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14. ABSTRACT
This research aims to address the needs of far forward Wounded Warriors in the era of prolonged field care (PFC) by developing a field-polymerizable hydrogel that can be readily applied in austere environments that enables sustained release of antibiotics, analgesics, and hemostatic agents over a five-day period. Following the optimization of our hydrogel system, we have analyzed therapeutic release in vitro and verified the presence of our therapeutics in the system using high-performance liquid chromatography and mass spectrometry. We have transitioned to in vivo studies using mouse models.

15. SUBJECT TERMS
None listed.

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1. INTRODUCTION:

This research aims to address the needs of far forward Wounded Warriors in the era of prolonged field care (PFC) by developing a field-polymerizable hydrogel that can be readily applied in austere environments that enables sustained release of antibiotics, analgesics, and hemostatic agents over a five-day period. This biochemical tool will be readily deployed in remote environments to improve antimicrobial, hemostatic, and pain outcomes at the point of injury in the setting of delayed evacuation.

2. KEYWORDS:

- Polymer Hydrogel
- Wound Sealant
- Antimicrobial
- Prolonged Field Care
- Wound Decontamination
- Extremity Blast Wounds

3. ACCOMPLISHMENTS:

Specific Aim 1 – Extend antibiotic, analgesic and hemostatic agent release profile through optimizing crosslink density of the hydrogel wound dressing

Major Task 1 – Synthesis of PEG-diacrylate macromers and hydrogels

Subtask 1 – Synthesis of PEG-diacrylate macromers of varying molecular weights, fabrication, and characterization of hydrogels. **Proposed Timeline: Months 1-12**

Percentage completion: 100%

Subtask 2 – Fabrication of hydrogels entrapping therapeutics, and characterization of release kinetics. **Proposed Timeline: Months 9-15**

Percentage completion: 95%

Subtask 3 – Leak pressure testing of hydrogels to ensure wound sealing. **Proposed Timeline: Months 6-12**

Percentage completion: 5%

Subtask 4 – Characterization the chemical and physical stability of the components under various environmental conditions. **Proposed Timeline: Months 9-12**

Percentage completion: 80%

Major Task 2 – In vitro assessment of released therapeutics

Subtask 1 – Molecular characterization of released therapeutics. Released drugs will be characterized by mass spectrometry, NMR, and/or other techniques to verify chemical structure. **Proposed Timeline: Months 12-18**

Percentage completion: 70%

Subtask 2- Efficacy of released antibiotics: we will use antibiotics released from hydrogels against E. coli (tobramycin) or S. Aureus (vancomycin) in a microdilution broth assay using standard protocols. **Proposed Timeline: Months 12-18**

Percentage completion: 0%

Subtask 3- Characterization of mechanical and thermal stability of components and hydrogels. **Proposed Timeline: Months 18-24**

Percentage completion: 75%

Specific Aim 2 – In vivo evaluation of PEG-diacrylate hydrogels in a) small and b) large animal models

Major Task 3 – In vivo assessment of wound dressing in mice

Subtask 1 – In vivo efficacy of wound sealant/dressing to prevent implant infection: Using the mouse model of infection after open wound, we will use non-invasive bioluminescence optical imaging (BLI) to longitudinally assess the efficacy of the wound dressing in eradicating bacteria from a contaminated tissue bed. **Proposed Timeline: Months 18-24**

Percentage completion: 70%

Subtask 2 – Immune response, toxicity: Assessing endpoints of neutrophil and macrophage recruitment (fluorescence imaging), renal function, and histology of kidney, we will assess local and systemic toxicity of the wound dressing delivery of TA, bupivacaine, and antibiotics (tobramycin or vancomycin). **Proposed Timeline: Months 24**

Percentage completion: 0%

Hydrogel Synthesis:

PEG-diacrylate (PEGDA) macromers of varying molecular weights were synthesized from PEG (M_n 575, 700, 2000, 3500, 4600) and used to fabricate hydrogels with different properties. Hydrogels were evaluated through observation of gelation and by characterizing mechanical properties with mass swelling ratios and elastic moduli. PEGDA 3350 was found to rapidly polymerize into hydrogels with ideal mechanical properties and extended therapeutic release profiles; as such, it was selected as the optimal macromer for the hydrogel system. We are currently working to further improve the polymerization time of our hydrogels by optimizing the methods used for PEGDA synthesis.

In Vitro Therapeutic Release:

We successfully transitioned from bupivacaine to lidocaine and were able to detect both in a similar manner. Therapeutic release of lidocaine/bupivacaine and vancomycin could be detected using a UV-Vis spectrophotometer. We developed a system using Fe(III)Cl to quantify tobramycin and tranexamic acid concentrations, in addition to a Cu(II)-based assay that can detect tobramycin using a UV-Vis spectrophotometer at high concentrations, which may be helpful in quantifying release from hydrogels delivering a combination of therapeutics. We have characterized vancomycin, lidocaine, tranexamic acid, and tobramycin release kinetics from hydrogels using PEGDA 3350. Vancomycin and lidocaine diffused out of the hydrogel over the span of 2-3.75 days, tobramycin released within 1 day, and tranexamic acid, the hemostatic agent, experienced burst release from the hydrogel within 2 hours. We are beginning to develop HPLC protocols to quantify drug release, using vancomycin for preliminary studies. We will then transition to quantifying the release of multiple drugs from a single hydrogel.

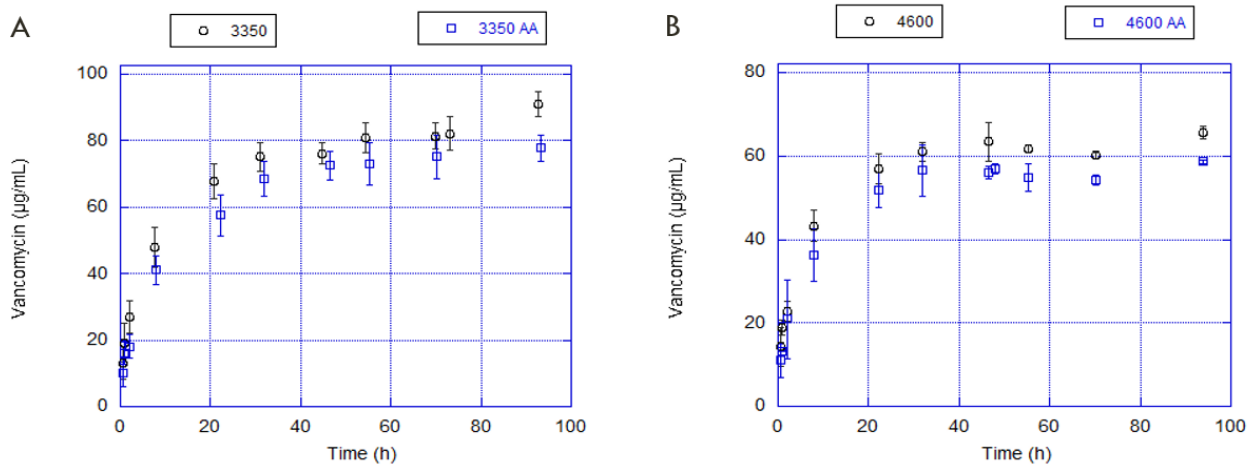


Figure 1. Kinetic release profiles of vancomycin from hydrogels synethsized using (A) PEGDA 3350 with (*blue*) and without (*black*) acrylic acid, and (B) PEGDA 4600 with (*blue*) and without (*black*) acrylic acid

In our attempts to further slow the release of therapeutics from the hydrogels, we incorporated charged acrylic acid to retard the diffusion of cationic drugs, primarily vancomycin and lidocaine. While hydrogels synthesized using lower molecular weight PEG-diacrylates (575 to 2000) displayed sustained release of vancomycin and lidocaine, higher molecular weight hydrogels showed only a slight improvement in drug release kinetics (Fig. 1).

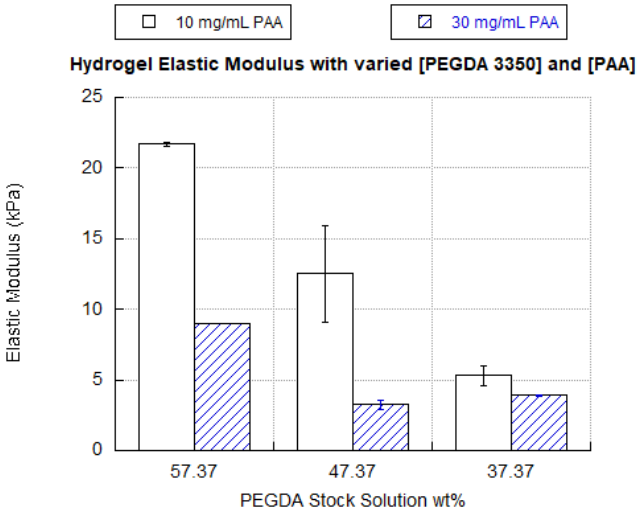


Figure 2. The decrease in hydrogel elastic modulus with the addition of sodium polyacrylate (PAA) can be counteracted by increasing the concentration of PEGDA added to system.

We also explored incorporating sodium polyacrylate (PAA) into the hydrogels to slow the release of therapeutic agents. By adding PAA to the system in its solid, powdered form—as would be the case in the final platform—hydrogels incorporating these chains were successfully synthesized. The ratio of PEG to PAA can easily be tailored to produce hydrogels of different stiffness (elastic modulus), therefore different therapeutic release rates (Fig. 2). Preliminary testing shows that adding 2 mg/mL of sodium polyacrylate to the hydrogel system may enable sustained vancomycin release over a 7-day period (Fig. 3).

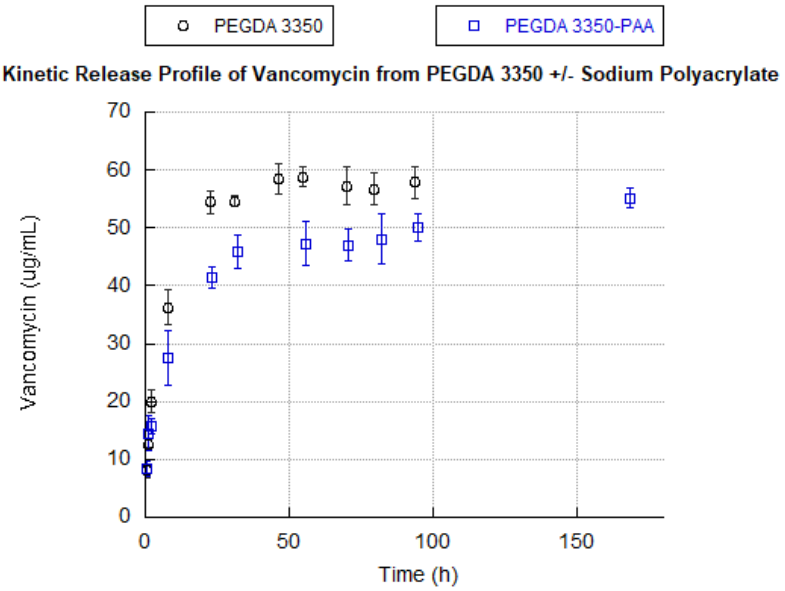


Figure 3. Adding 2 mg/mL PAA to the hydrogel system (*blue*) enables sustained release of vancomycin over 7 days, while the hydrogels without PAA (*black*) released vancomycin over approximately 2 days.

Elution experiments:

We have successfully optimized a method for the detection of the multiple drugs from our hydrogel system using HPLC. This method was adopted for the first *in vivo* preliminary studies. We used this to determine the amount of vancomycin remaining in the hydrogels after a 5-day implantation using mouse models. Analysis showed an average of 97.4% release of vancomycin *in vivo* (Fig. 4). We are currently transitioning into multi-drug studies where the current HPLC protocol will be used to quantify the release of multiple drugs from *in vitro* and *in vivo* models respectively.

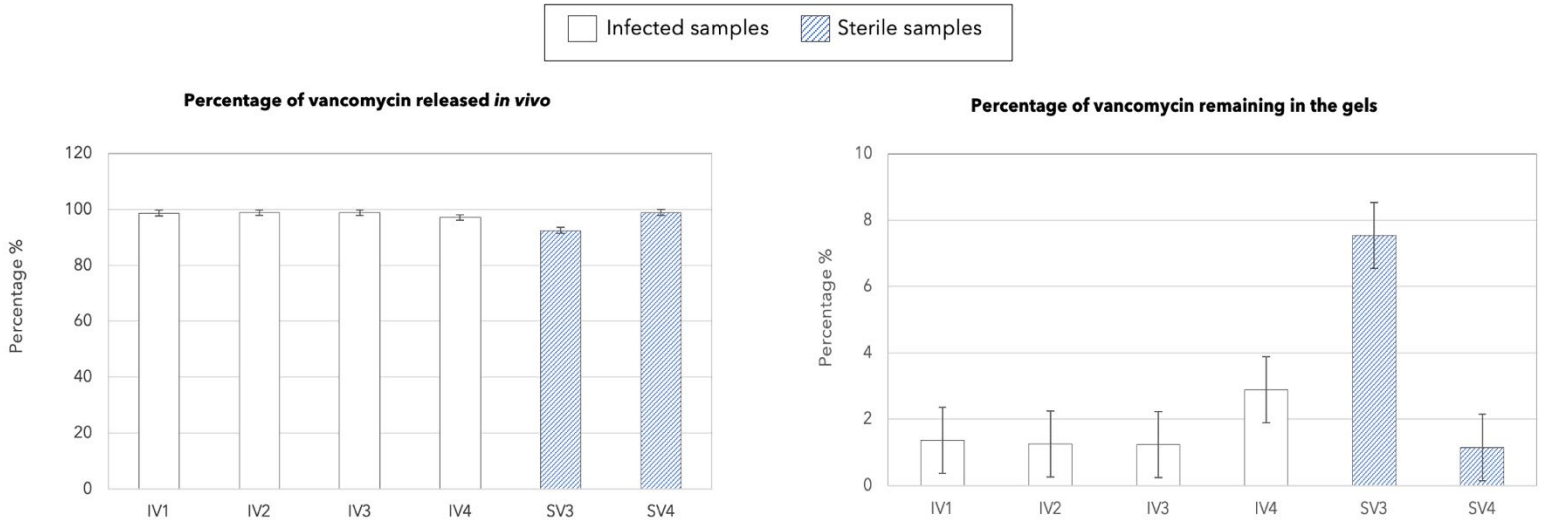


Figure 4. Amount of vancomycin released *in vivo* after a 5-day implantation. (A) Percentage released *in vivo*. (B) Percentage of drug remaining in the hydrogel system. IV – Gels implanted in the presence of *S. aureus.* SV – Gels implanted without *S. aureus.*

Multi-drug studies & assessment of released therapeutics:

To ensure that multi-drug loading does not affect the release of individual therapeutics, we studied the release of vancomycin from our hydrogel network containing all therapeutics using HPLC. Vancomycin and lidocaine only were detected and separated via our HPLC method; therefore, tranexamic acid and tobramycin will require colorimetric assays to be detected, as previously described (Fig. 5). Total vancomycin release was observed after 48 hours and mass spectrometry was used to ensure the efficacy of the separation method (Fig. 6). These data confirmed that multi-drug loading does not affect the release kinetics of individual therapeutics.

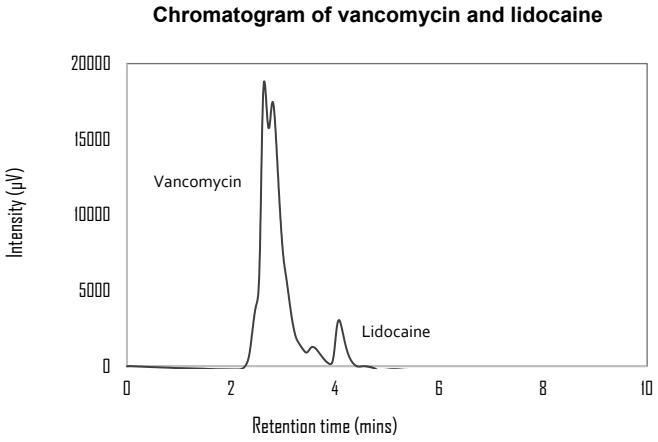


Figure 5. Detection and separation of vancomycin and lidocaine using HPLC. Retention times: Vancomycin; 2.6 – 3 mins. Lidocaine; 3.7 – 4.2 mins.

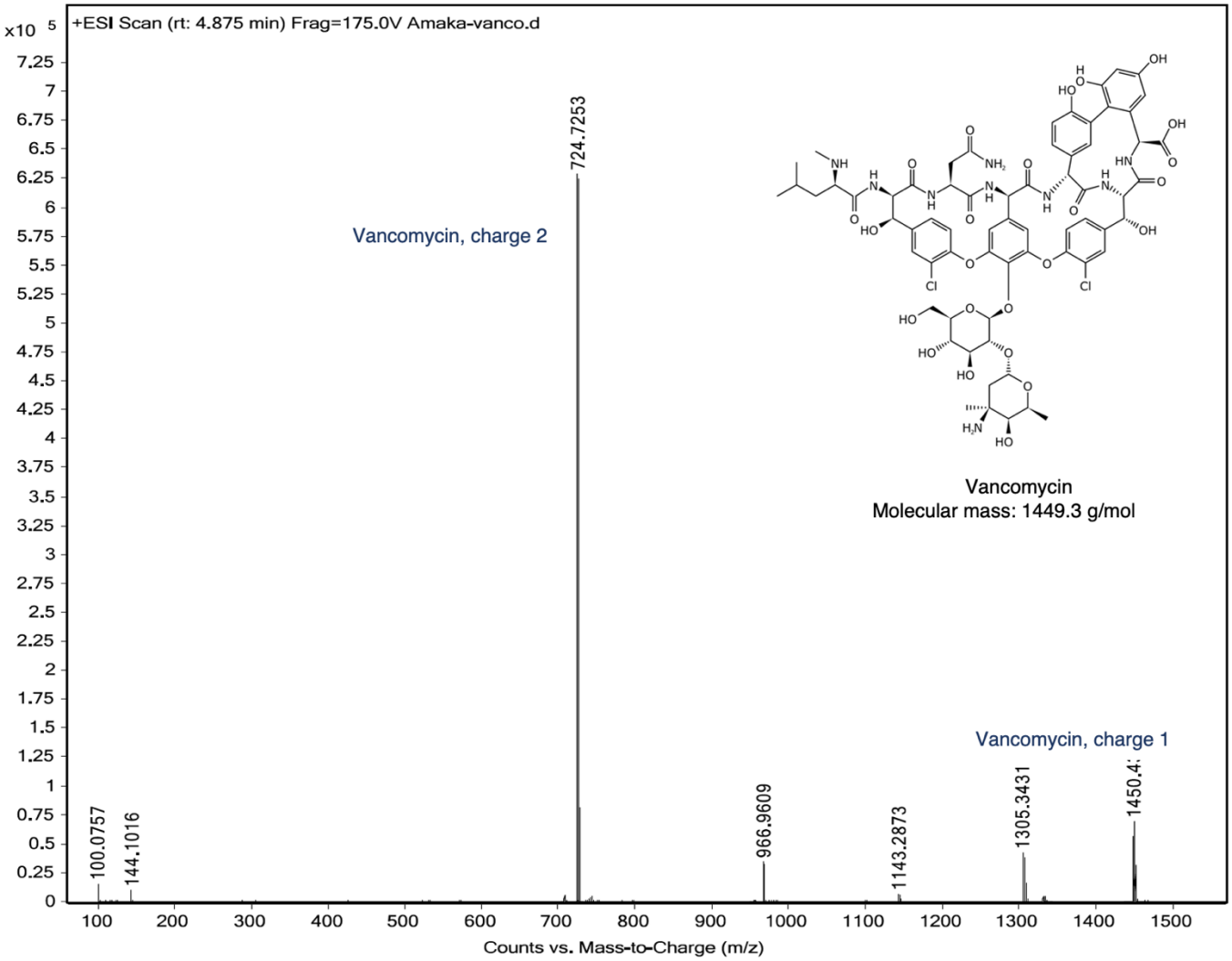


Figure 6: Mass spectrum (electron ionization) of vancomycin, isolated from a hydrogel system containing all therapeutics. *Sample collected post-HPLC separation*.

Stability Studies:

We stored the hydrogel components at an elevated temperature (110-115 °F) to characterize the physical and chemical stability of each. Samples were placed in an Eppendorf tube with no additional interventions (i.e. Parafilm around the cap), and placed in a pre-heated oven. Samples were characterized at 1 day, 1 week, 1 month, and 3 months using ^{1}H NMR and solubility and gelation studies. No degradation of PEGDA was observed while comparing the ^{1}H NMRs at each time point. Additionally, all samples were readily solubilized in water, indicating that they had not polymerized while stored at an elevated temperature. While the PEGDA 3350 and APS demonstrate a high degree of stability, the system's catalyst, TEMED, had completely evaporated within 1 week. This problem may be addressed by i) exploring alternative amine catalysts, ii) eliminating TEMED by increasing the amount of amine-containing drugs in gels, or iii) modifying final packaging with an improved seal.

Leak Pressure Testing:

We are currently developing the leak pressure testing set-up. Our system will consist of PTFE tubing connected to a syringe pump. The tubing, with a puncture through one side to emulate a blast wound, will be housed within a halved, rigid cylindrical casing filled with collagen gel on the side opposite the puncture. Our hydrogel system will be injected into the puncture site, allowed to solidify, and wrapped with a gauze bandage. The flow rate of the syringe pump will be gradually increased until a leak is observed.

In-Vivo Dosing:

We performed an in vivo experiment using our murine open fracture model. First, a titanium k-wire was place in a retrograde fashion into the right distal femur of the mouse in order to model a foreign body from a blast wound. Then, a lateral thigh incision was made, the femur was exposed and a lateral cortical defect over the distal femur was made using a rongeur to model a fracture. For each experimental group, an inoculum of 1x 10^{5} and 1 x 10^{7} of a bioluminescent strain of *S. aureus* (Xen36) was placed directly into the fracture site. Following inoculation, the hydrogel was placed into the wound. The experimental groups included: sterile control (2 mice), hydrogel only (infected control) (2 mice), hydrogel + 4 mg of vancomycin (8 mice). Outcomes included in vivo bioluminescence as a marker of bacterial burden on post-operative days (POD) 1-4, as well as soft tissue and metal foreign body colony forming units (CFUs) following sacrifice on POD 4.

The mean bioluminescence of the groups which received hydrogel + vancomycin was as low as the sterile control group at all time points. Conversely, the mean bioluminescence of the groups that received hydrogel only was significantly elevated at all time points (Fig. 5).

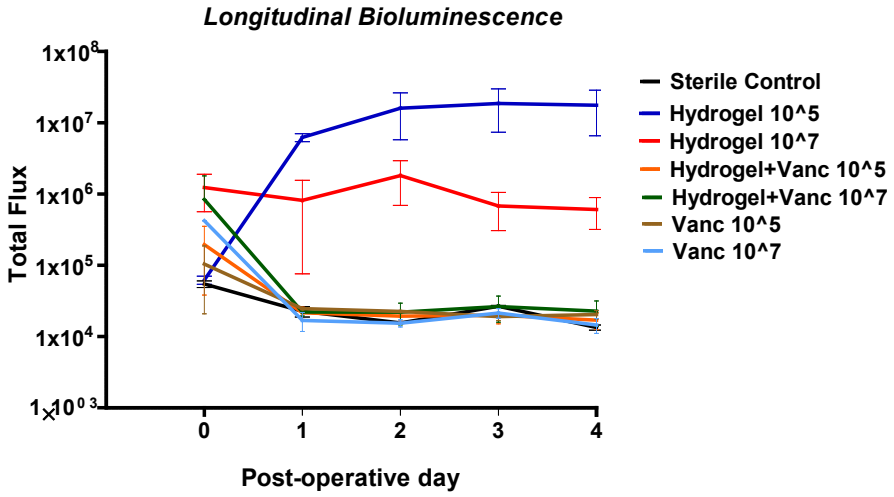


Figure 7. In vivo bioluminescent signal over time representing bacterial burden.

For POD 4 soft tissue CFUs, the hydrogel + vanc group had lower mean CFUs compared to the infected control group (Fig. 6-A). Similarly, the POD 4 metal foreign body CFUs were lower for the hydrogel + vancomycin group as compared to the hydrogel only group (Fig. 6-B).

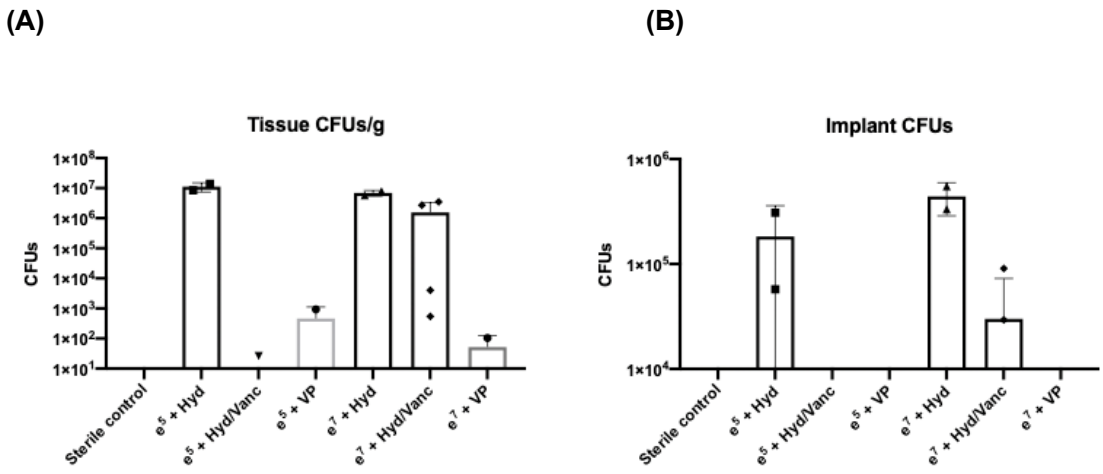


Figure 8. The average bacterial colony forming units (CFU) from the surrounding tissues (A) and implants (B).

In-Vivo Efficacy:

After the aforementioned dosing experiment, we proceeded with an inoculum of 1e5 CFUs S. *aureus* (Xen36). In addition, to provide limb stability to experimental mice, our titanium implant was lengthened by 4mm. In this experiment, we test the efficacy of hydrogel + 4mg vancomycin against the application of 4mg intrawound vancomycin powder. The aforementioned surgical procedures were performed. The experimental groups included: sterile control (2 mice), hydrogel only (infected control) (2 mice), hydrogel + 4 mg of vancomycin (8 mice) and 4mg intrawound vancomycin (8 mice). Outcomes included *in vivo* bioluminescence as a marker of bacterial burden on post-operative days (POD) 1-5, as well as soft tissue and implant colony forming units (CFUs) following sacrifice on POD 5 (Fig. 7).

There was not a significant difference in the mean bioluminescence between hydrogel + 4mg vancomycin and 4mg vancomycin powder. In addition, the bioluminescent curve for both of these treatment groups were as low as sterile control. The bioluminescent curve for the hydrogel only group was significantly elevated at all time points.

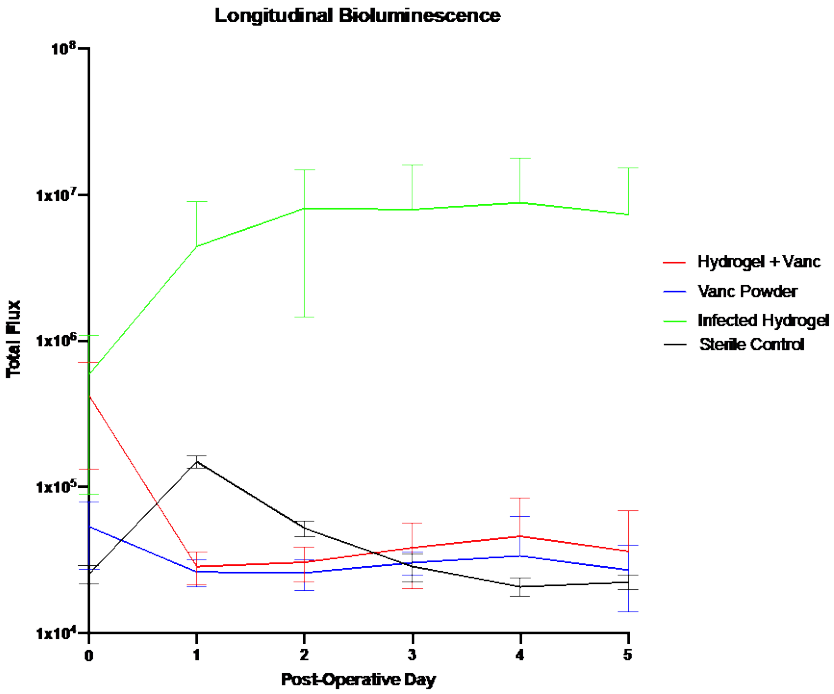
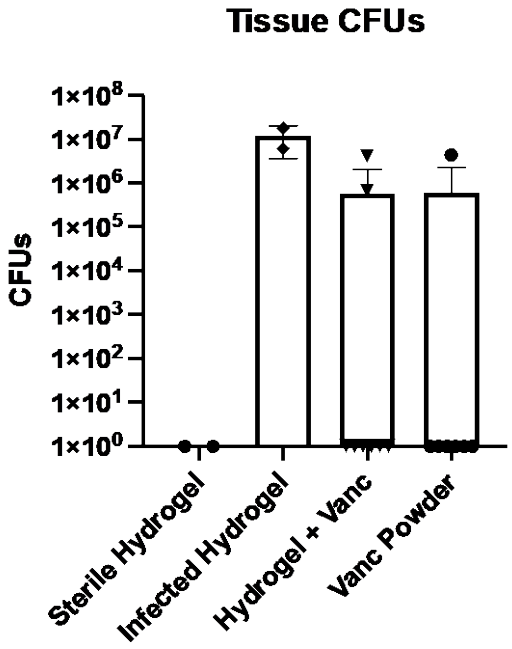


Figure 9. In vivo bioluminescent signal over time representing bacterial burden.

On POD5, tissue and implants were collected for CFUs. Both hydrogel + 4mg vancomycin and 4mg vancomycin powder had significantly lower tissue and implant CFUs compared to the infected hydrogel group. There was no significant difference in tissue or implant CFUs between the hydrogel + 4mg vancomycin and 4mg vancomycin powder groups. In addition, hydrogel + 4mg vancomycin prevented 6/8 tissue and implant infections. On the other hand, vancomycin powder prevented 5/8 tissue infections and 7/8 implant infections (Fig. 8).

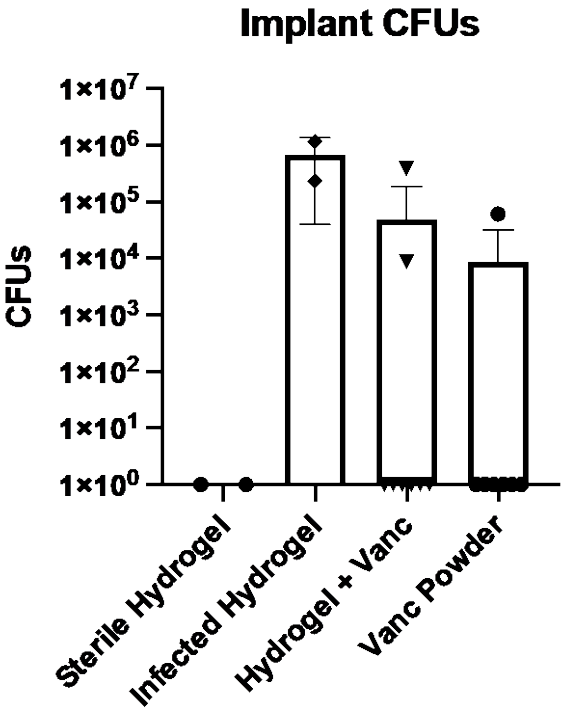
A).



B).

Sample	Tissue
Sterile Hydrogel	0/2
Infected Hydrogel	2/2
Hydrogel + Vanc	2/8
Vanc Powder	3/8

C).



D).

Sample	Implant
Sterile Hydrogel	0/2
Infected Hydrogel	2/2
Hydrogel + Vanc	2/8
Vanc Powder	1/8

Figure 10. Average tissue colony forming units (CFUs) (A) and binary tissue CFU growth (B). Average implant colony forming units (CFUs) (C) and binary implant CFU growth (D).

In-Vivo Vancomycin Elution Kinetics:

In this experiment, we want to determine the rate of vancomycin elution in our small animal model and see if the acidity of an infected wound environment affects the rate of drug elution. We performed an in vivo experiment using our murine open fracture model. First, a titanium k-wire was place in a retrograde fashion into the right distal femur of the mouse in order to model a foreign body from a blast wound. Then, a lateral thigh incision was made, the femur was exposed and a lateral cortical defect over the distal femur was made using a rongeur to model a fracture. For each experimental group, an inoculum of 1x 10^5 of a bioluminescent strain of *S. aureus* (Xen36) or sterile saline was placed directly into the fracture site. A biopsy punch was used to create identical cylinders of hydrogel containing 4mg of vancomycin and no drug. Following inoculation, the hydrogel was placed into the wound. The experimental groups included: sterile hydrogel (4 mice), sterile hydrogel + 4mg vancomycin (4 mice), infected hydrogel (6 mice) and infected hydrogel + 4 mg of vancomycin (6 mice). High performance liquid chromatography (HPLC) will be used to calculate the concentration of vancomycin remaining in each hydrogel on POD5.

Please see figure 4 above for HPLC data.

In-Vivo Development of a Polymicrobial Model of Infection:

In this series of 2 bacterial dosing experiments, we wanted to establish a polymicrobial model using MSSA (Xen36) and Pseudomonas (Xen41) to test the efficacy of multi-antibiotic loaded hydrogel. The aforementioned surgical procedures were performed. Experiment 1 groups included sterile control (1), E5(Xen36)E5(Xen41) (3), E5(Xen36)E4(Xen41) (3), and E5(Xen36)E3(Xen41) (3). Experiment 2 groups included sterile control (2), E3(Xen36)E2(Xen41) + Hydrogel (3), and E4(Xen36)E2(Xen41) + Hydrogel (3). Outcomes included *in-vivo* bioluminescence as a marker of bacterial burden on POD 1-5, as well as soft tissue and implant CFUs following sacrifice on POD 5. This experiment allowed us to refine selective media protocols to isolate either MSSA (TSB + 7.5%NaCL) or Pseudomonas (LB + 1ug/mL Vancomycin) for counting.

In experiment 1, all 3 mice that received E5 Pseudomonas expired on POD1. 2 of 3 mice receiving E4 Pseudomonas expired on POD1 and POD2, respectively. All 3 mice receiving E3 Pseudomonas survived the experiment (Fig. 9-A). In surviving mice, we obtained substantial CFUs from each respective organism indicating a robust infection (Fig. 9-B&C).

A).

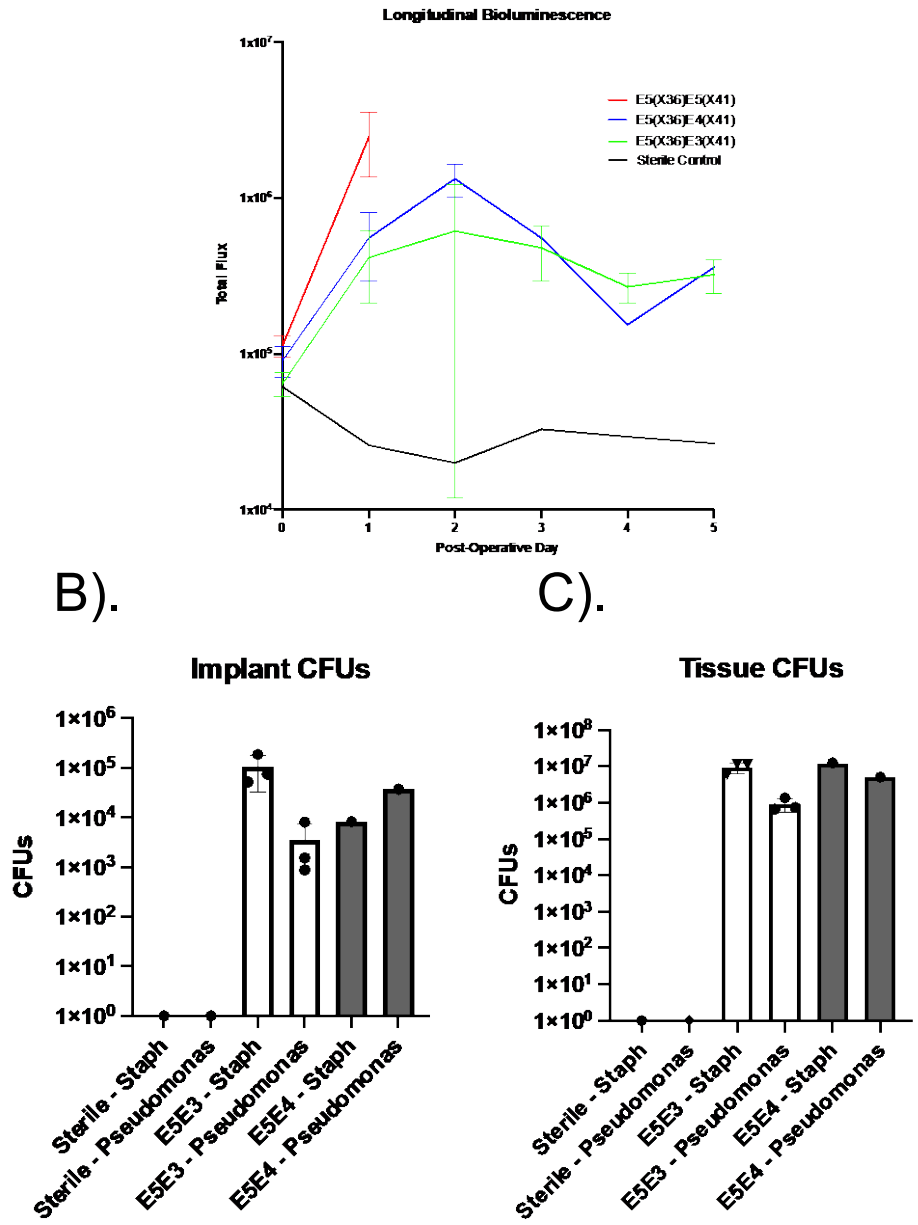


Figure 11. In vivo bioluminescent signal over time representing bacterial burden (A). Average implant colony forming units (CFUs) (B). Average tissue colony forming units (CFUs) (C).

E5 and E4 doses of Pseudomonas are lethal to mice in combination with E5 MSSA. Given the aggressive nature of Pseudomonas and its tendency to cause sepsis in this surgical model, we wondered if the addition of non-antibiotically loaded hydrogel will amplify infection in animals.

In experiment 2, 1 of 4 mice in the E3(Xen36)E2(Xen41) group expired on POD3. No mice expired in the E4(Xen36)E2(Xen41) group (Fig. 10-A). Despite lowering initial inoculum for both organisms, we were able to establish a robust infection (Fig. 10-B&C) with comparable CFUs to experiment 1 (Fig. 9-B&C).

A).

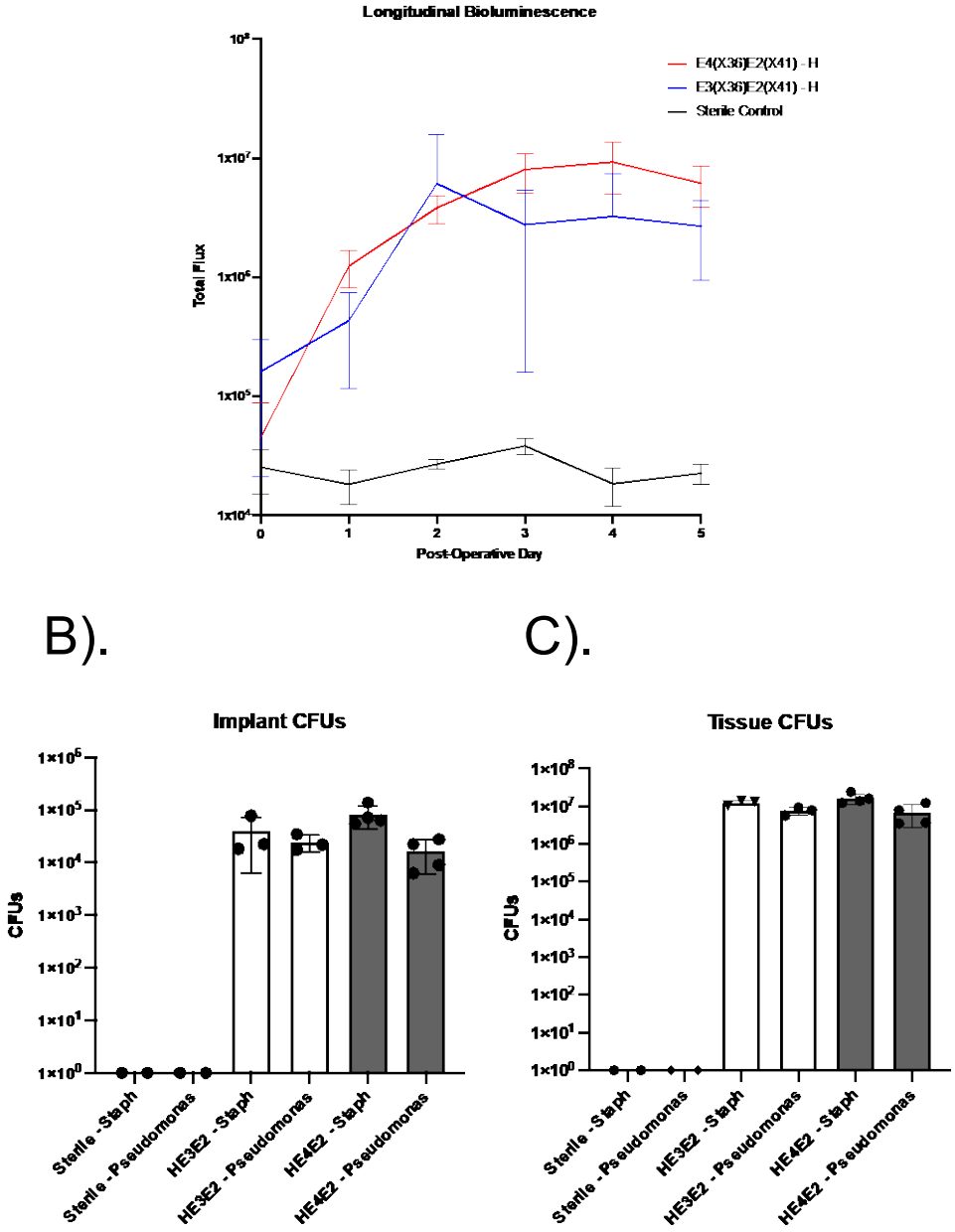


Figure 12. In vivo bioluminescent signal over time representing bacterial burden (A). Average implant colony forming units (CFUs) (B). Average tissue colony forming units (CFUs) (C).

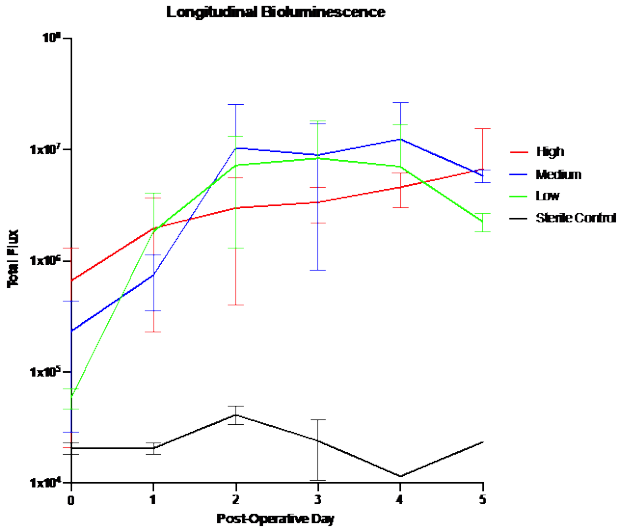
The chosen inoculum for future experiments will be E3(Xen36)E2(Xen41) considering that the addition of non-antibiotic impregnated hydrogel increases CFUs and burden of infection. E3 MSSA was chosen in anticipation of the addition of a third pathogen, *E. coli* (Xen14).

In Experiment 3, we added a third pathogen, *E. coli* (Xen14) to the experiment. The following inoculums/experimental groups were tested:

- Sterile Control – 2
- High - E3(X36)+E2(X41)+E3(X14) – 4
- Medium - E3(X36)+E2(X41)+E2(X14) – 4
- Low - E2(X36)+E2(X41)+E2(X14) – 4

Interestingly, in the low inoculum group one mouse expired on POD3 and POD5. In addition, one mouse in the medium inoculum group expired on POD5. No mice expired in the high inoculum group.

A).



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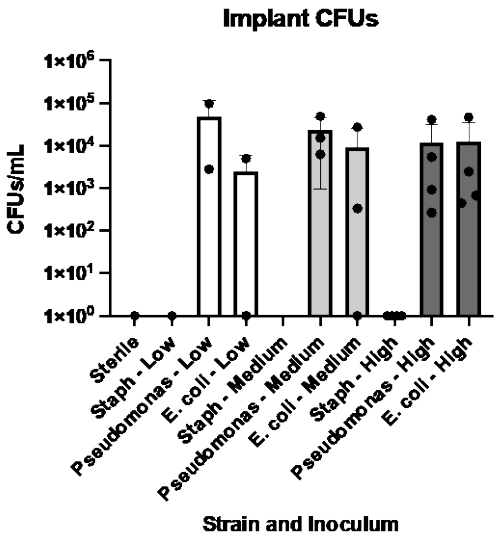

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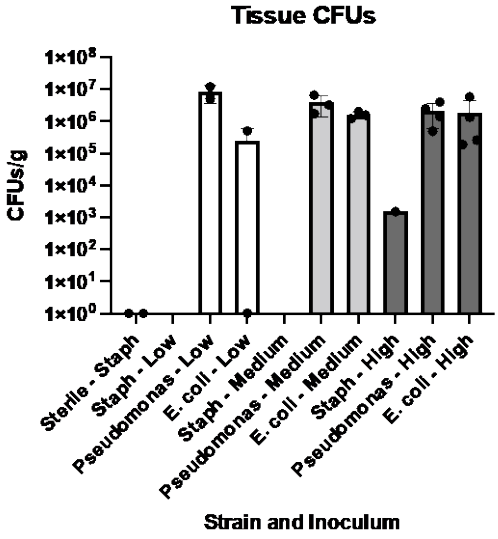


Figure 13. *In-vivo* bioluminescent signal over time representing bacterial burden (A). Average implant colony forming units (CFUs) (B). Average tissue colony forming units (CFUs) (C).

It is evident according to implant and tissue CFUs that lower inoculums of Xen36 *S. aureus* and Xen14 *E. coli* allow for more robust growth of Xen41 *P. aeruginosa*, leading to higher mouse mortality in the low and medium inoculum groups. Furthermore, the addition of a second gram negative pathogen, *E. coli*, allows for the both gram negative *P. aeruginosa* and *E. coli* to outcompete *S. aureus* with the exception of the high inoculum group. We will choose a final inoculum of E5(Xen36)E2(Xen41)E3(Xen14) allowing for a balance of E5 gram negative and E5 gram positive species.

In-Vivo Hydrogel Polymicrobial Efficacy:

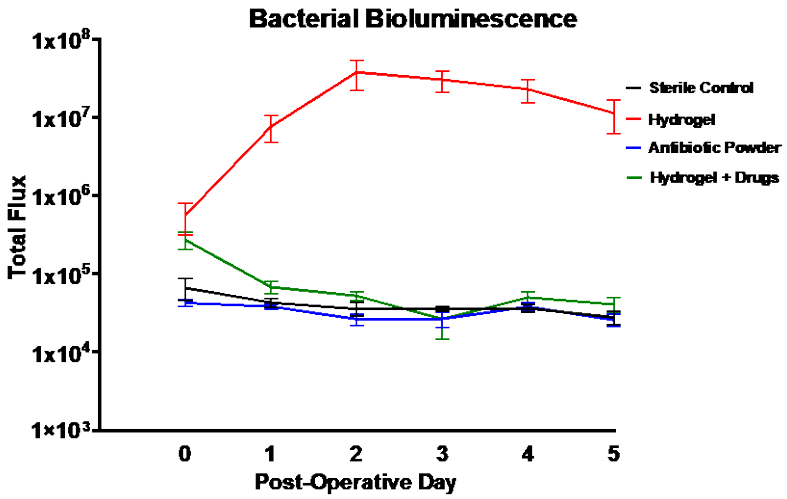
The goal of our first experiment was to identify the antimicrobial efficacy of our drug loaded hydrogel against our established E5(Xen36)E2(Xen41)E3(Xen14) polymicrobial animal model. Our experimental groups were as follows:

- Sterile Control – 2
- Infected Hydrogel – 4
- Intrawound antibiotic powder (vancomycin + tobramycin) – 4
- Hydrogel + Drugs (vancomycin + tobramycin + tranexamic acid + lidocaine) – 4

We intentionally loaded our hydrogel with all four agents to determine if multi-drug impregnation would interfere with antimicrobial efficacy. Please see drug concentrations below:

- Vancomycin: 4mg
- Tobramycin: 4.572mg
- Tranexamic acid: 5.56mg
- Lidocaine: 1.384mg

A).



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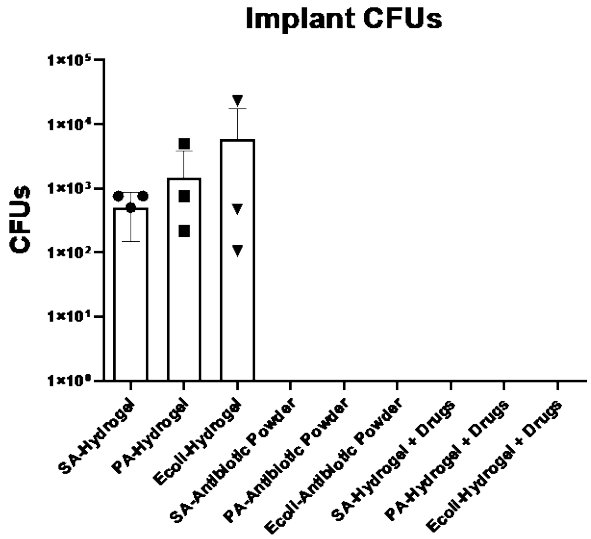

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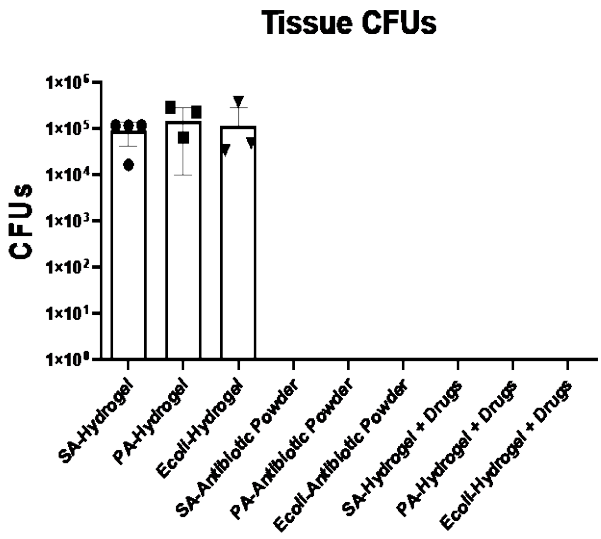

Figure 14. In-vivo bioluminescent signal over time representing bacterial burden (A). Average implant colony forming units (CFUs) (B). Average tissue colony forming units (CFUs) (C).

In Figure 14A, the antibiotic powder group lead to the immediate killing of bacteria in the wound as the bioluminescent signal in overlapping with sterile on POD0. In the drug loaded hydrogel group, one can observe a gradual killing of bacteria until the group meets sterile control on POD3. In Figures 14B and 14C, both antibiotic powder and drug loaded hydrogel eliminated polymicrobial CFUs completely by POD5, which is promising for our hydrogel. Our next polymicrobial efficacy experiment aims to increase our power and will also focus on renal toxicity, serum antibiotic levels, and antimicrobial susceptibility testing.

The next year, we plan to i) address the instability of TEMED stored in capped Eppendorf tubes; ii) begin characterizing the in vivo activity of the hydrogel wound dressing using a mouse model; and iii) complete the characterization of the chemical and physical stability of the components under various environmental conditions (extended storage, heat, etc.).

Results have been disseminated in a poster, entitled “Synthesis and Drug Release Kinetics of Robust Poly(ethylene glycol) Hydrogels for Field Dressings”, presented at UCLA’s Bioengineering Research Day.

IMPACT:

Nothing to Report. We cannot yet assess the impact of the science on the principle discipline.

What was the impact on other disciplines?

Nothing to Report. We cannot yet assess the impact of the science on the other disciplines.

What was the impact on technology transfer?

Nothing to Report. We have not yet initiated technology transfer.

What was the impact on society beyond science and technology?

Nothing to Report. We cannot yet assess the impact on society.

5. CHANGES/PROBLEMS:

In developing our small-animal polymicrobial model, the proposed E8 inoculum of 3 microbial agents are not feasible in our open fracture model as mice consistently perish during experiments. We have found that lower inoculums decrease lethality, but do not compromise intensity of soft tissue and bone infection.

While we were concerned about laboratory closures related to COVID, we began the funding period by taking a mathematical computational analysis approach to the PEG synthesis Subtask. We used computer programming to predict elusion criteria of several different hydrogels to see if we could predict optimal candidates for synthesis in the case we could not initiate work in the lab due to work restrictions. Given that we were in fact able to begin in person lab work, these computational models have served as supplemental data rather than a change of approach. In fact, they have helped guide synthesis plans and are in part responsible for why we are ahead of schedule.

We continue to have concern about potential COVID related laboratory closure but we have implemented work SOPs that allow experiments to proceed with one person working at any given time. This has been in concordance with UCLA policy to minimize potential COVID exposures.

Changes that had a significant impact on expenditures

We have had no changes in expenditures at present.

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

Nothing to Report.

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals.

Nothing to report. As vertebrate animal use may be initiated earlier than Month 18 as proposed (Major Task 3, Subtask 1), we have completed all Institutional and DOD Animal Care and Use Documentation. We currently expect no delays in transitioning to animal work one Major Task 1 and 2 are complete.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	Nicholas Bernthal
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0003-3338-5878
Nearest person month worked:	24
Contribution to Project:	Oversees and organizes laboratory meetings; guides conception and evaluation of PEGDA and hydrogels based on clinical needs; design and preparation of upcoming animal studies.
Funding Support:	

Name:	Andrea Kasko
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0003-2355-6258
Nearest person month worked:	24
Contribution to Project:	Oversees synthesis of PEGDA and hydrogels; evaluation of drug release
Funding Support:	

Name:	Elizabeth Pumford
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	0000-0001-8036-627X
Nearest person month worked:	19
Contribution to Project:	Synthesis of PEGDA and hydrogels; evaluation of drug release
Funding Support:	

Name:	Amaka Enueme
Project Role:	Graduate Student

Researcher Identifier (e.g. ORCID ID):	0000-0002-3337-0468
Nearest person month worked:	13
Contribution to Project:	Synthesis of PEGDA and hydrogels; evaluation of drug release
Funding Support:	

Name:	Zeinab Mamouei
Project Role:	Postdoctoral Research Fellow
Researcher Identifier (e.g. ORCID ID):	0000-0003-2898-1544
Nearest person month worked:	24
Contribution to Project:	In vivo hydrogel experiments using mouse models
Funding Support:	

Name:	Christopher Hamad
Project Role:	Postdoctoral Research Fellow
Researcher Identifier (e.g. ORCID ID):	0000-0002-8896-9857
Nearest person month worked:	14
Contribution to Project:	In vivo hydrogel experiments using mouse models
Funding Support:	

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

8. SPECIAL REPORTING REQUIREMENTS

None

9. APPENDICES:

None