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TITLE: Minimally Invasive VAC Therapy with Instillation for Treating Infected Skin-Implant Interfaces in Percutaneous Osseointegrated Devices

PRINCIPAL INVESTIGATOR: Jay Agarwal, MD

CONTRACTING ORGANIZATION: University of Utah, Salt Lake City, UT

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Minimally Invasive VAC Therapy with Instillation for Treating Infected Skin-Implant Interfaces in Percutaneous Osseointegrated Devices

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6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Percutaneous osseointegrated (OI) prosthetics are a superior alternative to socket-type prosthetics. Sadly, the weak link of this OI technology is high and re-occurring infections that originate from the implant post-exit sites. One potential method for treating infected tissue locally is the direct application of negative pressure wound therapy with instillation (NPWTi) at the implant exit site. Thus, this proposal's overall goal is to successfully develop an NPWTi treatment plan for infected skin-implant interfaces of percutaneous OI devices. We have designed, developed, and fabricated all necessary implants and tools during this reporting period and obtained the required institutional approvals. We have now performed initial surgery on a group of 20 rats. They are currently being inoculated with bacterial for starting the NPWTi treatments.

15. SUBJECT TERMS

Percutaneous osseointegrated implants, Infection, Negative pressure treatment with antibiotic instillation, NPWTi

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1. INTRODUCTION:

Even after thirty years of limited clinical use in certain European countries, the most percutaneous osseointegrated (OI) prosthetics for amputees are not FDA approved for use in the US, due to, in part, the potential of infection that originates at the percutaneous device exit-site/stoma. It is believed that there is an insufficient epidermal cell-to-device integration, which leads to a sinus tract that is a *locus* for bacterial colonization. As this implant system has a percutaneous post (exoprosthesis), which allows direct access to the skeletal system via an intramedullary implant (endoprosthesis), if any superficial infection is left untreated or inadequate treated, there is a high probability of bone infection and subsequent implant failures could occur. Clinical reports indicate that most of these percutaneous devices could become infected during their lifetime. These infections are usually superficial and can readily be treated with appropriate antibiotics. However, some inadequately treated and frequently recurring infections could lead to chronic bone infection (i.e., osteomyelitis). Clinically, bone necrosis and osteomyelitis have been observed in some severe diseases that could result in implant loosening. Thus, it is recommended to treat the infections early in order to prevent catastrophic implant failures later.

Moreover, antimicrobial resistance is a global health challenge, and we have no long-term solutions. A constant and systemic overuse/misuse of antibiotics in these OI populations could increase their antimicrobial resistance and related complications. Also, if the biofilm is to be formed on the implant surface, it will be near impossible to eradicate them and can result in reoccurring chronic infection. To prevent a chronic state of recurrent infections at the stoma, we proposed a local treatment of the infected site with negative pressure wound therapy with instillation (NPWTi), where antibiotics or antiseptics are delivered locally. This technique allows injecting antibiotics or antiseptics with pre-selected indwell times directly to the infection site, avoiding systemic toxicity.

Therefore, this study is designed to successfully test the efficacy of NPWTi therapy in treating the infected skin-device interfaces of percutaneous OI devices. The efficiency and effectiveness of the treatment are proposed to be tested in two translational animal models. Study 1 will utilize a rat model to determine the treatment frequency, while Study 2 will use a pig model to validate the treatment protocol.

There are two aims of this study:

- Specific Aim 1 is used to compare the effectiveness of a commercial NPWTi to systemic IP injection of broad-spectrum antibiotics for treating infected skin-percutaneous device interfaces in a subdermal rodent model.
- Specific Aim 2 is used to confirm the efficacy of NPWTi therapy for treating infected skin-percutaneous OI device interfaces in a bone-anchored porcine model.

2. KEYWORDS:

Osseointegrated percutaneous implants, OI prosthetics, Negative pressure wound therapy, Antibiotics instillation, Amputees, Alternative to socket prosthetics.

3. ACCOMPLISHMENTS:

What were the major goals of the project?

What was accomplished under these goals?

Task 1 (Completed and reported in Year 1 report): Implant Design and Manufacturing (One hundred percent (100%) of this task is now completed)
All of the implants that are needed for the animal studies described in Tasks 3 and 4 are now designed and fabricated. Implants were designed in-house and fabricated elsewhere.

Task 2: Institutional Approvals (One hundred percent (100%) of this task is now completed)
The proposed research required two separate animal study protocols. In order to carry out the animal studies, we required three different regulatory approvals from local and federal committees.

Primary goals for Years 1 & 2 are:

1. Task 1: Design, manufacture, and sterilize implants – 100% completed (June 2021)
2. Task 2: Acquiring institutional approvals – 100% completed (March 2021)
3. Task 3: To compare the effectiveness of a commercial NPWTi with the systemic IP injection of broad-spectrum antibiotics for treating infected skin-percutaneous device interfaces in a subdermal rat model – 20% completed
4. Task 4: To confirm the efficacy of NPWTi therapy for treating infected skin-percutaneous OI device interfaces in a bone-anchored porcine model – 5% completed

Milestone achieved
There were four deliverables for this reporting period; both are now achieved.

- Achieved - two milestones
  1. Implants for *in vivo* study (3 months)
  2. Institutional approvals for *in vivo* studies (6 months)

- Delayed - 2 milestones.
  1. Completion of rat surgeries: n=20 is now completed (40 more surgeries to perform, expected completion date -Dec 2022)
  2. Completion of necropsy: n=1 necropsy is performed (5 more surgeries to perform, expected completion date - Jan 2023)

Additional work completed

- Developed two surgical protocols
  1. Inoculation method for infecting the percutaneous stoma
  2. Pig forehead surgical technique

Reported in Year 1 – Completed tasks

Task 1: Implant Design and Manufacturing (One hundred percent (100%) of this task is now completed) All of the implants that are needed for the animal studies described in Tasks 3 and 4 are now designed and fabricated.

- Implants were designed in-house. The final engineering drawings and design specifications for rodent implants were sent to an independent implant manufacturer (Thortex, Inc.) for fabrication. All of these implants have now been manufactured; we received ~80 implants to date.

We previously reported the completion dates in Year 1 and other quarterly reports.

Task 2: Institutional Approvals

We needed to obtain three approvals. They were:

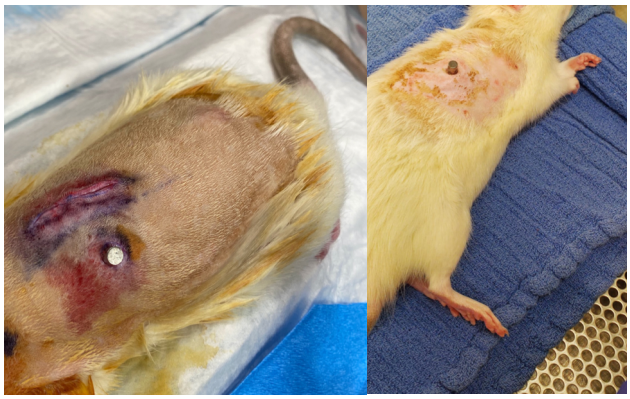
1. University of Utah School of Medicine,
2. Salt Lake City Department of Veterans Affairs (Local ACORP), and
3. Department of Army Animal Care and Use Review Office (ACURO).

We previously reported the dates of approvals in Year 1 and other quarterly reports.

Achievements in Year 2

Task 3: Guinea pig infection model (Specific Aim 1; on-going)

This task is designed to test Aim 1, which is to compare the effectiveness of a commercial NPWTi to systemic IP injection of broad-spectrum antibiotics for treating infected skin-percutaneous device interfaces in a subdermal rodent model. However, the unavailability of the nude guinea pig has driven us to develop an infection model in rats. In our previous reports, we have informed a delay in this task due to sourcing the animals and negotiating a contract with KCI to obtain their clinically used NPWTi device. This task is further behind schedule because there were delays in purchasing the Animal Biosafety Level 2 (ABSL-2) housing units. All necessary facilities and equipment are now sourced. This study is now progressing, with 12 animal surgeries being completed.



(a) (b)

Figure 1: The position of implant at surgery (a) and the implant exit site at 4-week post-surgery (b) healing.

Surgeries: After obtaining the minor amendment approval from VA ACORP to change the animal model, 12 rats were subjected to one-stage sterile surgeries. During the surgery, animals were anesthetized using vaporized isoflurane. Each rat was placed in a sternal recumbent position, shaved, and prepared for aseptic surgery using.

Surgery using a standard cleaning procedure with Betadine® Surgical Scrub, 70% alcohol, and Betadine® Surgical Solution. A single midline skin incision (approximately 4 cm in length) was made caudal to the scapula direction and about 10 mm left of the spinal column. A subdermal pocket was created on the right site using the blunt dissection technique for implanting the subdermal barrier. A 4-mm biopsy punch was used to create a percutaneous opening (2.5 cm from the scapula and 10 mm right of the spinal column), and a percutaneous post was allowed to protrude through the skin (Figure 1). Each rat was dosed with a single subcutaneous injection of long-lasting carprofen as post-operative analgesia. The incision line was then sutured closed using 4-0 resorbable sutures; rats were recovered and allowed to ambulate for four weeks. If the animal exhibited any signs of pain or distress, further analgesia treatment was given after consultation with the attending Veterinary Surgeon.

Today, all animals reached the 4-weeks post-implant healing period without any incidence of infection and are now subjected to infection induction (Figure 2).

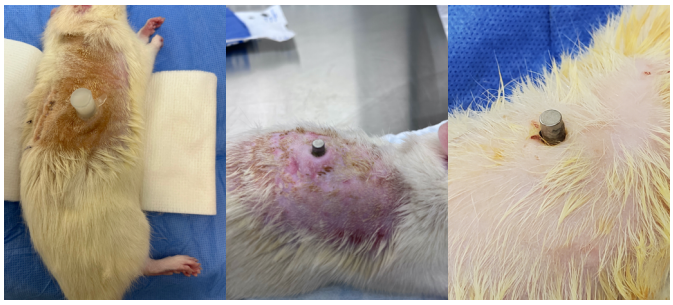
Infection Induction: Four weeks following the first surgery, animals were subjected to bacterial inoculation at the post. For this, 10^{10} CFU of *Staphylococcus aureus* (Seattle 1945 strain) was prepared freshly from certified frozen stock to be inoculated to the periprosthetic site. On the day of inoculation, a well was attached over the post, and 1 ml of bacteria prepared with sterile PBS was placed in the well. The animal was maintained under anesthesia for 1 hour (Figure 2). Rats were dressed with a base dressing that contained a further 1 ml of 10^{10} CFU of *Staphylococcus aureus* at the exit site with experimental bacteria to keep other potential environmental pathogens from contaminating the percutaneous area.

Post inoculation, they were observed for signs of infection once daily. The degree of inflammation, redness, discharge/exudate, infection, granulation tissue, and ulceration were graded using an adapted 5-tier Holger's grading metric (below).

Score	Holger's classification
0	<1 mm redness/no reaction
1	>1 mm redness, reddish discoloration around skin surrounding the implant
2	Moist surface around the skin surrounding the implant
3	Formation of granulation tissue around implant
4	Extensive soft tissue reaction requiring removal of implant

Location	*CFU/g sample*
Interface region	$1.9x10^6$
Skin	$3.0x10^6$
fat	$1.4x10^5$
periprosthetic	$6.4x10^5$

Table 1: Bacterial count



(a) (b) (c)

Figure 2: A set of representative photographs showing the bacterial inoculation (a). Three days after inoculation showed infection at Grade 2 (b). A week after the bacterial inoculation, i.e., at necropsy, showing resolved infection (c).

Only one animal reached its respective study endpoint, which was euthanized, and samples were collected for histology, bacterial enumeration, and RNA sequencing studies. The number of bacteria within the known weight of tissue was calculated and reported in Table 1. For this bacterial count study, a known weight of periprosthetic tissues was collected from the periprosthetic tissue. Aseptically, the known volume (1:10 weight/volume ratio) of liquid thioglycollate medium was added. Submerged tissue samples will be disrupted using a homogenizer for 90 s. Aliquots of 1 ml were prepared for molecular methods and frozen, but the rest of the sample was used for routine enumeration culture assays using serial dilution techniques. These bacteria are currently undergoing phenotype determination to confirm the strain. Thus, after each treatment, a number of bacteria within a gram of tissue will be used as the primary outcome measure.

Preparing for NPWTi treatment: Post-infection induction (1-week post-inoculation), we are preparing to treat Treatment 1 group animals are going to be treated with NPWTi (1 hour of instillation with Cefazolin, followed by 5 hours of topical NPWT therapy for a week). For this treatment, the percutaneous post site was dressed to achieve an airtight seal (Figure 3) and connected to a vacuum pump for 6 hours daily for a week. This is no-going, and its results will be reported at a later time point.

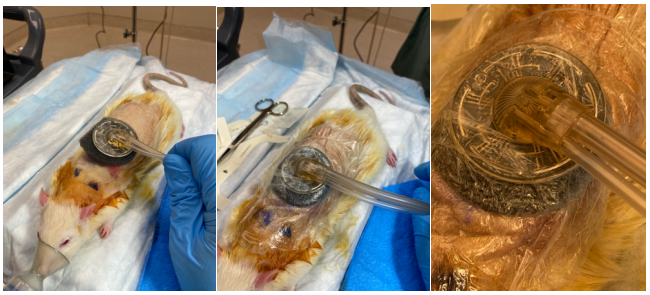
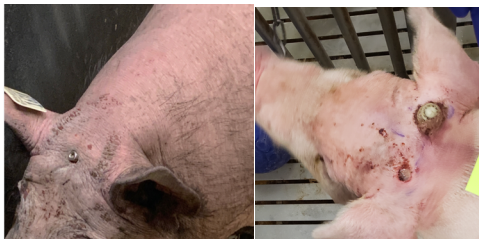


Figure 3: A set of photographs showing the NPWTi application to the implant exit site.

Task 4: Bone anchored pig forehead model – (Specific Aim 2; on-going)

This task is aimed to confirm the efficacy of the NPWTi therapy protocol developed in Task 3 in a bone-anchored porcine model (clinically relevant pig model). Previously, we have reported the failure with this model since the frontal skull had too many intra cranial sinuses. Now we have moved the implant to the back of the skull (i.e., (i.e., occipital bone) and implanted it in two animals (two implants each, Figure 4 (ii)). The outcome of this pilot study will be reported at a later date.



(i) (ii)

Figure 4: *(i) - A representative set of images showing a skin-penetrating implant that was placed on the pig forehead (i) and* occipital bone *(ii).*

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

Graduate student training – During the Q2-Q4 Y1, Samantha Style and Clark Neilson have received graduate student training through this study. Also, both of them are trained to perform animal surgeries, daycare and necropsies. Also, they are being trained on tissue processing, post nectropsy.

How were the results disseminated to communities of interest?

Nothing to report

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

We are planning to perform surgeries described in Task 3 during the next reporting period, while continue to monitor the pigs.

4. IMPACT:

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

Nothing to report

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

8. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Based on the pilot pig study, we may want to change the surgeries for pigs from a two-stage to a single-stage process. If effective, we plan to submit changes to the protocol to both local and ACURO for further approvals.

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

- Due to COVID restrictions, acquiring the surgical time and space for the animals are difficult, which may delay our milestones. Based on the current progress with the surgeries, tasks 3 and 4 are delayed by 6-9 months. We are planning on applying for a no-cost extension for one year.

Changes that had a significant impact on expenditures

- Delays in the large animal study and increasing costs for supplies, animals, and daycare are expected to impact the expenditures.

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

Significant changes in use or care of human subjects

N/A

Significant changes in use or care of vertebrate animals

N/A

Significant changes in use of biohazards and/or select agents

N/A

8. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to report

Books or other non-periodical, one-time publications.

Nothing to report

Other publications, conference papers and presentations.

Nothing to report

- **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

8. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

1. Name: Jay Agarwal, MD
   Project Role: Principal Investigator
   Research Identifier (ORCID ID): 0000-0002-1209-6703
   Nearest person month worked: 0.6 Months
   Contribution to Project: Dr. Agarwal reviewed all of the animal protocols, helped with surgical technique development and reporting.

2. Name: Sujee Jeyapalina, PhD
   Project Role: Principal Investigator
   Research Identifier (ORCID ID): 0000-0002-2199-7191
   Nearest person month worked: 3.0 Months
   Contribution to Project: Dr. Jeyapalina has prepared all of the institutional approval documents and reports, designed and acquired engineering drawings, and contributed to the methodology developments and project management, surgeries and related tasks.

3. Name: Clark Nielson
   Project Role: Graduate student
   Research Identifier: N/A
   Nearest person month worked: 4.0 - 6.0 Months
   Contribution to Project: Mr. Nielson is responsible for acquiring equipment and supplies for the study and has taken a lead role in optimizing the procedures. He also helps with animal surgeries, daycare, and related tasks.

4. Name: Samantha Steyl
   Project Role: Postgraduate research assistant
   Research Identifier: N/A
   Nearest person month worked: ~3.0 Months
   Contribution to Project: Ms. Steyl developed methodologies for optimizing and delivery of bacteria to the percutaneous implant exit site reproducibly.

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

No

What other organizations were involved as partners?

Facilities: Department of Veterans affairs, Salt Lake City Health Care System.

In-kind Support – KCI will loan their NPWTi devices

8. SPECIAL REPORTING REQUIREMENTS

Minimally Invasive V.A.C. Therapy with Instillation for Treating Infected Skin-Implant Interfaces in Percutaneous Osseointegrated Devices
OR190083/W81XWH2010606

PI: Jay Agarwal, MD **Org:** University of Utah /Isabella Johnsen **Award Amount:** $749,755

Study/Product Aim(s)

We hypothesize that superficially infected skin-device interfaces of percutaneous OI devices can be efficiently and effectively treated with negative pressure wound therapy with antibiotic instillation (NPWTi).

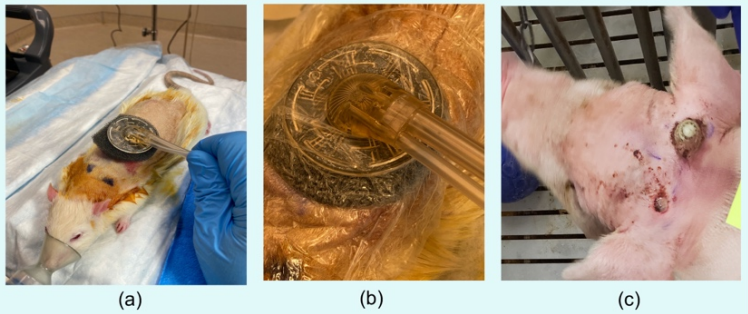
This will be tested using two aims:

Specific Aim 1 will compare the effectiveness of NPWTi with the systemic IP injection of broad-spectrum antibiotics for treating infected skin-percutaneous device interfaces in a subdermal guinea pig model.

Specific Aim 2 will confirm the efficacy of NPWTi therapy for treating infected skin-percutaneous OI device interfaces in a bone-anchored porcine model.

Approach

Our approach includes a two-stage surgery to implant percutaneous devices in a guinea pig/pig model, followed by an inoculation of 10^8 CFU of Seattle 1945 *Staphylococcus aureus* directly into the periprosthetic tissues to induce infection. Post-infection, each animal will be treated with a combination of NPWT therapy with or without antibiotic instillation. An effective treatment protocol will then be considered for human applications.



(a) (b) (c)

Figure: A set of photographs showing the NPWTi application to the implant exit site (a) and (b). The new implantation occipital bone site in pig (c).

Accomplishment: (1) Institutional approvals for animal studies (100% completed); (2) – Completed implant designs (100% completed); (3) – Completed surgeries (20%); (4) – Implant manufacturing (100 % completed). (5) – rat infection model development (20 % completed). (6) – Development of pig skull surgeries (50%)

Timeline and Cost

Activities CY	21	22	23
Completion of guinea pig surgeries			
Completion of data analysis			
Completion of pig surgeries and analysis			
Manuscript preparation and submission/clinical translation			
Estimated Budget ($K)	**$290**	**$239**	**$220**

Updated: 01 June 2022

Goals/Milestones (Example)
CY20 Goal – IACUC approvals
✓ Local and DOD approvals
CY21 Goals – Completion of guinea pig study
☐Completion of guinea pig surgeries
☐Collect data/analyses
CY21 Goal – Define the treatment protocol
☐ Submission of manuscript
CY22 Goal – Confirm the treatment protocol in pigs
☐ Completion of pig surgeries and analysis
Comments/Challenges/Issues/Concerns
• N/A
Budget Expenditure to Date
Projected Expenditure: $749,755
Actual Expenditure: ~$451,527 (including WIVR sub-contract)