyclic lipopeptide/polymer nanocomplex in human plasma using RP-HPLC and MALDI-TOF MS.
*Subtask 3:* Optimize the viscosity of the cyclic lipopeptide/graft copolymer nanocomplex formulations for topical delivery
**Major Task 6:** Assess antimicrobial activity and toxicity of cyclic lipopeptide/polymer nanocomplexes.
*Subtask 1:* Assess cyclic lipopeptide/polymer complexes in vitro antimicrobial/antibiofilm activities and toxicity.
*Subtask 2:* Based on the data from Aims 1 and 2, prepare optimal selected cyclic lipopeptide/polymer formulation in large quantity.
*Specific Aim 3:* Assess selected cyclic lipopeptides therapeutic potentials using porcine wound infection model.

**Major Task 7:** Evaluate therapeutic potential of lipopeptides in porcine partial thickness wound infection model
*Subtask 1:* Assess efficacy of selected lipopeptides using deep partial thickness infection prevention model.
*Subtask 2:* Assess efficacy of lipopeptide/polymer nanocomplexes using deep partial thickness infection prevention model.
*Subtask 3:* Assess effects of lipopeptides on bacterial virulence in vivo
**Major Task 8:** Assessment of lipopeptides anti-biofilm potential in vivo using full thickness biofilm elimination model.
*Subtask 1:* Assess efficacy of selected lipopeptides and lipopeptide/polymer nanocomplexes in porcine full thickness biofilm-associated elimination model.
**Major Task 9:** Effects of lipopeptide formulations on wound healing
*Subtask 1:* Assess effects of selected lipopeptides and lipopeptide/polymer nanocomplexes on wound healing.
*Subtask 2:* Histological and visual assessment of wound tissue.
*Subtask 3:* Molecular assessment of wound tissue.

b) Accomplishments under the goals

Along with Torrey Pines we have accomplished the following:

1. Obtained the IACUC and Animal Care and Use Review Office (ACURO) approval
2. We published the following publication: N. Bionda, R. M. Fleeman, C. de la Fuente-Núñez, M. C. Rodriguez, F. Reffuveille, L. N. Shaw, I. Pastar, S. C. Davis, R. E.W. Hancock, P. Cudic, Identification of novel cyclic lipopeptides from a positional scanning combinatorial library with enhanced antibacterial and antibiofilm activities, *Eur. J. Med. Chem.* 2016 Jan 27;108:354-63. doi: 10.1016/j.ejmech.2015.11.032..
3. Submitted an abstract entitled “Tryptophan and arginine rich antibacterial cyclic lipopeptides” for the 2016 MHSRS meeting.
4. Completed Major Task 1, Subtasks 1 and, 3.
5. Completed Major Task 1, Subtasks 2 and 4.
6. Completed Major Task 2, Subtasks 1 and 2
7. Completed Major Task 3
8. Almost completed Task 4-6 (Synthesize graft copolymers and their cyclic lipopeptide nanocomplexes). Due to Torrey Pines subcontract (Dave Davore: Rutgers) running out of money to complete this task, we have continued work with a DMSO delivery system.
9. Presented at the 28$^{th}$ Annual Meeting of the Wound Healing Society with the Symposium on Advanced Wound Care (SAWC). Georgia World Congress Center in Atlanta, Georgia, A pril 13-17, 2016
10. Completed Task 7, Subtasks 1 and 2 (Task 3 almost complete)
11. Currently working on Task 8 and Task 9 will start in early 2019.

**Significant Results and Key Outcomes**

**Major Tasks 1-6 (see report from partnering PI, Richard Houghten [Torrey Pines])**

Over the past year Torrey Pines has continued to optimize the lipopeptides by introducing hydrophobic amino acid residues such as $N^{\alpha}$-Fmoc-D-tryptophan, $N^{\alpha}$-Fmoc-L-tryptophan, Fmoc-D-phenylalanine and Fmoc-L –phenylalanine in the macrocycle. Along with their subcontractor, David Devore (Rutgers) they formulated the optimal cyclic lipopeptides into nanocomplex formulations (see Torrey Pines report) in order for us to study their *in vivo* activity.

**Major Task 7: Evaluate therapeutic potential of lipopeptides in porcine partial thickness wound infection model**

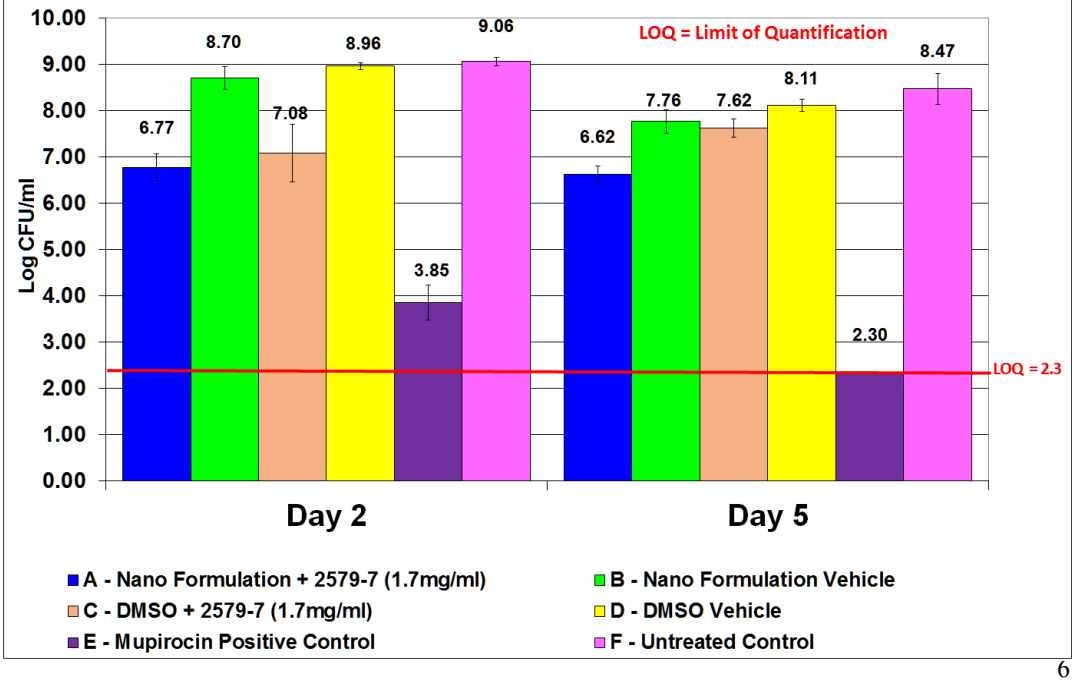
**Subtasks 1-3: Assess efficacy of lipopeptides using deep partial thickness infection prevention model (prior to biofilm formation) and sssessment of lipopeptides on bacterial virulence in vivo.**

Since combat wounds can easily become infected and initial treatment is important we used our deep partial thickness infection model. In this model we determined whether early treatment (within 20 minutes) with the nancomplex formulations could enhance the antimicrobial efficacy of selected cyclic lipopeptide(s) as compared to DMSO delivery. We initially examined 1.7mg/ml of peptide and then subsequently were able to increase the concentration of the peptide to 7mg/ml (which is maximum capacity of the current nanoformulation).

Briefly, we created forty-eight partial thickness wounds (10x7x0.5mm) on the back of a pig with an electrokeratome. Wounds were inoculated with 25 µl of the suspension $10^6$ suspension of MRSA that was scrubbed into each wound. Eight wounds were assigned to one of the following treatment groups: A) nanoformulation + peptide 2579-7 (1.7 or 7mg/ml), B) nanoformulation vehicle, C) DMSO + peptide 2579-7 (1.7 or 7mg/ml), D) DMSO vehicle, E) Mupirocin positive control and F) untreated control. All treatments including untreated were covered with a polyurethane film dressing to prevent possible cross contamination. Wounds were treated daily and four wounds from each treatment group were recovered from each treatment group on days 1 or 2 and 5 post treatment using an established scrub technique, selective media and spiral plater system. All plates were incubated aerobically overnight (24 hours) at 37°C, after which the number of viable colonies were counted.

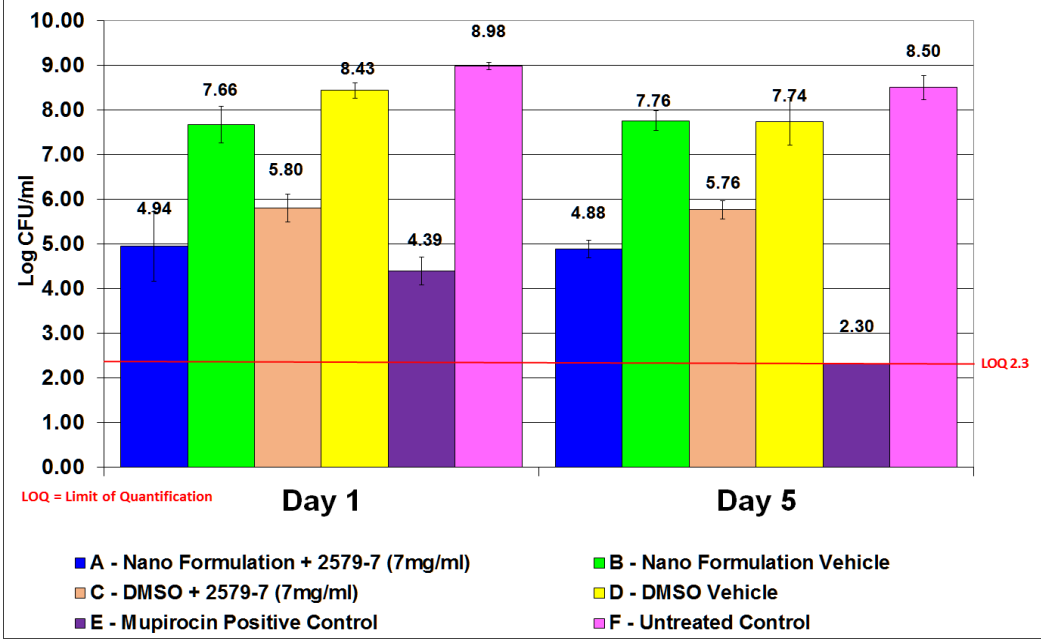
As seen in Figure 1 on Day 2 the Nanofomulation with peptide at 1.7mg/ml was able to reduce the MRSA counts by 2 and 2.3 log CFU/ml as compared nanoformulation vehicle and untreated control respectively. The Nanofomulation with peptide was also able to reduce the MRSA counts by 0.3 log CFU/ml and 1 log CFU/ml on days 2 and 5 respectively as compared to DMSO + peptide thus showing an additional benefit of the delivery system. We did see that our positive control mupirocin showed the hightest reduction in MRSA counts with 3.85 and 2.3 log CFU/ml on days 2 and 5 respectively. Note that the limit of quantification (LOQ) of the spiral plater is 2.3 log CFU/ml.

**Figure 1: Methicillin Resistant Staphylococcus aureus (MRSA) counts**



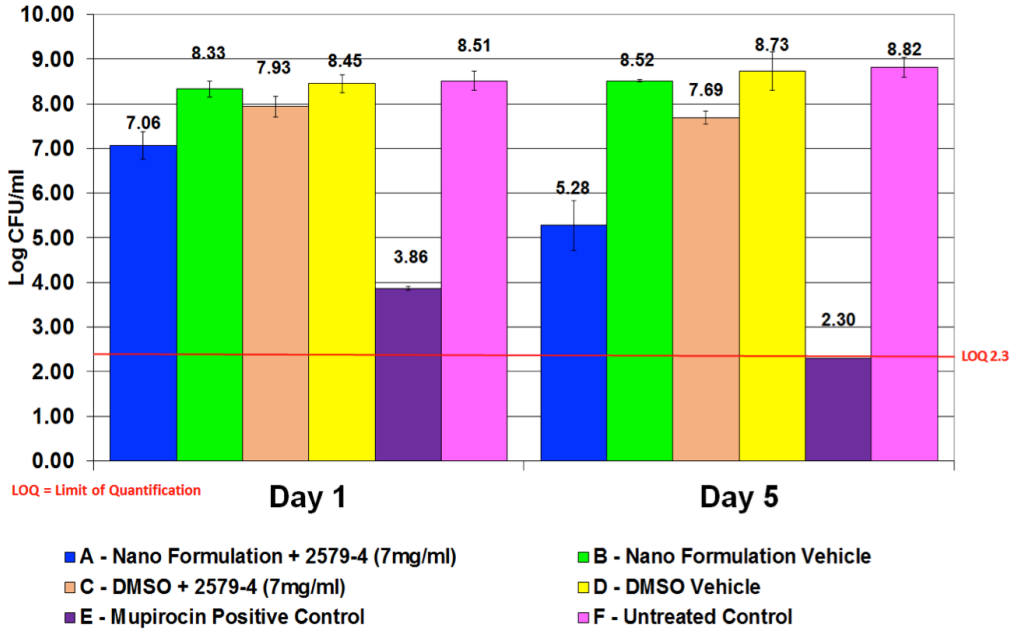
The results from the animal that was treated with the higher peptide formulation can be seen in Figure 2 below. On day 1 wounds which were treated with the peptide in the nanoformulation had a 2.72 log CFU reduction of MRSA as compared to nanoformulation alone and a 0.86 log CFU reduction compared to peptide in DMSO. Topical murpirocin again had the lowest MRSA counts on this day (4.39 log CFU/ml). On day 5, topical muripocin continued to have the lowest MRSA counts. The next best results on day 5 for MRSA reduction was found with the peptide nanoformulation (4.88 log CFU/ml). There was a decrease of almost a log as compared to peptide in DMSO and almost a 3 log reduction as compared to both vehicles (nanoformulation and DMSO alone). As expected the untreated control wound counts remained high (above 8.5 log CFU/ml). As compared to the previous animal with the same peptide at a lower concentration (1.7mg/ml – see Figure 1), the higher concentration (7mg/ml) was over 2 log CFU/ml more effective when formulated in the nanoformulation.

**Figure 2: Methicillin Resistant Staphylococcus aureus (MRSA) counts**




This past year we continued the *in vivo* antimicrobial analysis and performed additional animals with optimal cyclic lipopeptides in nanoformulations. The below data shows the microbiology efficacy of the other leading peptide (2579-4) using the same methodology.
As seen in Figure 3, on day 1 wounds which were treated with topical mupirocin had the lowest MRSA counts (3.86 log CFU/ml) followed by the nanoformulation +peptide 2579-4 which showed an MRSA count of 7.06 log CFU/ml. The nanoformulation + 2579-4 showed enhanced antimicrobial activity as compared to the DMSO + 2579-4 by almost a log (0.87 log CFU/ml). The nanoformulation + 2579-4 had over a log reduction(1.27 log CFU/ml) as compared to its own vehicle.
The highest MRSA counts were found with the untreated control and DMSO vehicle with 8.51 log CFU/ml and 8,45 log CFU/ml, respectively. On day 5 similar trends were seen with all treatment groups with topcial mupirocin

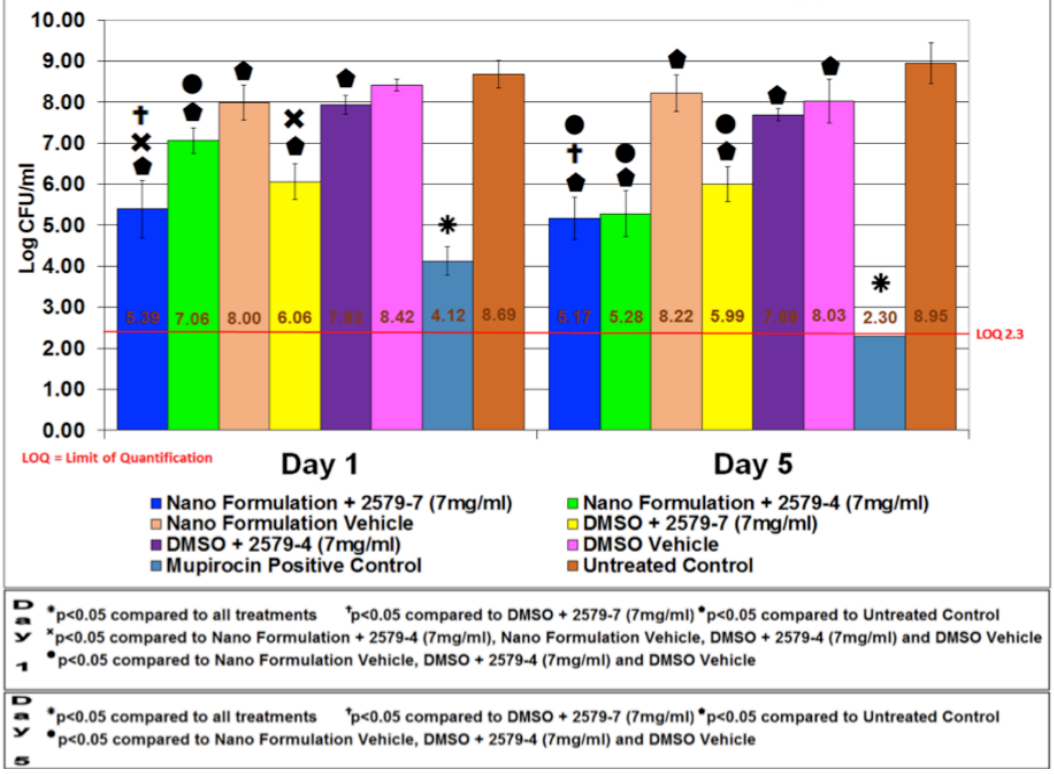
**Figure 3: Methicillin Resistant Staphylococcus aureus (MRSA) counts**



having the lowest MRSA counts. A 1.56 and 1.78 log CFU/ml reduction of MRSA was seen from Day 1 to Day 5 with murpicin and Nanoformulation + 2579-4.

The above data was then combined with previous animals which also assessed the other leading peptide candidates (2579-7 and 2579-4 at 7mg/ml). Statistical analysis was performed on the data and is presented below. As seen in Figure 4 below the topical mupirocin was the most effective treatment against MRSA followed by Nanoformulation + 2579-7 and Nanoformulation + 2579-4, respectively. Nanoformulation 2579-7 was found to be statistically more effective than Nanoformlation + 2579-4 on day 1. Both of the nanopeptide peptide formulations were found to have enhanced antimicrobial activity as compared DMSO delivery. The untreated control wounds and vehicles (nanoformulation and DMSO) had the highest MRSA counts (Days 1 and 5).

**Figure 4: Combined MRSA counts with statistical analysis**



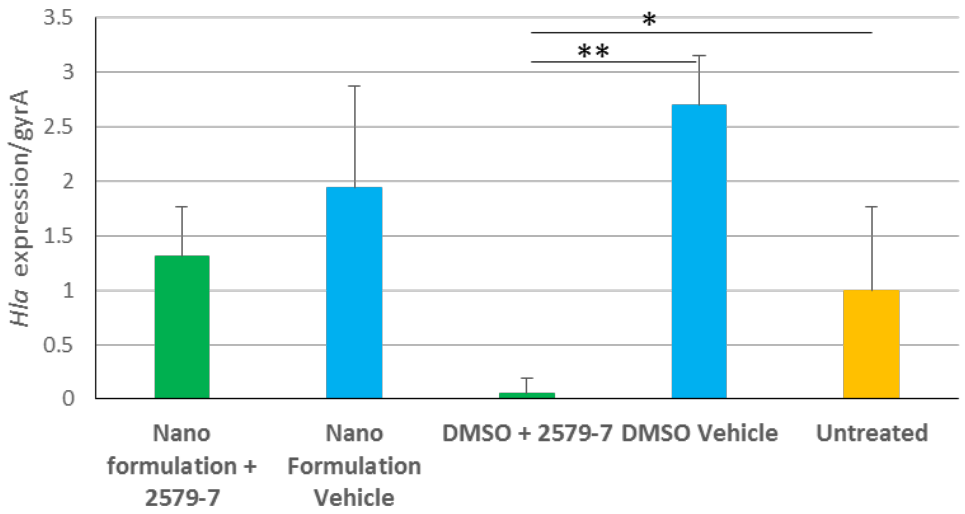

## Virulence Analysis

In additional to bacterial counts, we have assessed the ability of the formulations to influence virulence factors which are produced by the bacteria. We previously reported relative expression of the MRSA virulence factor Panton-Valentine leukocidin (*pvl*) in wound samples and found it to be upregualted as compared to vehicle control. This year we have assessed two additional virulence factors.

Based on the previously performed CFU determination and observed reduction of MRSA load we analyzed expression of the additional virulence factors in wounds treated with 2579-7 incorporated in nano-formulation or dissolved in DMSO. Total RNA was extracted from all infected and treated tissue, vehicle treated tissue samples as well as untreated infected wounds. cDNA was generated with Reverse transcription and DNase treatment using Quantitect Reverse Transcription Kit (Qiagen) followed by qPCR (IQ Supermix, Quanta). Gene expression quantification was performed in triplicates using the CFX96 real-time PCR system (Bio-Rad). A mock reaction without addition of reverse transcriptase ensured that amplification was specific for mRNA, while DNA was completely eliminated. We determined relative expression of the MRSA virulence factors alpha hemolysin (*hla*) and staphylococcal protein A (spA) in wound tissue collected at day 5. Alpha-hemolysin, also known as alpha-toxin, is the major cytotoxic agent released by *Staphylococcus aureus* and the first identified member of the pore forming beta-barrel toxin family. The hla protein monomer forms heptameric units on the cellular membrane to form complete beta-barrel pore. This structure allows the toxin to perform its major function, development of pores in the cellular membrane, eventually causing cell death. *Hla* also plays a role in *S. aureus* invasion through human epidermal keratinocytes. Our data shows suppression of this virulence factor in wounds treated

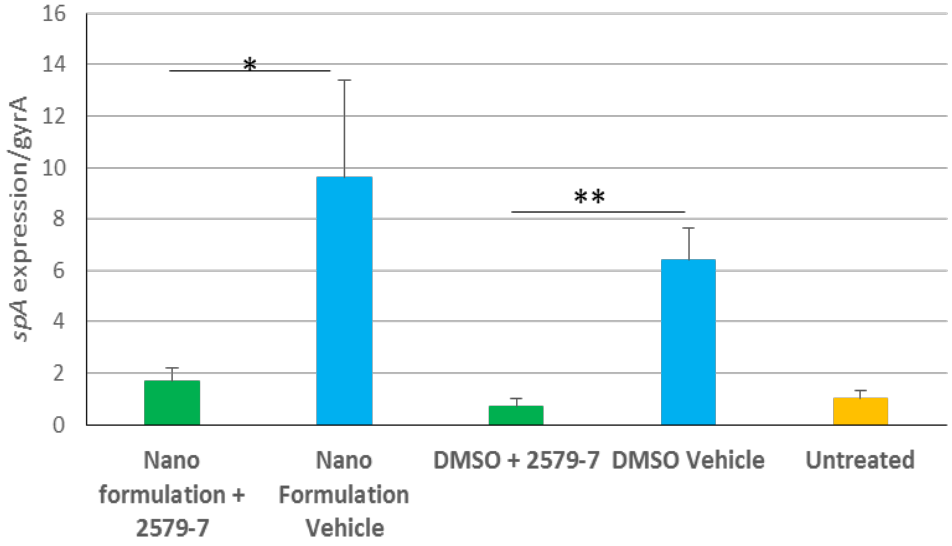
with peptide 2579-7 dissolved in vehicle compared to vehicle alone and also untreated control. Treatment with the peptide 2579-7 delivered in nano-formulation resulted in a trend of *hla* suppression compared to nano-formulation vehicle (Figure 5). This data suggest that peptide 2579-7 has the ability to alter expression of alpha hemolysis and reduce cytotoxic effect of MRSA.

**Figure 5. Hla expression**




We also assessed gene expression analyses for the *spA*, known for its role in modulation of the host immune system. SpA inhibits the normal mechanisms of adaptive immunity by binding host antibody molecules in the Fc region, which is the opposite direction foreign particles are usually bound. This makes Staphylococcus a "wolf in sheep's clothing" by tricking the mammalian immune system into recognizing the bacterial cell as one of its own cells. Another pathogenic property of SpA is its ability to bind to and activate the TNFα receptor, which leads to prolonged inflammation. Gene expression data have shown that treatment with 2579-7 resulted in suppression of *spA* compared to levels detected in wound treated with either of vehicles, nano-formulation or DMSO. No significant suppression of spA was observed compared to untreated control, while both DMSO and nano-formulation resulted in increased expression of this virulence factor (Figure 6). In summary peptide 2579-7 has shown potential to suppress MRSA virulence through modulation of *hla* and *spA*. Increased concentration of 2579-7 may result in more efficient antimicrobial effect and further reduction of pathogen's virulence.

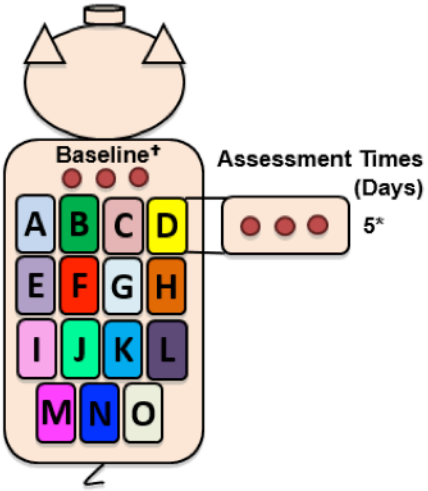
**Figure 6. SpA expression**



During running the above studies, Torrey Pines completed additional *in vitro* analysis of new peptides (See Partnering PI report) and found that 4 that had similar or higher antimicrobial activity as compared to previously examined peptides. Since Dr. Davore (Torrey Pines subcontractor) had ran out of funds to complete the nanoformulations for the remaining *in vivo* work, we decided to perform a pilot study using the 4 most active peptides (at various concentrations) using DMSO as the vehicle against biofilm bacteria (treated after 24 hours of inoculation). Task 8 of this proposal is to examine the ability of the peptides against biofilm associated bacteria. The rational for performing these studies was if a soldier in the field sustained a wound and it is not treated within 24 hours. Our group and others have shown that once bacteria are in the biofilm state, they are much more difficult to treat. We used our porcine full thickness wound model against a military isolate of *Pseudmonas aurginosa* (PA-09-10: Lee Canio, MD, US Army Institute of Surgical Research, Fort Sam Houston, TX).

Briefly, forty eight (48) full thickness wounds were made on the paravertebral and thoracic area with an 8mm punch biopsy. Wounds were inoculcated with $10^6$ log CFU/ml and covered for 24 hours to allow for biofilm formation. Three (3) wounds were assigned to each treatment group and three wounds were used as baseline (recovered after 24 hour biofilm formation and prior to treatment). The experimental design and timeline are below (Figure 7).

**Figure 7. Experimental Design**




† Baseline wounds were recovered 24 hrs after biofilm formation / prior to treatment

* Wound were recovered 5 days after treatment application

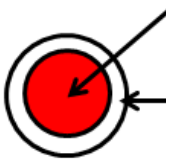
**Treatment Groups**

A. DMSO + 2612-8.1 (5 mg/ml)
B. DMSO + 2612-8.1 (10 mg/ml)
C. DMSO + 2612-8.1 (20 mg/ml)
D. DMSO + 2612-8.2 (5 mg/ml)
E. DMSO + 2612-8.2 (10 mg/ml)
F. DMSO + 2612-8.2 (20 mg/ml)
G. DMSO + 2612-8.13 (5 mg/ml)
H. DMSO + 2612-8.13 (10 mg/ml)
I. DMSO + 2612-8.13 (20 mg/ml)
J. DMSO + 2612-8.16 (5 mg/ml)
K. DMSO + 2612-8.16 (10 mg/ml)
L. DMSO + 2612-8.16 (20 mg/ml)
M. DMSO Vehicle Control
N. Silver Sulfadiazine Positive Control
O. Untreated control

**Assessment Methods**

Three (3) wounds were biopsied 10mm punch biopsy per group on day 5 post treatment application (see figure 4). The punch biopsy (10mm) was taken around entire wound and removed down to subcutaneous tissue in order to evaluate bacteria on wound edges, bed and surface - see figure 4. Biopsies were weighed then immediately placed in 1 ml of All Purpose Neutralizing Solution. The sample was combined with an additional 4 ml of Neutralizing Solution and homogenized in a sterile homogenization tube (Tenbroeck Tissue Grinder). Serial dilutions were made and scrub solutions were quantified using the Spiral Plater System as described above. Pseudomonas Agar-base with CN supplementation was used to isolate PA from the wounds.

**Figure 8**

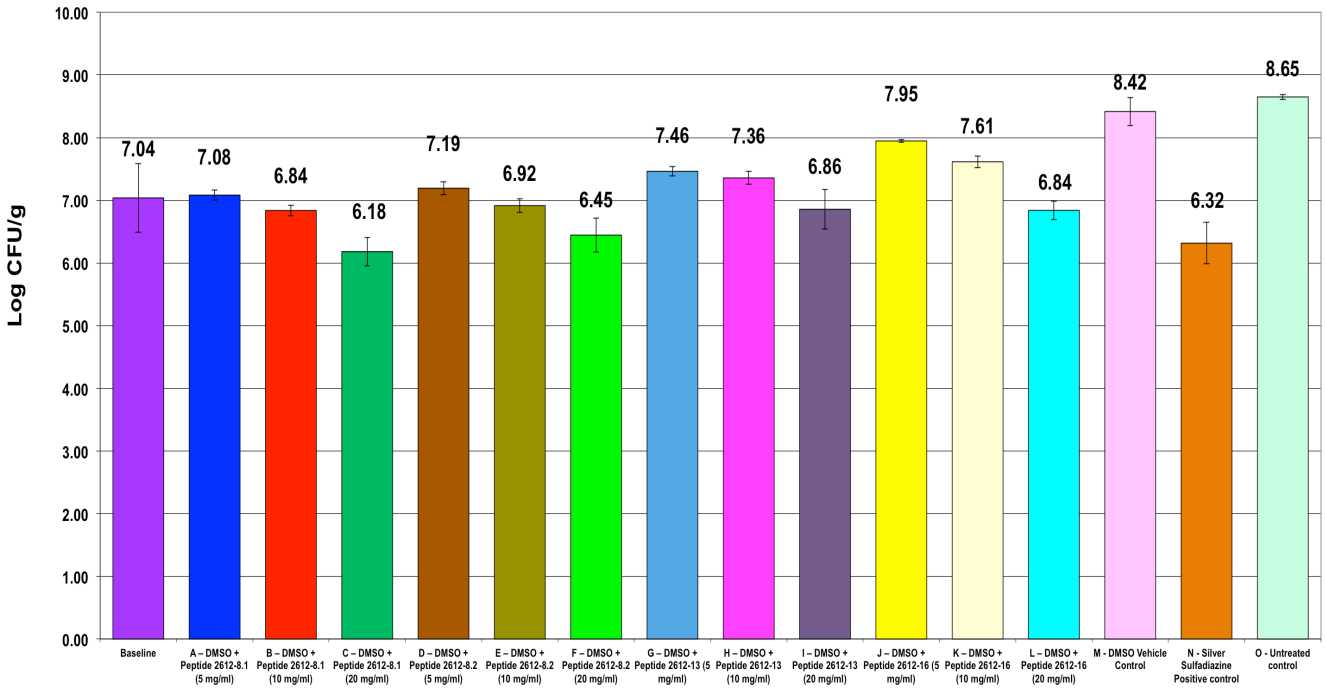


8mm punch wound

10mm punch was taken around the entire wound down to subcutaneous tissue to evaluate bacteria on wound edges, bed and surface.

All peptides showed a dose response effect against PA (Figure 5). The lowest PA counts were found in wounds that were treated with peptide 2612-8.1 at 20 mg/ml (6.18 log CFU/g). This was followed by positive control (silver sulfadiazine) which had 6.32 log CFU/g. The next most effective peptide was peptide 2612-8.2 (20 mg/ml) which had PA counts of 6.45 log CFU/g. The highest concentration (20 mg/ml) of peptides 2612-16 and 2612-13 had similar PA counts with 6.84 log CFU/g and 6.86 log CFU/g, respectively. The untreated control and DMSO treated wounds had the highest PA coutns with 8.65 log CFU/g and 8.42 log CFU/g, respectively. The most effective peptide (2612-8.1) was 2.47 log CFU/g lower than untreated control and 2.24 log CFU/g lower than DMSO vehicle control.

**Figure 9: *Pseudomonas aeruginosa* Recovery**



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

- *Nothing to Report*

a) **How were the results disseminated to communities of interest?**

Presented at the 28th Annual Meeting of the Wound Healing Society with the Symposium on Advanced Wound Care (SAWC). Georgia World Congress Center in Atlanta, Georgia, April 13-17, 2016.
We are currently working on the manuscript for the microbiology portion of this study which will be submitted for publication.

**WHS SESSION K: Concurrent Session:**
**Biofilms & Microbiomes**
**Friday April 15, 2016 2:15 p.m. − 3:15 p.m.**
**K3.01 - NOVEL CYCLIC LIPOPEPTIDES WITH ENHANCED ANTIBACTERIAL AND ANTIBIOFILM ACTIVITY AGAINST CHRONIC WOUND PATHOGENS**
I. Pastar[1], B. Williams[2], J. Gil[1], J. Valdes[1], A. Higa[1], M. Solis[1], P. Cudic[2], S. C. Davis[1]
*[1]University Of Miami Miller School Of Medicine, Dermatology And Cutaneous Surgery, Miami, FL, USA [2]Torrey Pines Institute For Molecular Studies, Port St. Lucie, FL, USA*

**What do you plan to do during the next reporting period to accomplish the goals?**

We currently have in house 4 animals that will be started in the next few weeks to finish our work on studying the most effective peptides against biofilm associated bacteria using our full thickness wound model. Both MRSA and PA animals will be completed.

In early 2019, after the above studies are completed, we will assess the wound healing effects of these peptides using our full thickness wound healing model. It should be noted that no signs of wound erythema (redness) that is indicative of an inflammatory response, has been seen with any of the peptides studied.

**Impact**

a) **What was the impact on the development of the principal discipline(s) of the project?**
Nothing to Report

b) **What was the impact on other disciplines?**
Nothing to Report

c) **What was the impact on technology transfer?**
Nothing to Report

d) **What was the impact on society beyond science and technology?**
Nothing to Report

**Changes/Problems**

a) **Changes in approach and reasons for change**

As mentioned in the last quarterly report, Dr. Devore (Rutgers) who is subcontracted by Torrey Pines told us that he no longer has the funds to complete the remaining animal work. We therefore plan to evaluate the remaining animals using DMSO as a vehicle and using higher concentrations of peptides to obtain better efficacy.

b) **Actual or anticipated problems or delays and actions or plans to resolve them**

The major delay in this grant was that Dr. Cudic resigned from Torrey Pines Institute (TPI) and it took several months to transfer the grant to Dr. Richard Houghten (found and CEO of TPI). Dr. Houghten has hired someone to perform the necessary work to complete the grant.

**Changes that had a significant impact on expenditures**
Change of partnering PI at Torrey Pines has produced some delays in the in vivo evaluations. All plans are now in place, including a sub-award to Dr. Cudic at Florida Atlantic University, to ensure the successful outcome of the proposed studies.

d) **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Nothing to Report

e) **Significant changes in use or care of human subjects**

Nothing to Report

f) **Significant changes in use or care of vertebrate animals**

Nothing to Report

**2. Significant changes in use of biohazards and/or select agents**

Nothing to Report

**3. Products**

Presented at the 28th Annual Meeting of the Wound Healing Society with the Symposium on Advanced Wound Care (SAWC). Georgia World Congress Center in Atlanta, Georgia, April 13-17, 2016.

**WHS SESSION K: Concurrent Session:**
**Biofilms & Microbiomes**
**Friday April 15, 2016 2:15 p.m. − 3:15 p.m.**
**K3.01 - NOVEL CYCLIC LIPOPEPTIDES WITH ENHANCED ANTIBACTERIAL AND ANTIBIOFILM ACTIVITY AGAINST CHRONIC WOUND PATHOGENS**

I. Pastar[1], B. Williams[2], J. Gil[1], J. Valdes[1], A. Higa[1], M. Solis[1], P. Cudic[2], S. C. Davis[1]
*[1]University Of Miami Miller School Of Medicine, Dermatology And Cutaneous Surgery, Miami, FL, USA [2]Torrey Pines Institute For Molecular Studies, Port St. Lucie, FL, USA*

**4. Participants & Other Collaborating Organizations**

**a) What individuals have worked on the project?**

| | |
|---|---|
| Name: | Stephen Davis |
| Project Role: | PI |
| Researcher Identifier: | |
| Nearest person month worked: | 12 months |
| Contribution to Project: | Professor Davis is overseeing all of the in vivo studies and works Torrey Pines and coordinates the efforts of the collaborators. |
| Funding Support: | Not applicable |

| | |
|---|---|
| Name: | Joel Gil |
| Project Role: | Laboratory Manager |
| Researcher Identifier: | |
| Nearest person month worked: | 12 months |
| Contribution to Project: | Joel Gil performs all surgery and treatments to the animals. He performs the assessments |
| Funding Support: | Not applicable |

| | |
|---|---|
| Name: | Jose Valdes |
| Project Role: | Research Assistant |
| Researcher Identifier: | |
| Nearest person month worked: | 12 months |
| Contribution to Project: | Jose assists with all procedures on the animal. Performs animal monitoring. |
| Funding Support: | Not applicable |

| | |
|---|---|
| Name: | Michael Solis |
| Project Role: | Research Associate |
| Researcher Identifier: | |
| Nearest person month worked: | 12 months |
| Contribution to Project: | Michael makes all microbiology media, assists with surgery and animal records |
| Funding Support: | Not applicable |

| | |
|---|---|
| Name: | Alex Higa |
| Project Role: | Research Associate |
| Researcher Identifier: | |
| Nearest person month worked: | 12 |
| Contribution to Project: | Alex assists will microbiology media, animal surgery and records |
| Funding Support: | Not applicable |

| | |
|---|---|
| Name: | Irena Pastar |
| Project Role: | Research Associate Professor |
| Researcher Identifier: | |
| Nearest person month worked: | 12 |
| Contribution to Project: | Perform the molecular virulence anlaysis |
| Funding Support: | Not applicable |

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

The only changes involved a PI change and hiring of a new postdoc following the resignations of Dr. Cudic.

**What other organizations were involved as partners?**

**Organization Name:** Torrey Pines Institute
**Location of Organization:** Port St. Lucie, Florida
**Partner's contribution to the project:** Collaboration

**Appendices**

none

# Novel cyclic lipopeptides for treating complicated wound infections

Log Number: W81XWH-15-1-0657; Task Title: Annual Technical Progress Report (09/21/2015-10/20/2018)
Award Number: DM140052

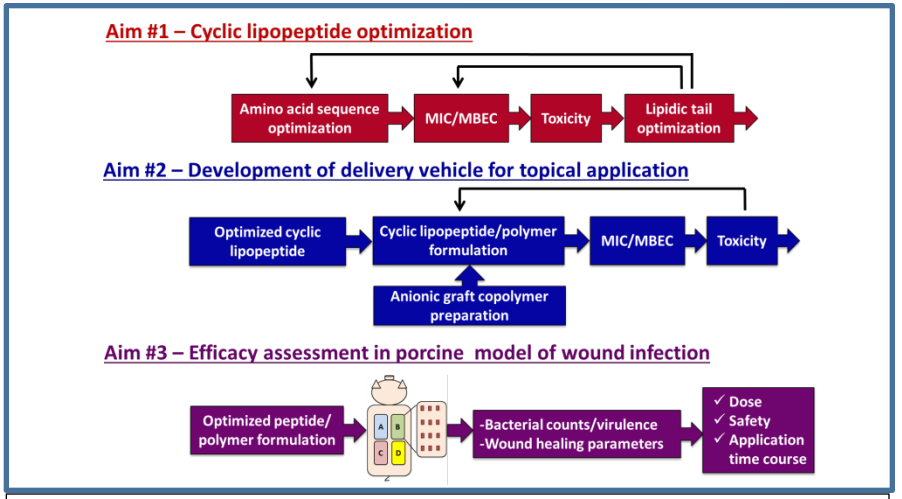
**PI:** Stephen Davis **Org:** University of Miami **Award Amount:** $654,583

## Study Aims

- **Aim 1.** Optimize lead cyclic lipopeptide structure using systematic and rational synthetic chemistry approach.
- **Aim 2.** Develop and optimize cyclic lipopeptide delivery system for topical application.
- **Aim 3.** Assess the therapeutic potentials of selected cyclic lipopeptides using porcine wound infection model.

## Approach

Our overall goal is to develop novel cyclic lipopeptide antibiotics for topical treatment of combat-related or trauma-induced wound infections. To achieve this goal we plan to: a) further improve the antimicrobial/antibiofilm activities of lead cyclic lipodepsipeptide and minimize nonselective toxicity by modifying its amino acid sequence and lipidic tail using a systematic and rational synthetic approach; b) develop an optimal delivery system for topical application based on hydrogels or polymers; and c) assess the therapeutic potentials of optimized peptide/polymer formulations in a porcine wound infection model.




Accomplishment: Identified key amino acids in the sequence of cyclic lipopeptide scaffold for antibacterial and antibiofilm activities.

## Timeline and Cost

| Activities CY | 15 | 16 | 17 | 18 |
|---|---|---|---|---|
| Optimize lead cyclic lipopeptide (Torrey Pines). | | | | |
| Synthesize large quantities of optimized cyclic lipopeptides for studies outlined in Aims 2 and 3. | | | | |
| Develop and optimize delivery system for cyclic lipopeptide topical application. | | | | |
| Assess therapeutic potentials of peptide/polymer formulations in a porcine wound infection model. | | | | |
| **Estimated Budget ($654K)** | | **$211** | **$219** | **$224** |

**Updated: 10/21/2018**

**Goals/Milestones**

**CY15 Goals** – Cyclic lipopeptide sequence optimization.
☑Synthesized cyclic lipopeptide analogs with different amino acid sequences.
**CY16 Goal** – Lipidic tail optimization, large scale synthesis and assessment of peptides *in vitro* activities.
☑Synthesized large quantities of peptides and assess their *in vitro* activities.
**CY17 Goal** – Development and optimization of delivery system.
☑Synthesized graft copolymers and prepare variety of cyclic lipopeptide/ polymer complexes.
**CY18 Goal** – Efficacy assessment in porcine model of wound infection.
☑Assessed therapeutic potentials of optimized peptide/polymer formulations using partial thickness porcine wound infection model (completed) and will be finished with full thickness model in the next few months (we have NCE due delayed due to change in partnering PI and nanoformulation).

**Comments**

Partnering PI reassignment has been completed. All plans and resources in place to successfully accomplish research goals.

**Budget Expenditure to Date**

Projected Expenditure: $654,583 / Actual Expenditure: $496,579