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TITLE: Optimizing Skin-Implant Interface of Osseointegrated Device

PRINCIPAL INVESTIGATOR: Jonathan Forsberg, MD, PhD

RECIPIENT: Henry M Jackson Foundation, Bethesda, MD

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The proposed scope work addresses bio-engineering strategies to enhance healing and functionality of osseointegrated devices for the person with an upper extremity amputation. Recognizing the role of the septal compartments at the hard tissue-soft tissue interface at the tips of appendages, the investigators are investigating methodologies to create a more durable interface. The approach is to apply a scaffold to mimic septal compartments, select and propagate stem cells with enhanced adhesive properties to promote healing and osseointegration and to prevent infection with antimicrobial coated metal device material. We have isolated, characterized and selected highly adhesive stem cells (MSCs) with plasticity for multilineage differentiation that have good scaffold ingrowth, intending them to be a population of cells that can be seeded into the scaffold and transplanted to the interface of the metal device. Animal studies are being designed and the IACUC protocol developed to measure outcome to test depth of tissue growth, preventing or limiting infection, metal-skin interface strength, and histopathology.

15. SUBJECT TERMS
None listed

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
					USAMRDC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**	Unclassified	11	**19b. TELEPHONE NUMBER** ***(include area code)***
Unclassified	Unclassified	Unclassified			

Standard Form 298 (Rev. 8-98)
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TABLE OF CONTENTS

1. INTRODUCTION: Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

The study addresses two focus areas of research with pressing clinical need: **1.** Optimization of the skin-implant interface for osseointegrated (OI) implants, and **2.** Prevention of infection at the skin-implant interface. The goal is to address both these issues while maintaining residual limb skin integrity and durability. We address the issue of skin-to-implant healing and attachment for osseointegrated (OI) prostheses by focusing on integration and durability of their microbially, mechanically and biologically challenging skin-to-implant interface. This study focuses on an OI prosthetic implant anchored in the long bone of a residual limb and exiting through the skin. Implant and soft tissue infections (29-38%) and implant loosening (13-29%) are common complications for both upper and lower extremity bone-anchored implants, resulting in revision surgeries and increased morbidity. These complications develop due to lack of a tight, impervious seal at the skin-percutaneous implant interface, resulting in exposure of soft tissue and vasculature, thereby increasing chances of infection as well as implant loosening. For both focus areas, we explore the possibility of creating a tight, durable skin-implant interface for OI implants using mesenchymal stem cells (MSCs) derived from naturally occurring porcine integumentary and connective tissues or human induced pluripotent stem (iPS) cells, which have the intrinsic potential to form an impervious seal at hard and soft tissue junctions. We hypothesize that comparative analysis of the differentiation and adhesion properties of naturally occurring cells of the integumentary system, present at hard and soft tissue junctions at the dermis, nails or hoof, periodontal ligament, adipose tissues, as well as iPS cells could enable us to engineer durable and impervious cell-based scaffolds for placement at the skin-implant interface.

2. KEYWORDS: Provide a brief list of keywords (limit to 20 words).

Osseointegration, implants, titanium, scaffold, MSCs, iPS cells, differentiation, adhesion, tissues, bone, cartilage, adipose, muscle, ligament, tendon, dermis

3. ACCOMPLISHMENTS: The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project?

Research-Specific Aims and Tasks	Mos.	Percent completed Site 2	Sub-site2 a
Administrative Aims and Tasks: 1. Establish subaward agreement between HJF and MMRF 2. Develop and sign USU-MMRF data sharing agreement 3. Recruit and hire support personnel a. Stem Cell Biologist (USU) b. Research Associate (USU) c. Stem Cell Biologist (MMRF-UMN)	1-4	100% 100% 100% a. 100% b: 100% c: 100%	

Specific Aim 1: Ex vivo culture (a) steer differentiation for human and swine MSC, iPS cells and mature site-specific (gingival and hoof/nail bed) cells to adhesive/epithelial phenotypes, (b) Characterize and rate the ingrowth of these cells into scaffold and their adhesive potential to metal substrate.	**1-24**	100%	X
Major Task 1: Develop cell culture	1-16	100%	
Subtask 1.1: In vitro isolation and characterization of *porcine* cells.	1-16	100%	
Subtask 1.2: In vitro development and characterization of *human* cells.	1-16	100%	X
Major Task 2: *In vitro*: evaluate cell adhesion to metal substrate	7-16	100%	X
Subtask 2.1a: Test porcine cells for adhesion	7-16	100%	X
Subtask 2.1b: Test human cells for adhesion	7-16	100%	X
Major Task 3: Scaffold development for cell growth and anchorage to underlying tissue.	5-24	100%	X
Subtask 3.1: Complete scaffold design (constructs) for "sleeve" & "transition designs	5-16	100%	X
Subtask 3.2: Seed and grow porcine cells on flat collagen sheets, assemble scaffolds	17-24	100%	
Subtask 3.3: Seed and grow human cells on flat collagen sheets, assemble scaffolds	17-24	100%	X
Major Task 4: Complete full statistical analysis, complete/submit 2-4 manuscripts.	18-24	80%	X
Milestone(s) Achieved: Characterization of 3-4 cell choices for optimal adhesivity - in vitro; submission of 2-4 manuscripts.	*1-24*	90%	*X*
Specific Aim 2: In vivo large animal (swine) testing of transdermal implants with and without subdermal cellular augmentation (SA2a), +/- septal /strain limiting scaffold (SA2b), and topical bacterial challenge (SA2c).	**25-60**	90%	X
Major task 5: (SA2a) Implant 8 implants/animal with "best of" **cells** from Specific Aims 1 and 2 x 10 animals at USU-Surgery	25-35	100%	X
Subtask 5.1: Implant in initial 5 animals with 3-4 types of cell augmentation per animal	25-27	100%	X
Subtask 5.2: Initial evaluation of skin integration - assessment of initial results; experiment modification as necessary.	28-29	5%	X

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project identify these dates and show actual completion dates or the percentage of completion.

What was accomplished under these goals?

Specific Aim 1: Ex-vivo culture: Characterize and rate the ingrowth of these cells into scaffold and their adhesive potential to metal substrate

Major Task 3: Scaffold development for cell growth and anchorage to underlying tissue.

The ex-vivo study to assess skin attachment onto titanium rods in the presence of cell-laden hydrogel was completed and the samples were provided to the University of Minnesota imaging center for tissue imaging to analyze cellular responses. The center has optimized the staining protocols and are in the process of collecting data which will complete the analysis and last piece of information needed for the manuscript.

Specific Aim 2: In vivo large animal (swine) testing of transdermal implants
Task 1: Testing the effect of MSCs for soft tissue attachment around transdermal implants.

Methodology:

1. For porcine MSCs: Cell source: Tendon-derived porcine MSCs
   Three groups were tested with n=4 in each animal (5 animals) with a total of 12 implants implanted in each animal. The three groups tested include: implant only, implant with GelMA hydrogel and implant with cell-laden GelMA hydrogel. The cells were cultured and maintained in accordance with the previously reported method. On the day of the surgery, 90% confluent cells were trypsinized, counted and resuspended in the GelMA macromer (10 wt%) at a cell density of 5 x 10^6 cells/ml along with 0.05% photo-initiator. The GelMA control was prepared the same way without the cells. The materials were maintained at 37°C in a dry heatblock until applied around the implant. Once the material was applied, the GelMA was polymerized by shining light at a λ=405nm for 3minutes where the light was held very close to the surface of the animal.

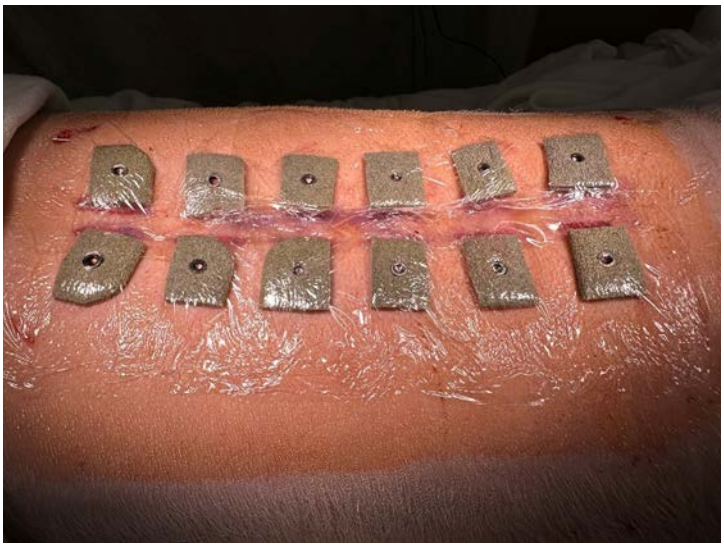

Figure 1: Representative image of the site of implantation and post-surgery site preparation.

2. For human MSCs: Cell source: Bone Marrow derived MSCs (BM-MSCs, commercially available from Lonza) and induced pluripotent stem cells derived MSCs (iMSCs) were used. To avoid immune rejection when using human stem cells into pigs, the pigs were treated with cyclosporine dosed at 15mg/kg on daily basis. The treatment started 24h prior to xenogenic transplantation and continued every day until explantation.

Four groups were tested with n=4 in each animal (6 animals) with a total of 12 implants implanted in each animal. The four groups tested include: implant only, implant with GelMA hydrogel, implant with BM-MSCs-laden GelMA hydrogel and implant with iMSCs-laden GelMA hydrogel. The cells were cultured and maintained in accordance with the previously reported method. On the day of the surgery, 90% confluent cells were trypsinized, counted and resuspended in the GelMA macromer (10 wt%) at a cell density of 5 x 10^6 cells/ml along with 0.05% photo-initiator. The GelMA control was prepared the same way without the cells. The materials were maintained at 37°C in a dry heatblock until applied around the implant. Once the material was applied, the GelMA was polymerized by shining light at a λ=405nm for 3minutes where the light was held very close to the surface of the animal.

The implants and the surrounding tissues were excised after 12 weeks, fixed in 10% neutral buffered formalin (NBF) solution for 48 hours and then stored in 70% ethanol at 4°C for histological analysis.

Manuscripts

- The manuscript on the use of iMSCs-laden scaffolds for epithelial attachment onto metal abutment for improved performance of osseointegrated devices is under preparation.

Describe the Regulatory Protocol and Activity Status (if applicable).

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

TOTAL PROTOCOLS: *State the total number of human use protocols required to complete this project (e.g., 5 human subject research protocols will be required to complete the Statement of Work."). If not applicable, write "No human subjects research will be performed to complete the Statement of Work."*

TOTAL PROTOCOLS: 1. Human cells collected for the SOW were part of Dr. Jakub Tolar's lab protocol entitled 'Comparison of normal skin biology to the biology of diseased skin' (STUDY00001939, University of Minnesota), **exempt for IRB approval ("WORKSHEET: Exemption (HRP-312)**" as 'Research involving the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens if the information is recorded by the investigator in such a manner that subjects cannot be identified, directly or through identifiers linked of the subjects.' This exempt letter to HRPO has been forwarded to HRPO.

(b) Use of Human Cadavers for Research Development Test & Evaluation (RDT&E), Education or Training

TOTAL ACTIVITIES:

- *No RDT&E, education or training activities involving human cadavers will be performed to complete the Statement of Work (SOW)."*

(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."

TOTAL PROTOCOL(S): 2

PROTOCOL (2 of 2 total):

Protocol [ACURO Assigned Number]: N*ot yet available*

Title: *Porcine implant study to optimize Skin-Implant Interface of Osseointegrated Devices.*

Target required for statistical significance: *At 80% power and an accepted error of 0.05,if the variable has a standard deviation of 25% of the mean (assumption), then a 9 sample size would allow us to statistically find a significant difference >33% between the two compared groups. If the sample size would be of 12, then the significant difference detectable at this power would be >29%. Thus, the power difference between a 12 and 9 sample size is not enough to justify a larger number of animals.*

Target approved for statistical significance: *In progress*

Protocol ID: 2112-39687A

Principal Investigator: Gorr, Sven-Ulrik

Protocol Title: The development and characterization of a soft-tissue attachment to a transdermal skin-implant interface in a swine model of osseointegration

Approval Date: May 06, 2022

ACURO approval: July 5, 2022

Protocol ID: 2112-39687A; Amendment/Modification 1

Principal Investigator: Gorr, Sven-Ulrik

Protocol Title: The development and characterization of a soft-tissue attachment to a transdermal skin-implant interface in a swine model of osseointegration

Approval Date: January 30, 2023

ACURO approval: April 4, 2023

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

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

- A no-cost extension is requested to complete the histology of samples from both cohorts, data analysis and writing of reports and publications.

4. Impact:

5. Products:

Nothing to report

6. Participants & Other Collaborating Organizations

What individuals have worked on the project?

Provide the following information for: (1) Project Directors (PDs)/ PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort).

Describe how this person contributed to the project. If information is unchanged from a previous submission, provide the name only and indicate "no change."

Name: *COL Benjamin K. Potter, MC, USA*
Project Role: *Principal Investigator*
Researcher Identifier (e.g. ORCID ID): 0000-0002-8771-0317
Nearest person month worked: *1*
Contribution to Project: COL Potter responsible for overall project coordination.

Name: *Thomas A. Davis, PhD*
Project Role: *Associate Investigator*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *1*
Contribution to Project: Oversight of porcine in vitro and in vivo studies conducted at USU

Name: *Joan Bechtold*
Project Role: *Site PI- MMRF*
Researcher Identifier (e.g. ORCID ID): 0000-0002-7090-4270
Nearest person month worked: *1*
Contribution to Project: Responsible for overall project coordination at subaward site(s).

Name: *Sven-Ulrik Gorr, PhD*
Project Role: *sub-site PI*
Researcher Identifier (e.g. ORCID ID): 0000-0002-7855-4949
Nearest person month worked: *1*
Contribution to Project: Oversight of iPSC in vitro studies and animal studies conducted at UMN.

Name: *Isha Mutreja. PhD*
Project Role: *Investigator*
Researcher Identifier (e.g. ORCID ID): 0000-0002-8998-7563
Nearest person month worked: *1*
Contribution to Project: Performance of iPSC in vitro studies and cell adhesion studies conducted at UMN.:

Name: *Liz Pluhar. DVM, PhD*
Project Role: *Investigator*
Researcher Identifier (e.g. ORCID ID): 0000-0002-7881-5061
Nearest person month worked: *1*
Contribution to Project: Performance of implant surgeries at UMN

7. Changes/Problems:

a. Actual Problems or delays and actions to resolve them

The implants used for this study were redesigned and required a modified research plan which allowed for more implants per animal and reduction of the number of animals used.
The regulatory implications of this change is described below.

Due to the need for additional regulatory review, we were not able to hold our spot at the UMN Experimental Surgical Services. Instead we were referred to a different surgical service at the University, APIC, which has better availability. The project surgeries were completed with APIC at no additional cost to the project.

b. Anticipated Problems/Issues

Provide a description of anticipated problems or issues that have a potential to impede performance or progress. Also provide course of actions planned to mitigate problems or to take should the problem materialize.

Nothing to report

8. Special Reporting Requirements:

Quad Charts: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.