Smooth Percutaneous Bone Anchored Implants - An Iliac Crest Pig Model

Applicant: [redacted]

Date: March 15, 2016

Faculty Mentor: [redacted]

Department: Bioengineering

Statement of the problem/topic of the research or creative work

Standard of care is rapidly changing for those who face limb amputation due to the introduction of percutaneous osseointegrated prosthetic implants. The new method of attaching an artificial limb directly to the skeleton brings with it the promising potential for improved gait and functionality, faster donning and doffing of the prosthesis, and reduced wearer fatigue. These techniques incorporate the use of a titanium rod, inserted into healthy bone tissue using a formed stoma. In order to improve standard of care alongside patient outcomes, it becomes necessary to address the inherent problems associated with device related infections. Epithelial down-growth (ED) and lack of skin-to-device integration has long been associated with device related infections. ED occurs when the basal cells of healthy tissue fail to adhere to the surface of the titanium device while continually promoting wound healing around the device. These cells inherently grow proximally to the surface of the implant, which inevitably creates a sinus tract around the implant similar to a pocket, this location becomes a breed where bacterial colonization and growth can occur. In certain cases, it will induce superficial and chronic infection episodes along with subsequent revision surgeries, and the inherent antibacterial drug treatment regimens. Research studies in this area have shown that fibrous tissue adhesion can be improved using porous materials and biomimetic surface coatings. Ongoing research in our lab is aimed at optimizing the coating integrity of a fluorhydroxyapatite[1] (FHA) which will help characterize the aforementioned. In human trials conducted in European countries, it has been indicated that using smooth implants can also facilitate the formation of a stable stoma, which helps to prevent infection at longer implant *in situ* periods. Although it was a general consensus that epithelial tissue migrates fast and adheres to the bone surface, to date, this has not been definitively proven in any translational models. It is quite probable that when epithelial tissue migrates to the

periosteal surface (outer membrane that covers the bone surface), which is mainly a cellular layer, cell-to-cell connection may establish between the epithelial and differentiated stem cells found in the periosteal membrane. This may mark the end of soft tissue healing and the formation of a stable stoma. Currently, a similar implant system is being used at the SLC VA hospital in an FDA approved 10-patient study to understand the efficacy of this system. Based on this trial, a larger clinical trial is expected to rehabilitate amputees with this novel implant system. Given this, it is also pertinent to understand the precise location of healthy tissue attachment in an osseointegrated implant system in order to devise new implant systems and techniques for improved patient outcomes. We believe that when epithelial down growth occurs, the healthy cells are adhering to the bone periosteum near the bone implant interface and are restoring the protective function of epithelium. Although a nidus may form between the implant surface and skin, having the protective epithelial barrier is paramount importance when preventing infection. It is hypothesized that when epithelial down-growth occurs, a stable stoma is formed by the attachment of healthy cells to the periosteal surface. This hypothesis will be tested in a pig ilium model.

Relevant background/literature review

In the United States, it is estimated that the need for prosthetic limb devices will triple by the year 2050[2]. Given the increasing rate of diabetes, which is expected to double by 2030, and other factors of limb loss such as trauma, dysvascular disease, and cancer[2]; it is evident that current standard of care practices can use definite and measurable change to improve the quality of life of those affected by this condition. Traditional socket suspension methods do not provide viable solutions for many who have need of prosthetic assistance. “Recurrent skin infection and ulceration in the socket contact area, a short residual limb, volume fluctuation of the residual

limb, soft tissue scarring, extensive skin grafting, or socket retention problems due to excessive perspiration," causes many to be unable to use conventional socket prosthesis[3]. As an alternative to traditional socket suspension of prosthetic attachment systems, Rickard Brånemark and his colleagues developed an osseointegrated prosthetic device in the year 1990[4]. They were able to demonstrate clinical applications of the rudimentary osseointegrated design[4]. Standardization of this treatment method didn't occur until almost nine years later in 1999 when OPRA protocol was released and subsequently used as the new treatment method. Although results were promising at the time; patients incurred superficial infection once every two years, and six of the 51 patients had deep infections that resulted in the complete removal of one transfemoral device[5]. Device related infection is one of the major risks associated with this implantation technique, and ultimately, it has resulted in the ongoing studies aimed at addressing these concerns. As mentioned previously, one of the major reasons for infection is the lack of epithelial cell-to-implant integration at the skin-implant interface, which forms a pocket, or nidus.

Epithelial cell migration results as part of a normal wound healing process when tissue is disrupted or damaged, and until then, epithelial-mesenchymal cell junctions are disassembled, apico-basal polarity is lost, and migratory capabilities are enhanced[6]. This "flow," or migration, of epithelial cells is what accounts for normal skin healing and wound closure at the injury site to create the skin barrier, which protects against infection. However, with the introduction of a percutaneous osseointegrated device, the skin demonstrates the inability to reform the necessary amount of cell-cell junctions that effect complete tissue remodeling and wound closure around the surface of the device. An *in-vivo* pig back model will allow us the ability to analyze the

inherent properties of epithelial down growth and location of attachment to resolve nidus formation and prevent ongoing infection.

Specific activities to be undertaken and a timetable allotted for each activity

I will take part in each phase of this translational study and the eventual conclusion studies to follow. The surgeries for implanting our devices will take place in one stage. Surgery will involve the implantation of two bone-anchored sub-dermal devices into 3 pigs, specifically placed in the right and left ileums. Each group will receive identical Titanium devices. During the surgery, the periosteal membrane in one of the implant sites will be stripped out in each pig. Cancellous bone screws will be anchored to the iliac crest and then extend through the epithelium, which will allow the desired quantification of bone healing and integration as well as the ED outcomes. Percutaneous components will be polished smooth to accelerate the ED. This surgery will be done by a trained Orthopaedic Surgeon (Dr. [redacted]) and I will be assisting him during the surgery. The animals will be allowed to ambulate freely for a period of 16 weeks. Each week, the animals will undergo a strict cleaning regimen in which the implant exit sites will be cleaned with a soft shaving brush soaked in mild soapy water, followed by rinsing with warm water. After drying, the site will be covered with gauze and a protective "Tegaderm™" covering. This process will continue until the study endpoint.

At necropsy, the implant and surrounding tissues will be harvested, which marks the beginning of the next phase of analysis. Using several established analytical and histological techniques, we will be able to quantify and analyze the tissue integration in relation to the implants of each sample. Bone-implant integration will be quantified using radiographic techniques (X-ray and MicroCT). Skin-implant integration and epithelial down-growth will be quantified using histological techniques. The histological preparation of soft tissue will be done by embedding the dehydrated specimens in polymethylmethacrylate (PMMA). The next stage

[redacted]

will involve the immunohistochemical analysis using a few different strategies to examine specific cell types and the protein expression involved in tissue remodeling. Using a cryostat, each sample will be sectioned and subsequently stained using standard immunohistochemistry techniques for anti- and pro-inflammatory markers, collagen, and other indicators of the implants integration with epithelial cells. Using a confocal microscope, I will analyze the samples to compare the relative quantities of each marker, which will help in determining the success of integration of the epithelial tissue with the periosteal surface in promoting the stable stoma. This will test the proposed hypothesis. We will also be able to determine from these analyses, whether the tissue is forming cell-cell junctions at the periosteum. Expected time-line for this proposed work is given below.

Tasks	1-3 months	4-6 months	7-9 months	10-12 months
Animal surgeries				
Animal day care				
Necropsies				
Radiographic/CT analyses				
Immunohistochemistry				
PMMA embedding				
Standard histology				

Relationship of the proposed work to the expertise of the faculty mentor

Dr. [redacted] has dedicated her work and expertise to percutaneous devices, biomaterial innovation, and development in specific relation to bio-mimetics, nano-scale and micro-scale surface technology for the purpose of promoting better cellular attachment and biocompatibility for medical devices used in many aspects of clinical medicine. Her specific expertise in these areas has provided the ability to conduct many preclinical research and antimicrobial trials that have led to developments in standard of care practices that dramatically improve device implant related outcomes. The great majority of her published work is directly

related to the percutaneous device. This project is another method of understanding the wound healing process of epithelial tissue to improve device related infection, which is funded by the Department of Defense. This project directly applies to her ongoing research in this area and will help to further our understanding of how to prevent infection and facilitate cellular adhesion.

Relationship of the proposed work to the student's future goals

I am currently in the process of completing a degree in Biomedical Engineering at the University of Utah as a student researcher and scientist. I have a particular interest in medical device development and research as it pertains to the improvement of current methods and techniques. I desire to complete a Masters level degree in the area of Biomedical Engineering, and it is therefore necessary to gain the research experience needed to understand common problems associated with biomedical devices such as infection, aseptic loosening and tissue degradation. By working in this lab with Dr. [redacted] I will develop the ability to conduct meaningful trials whose aim is to provide solutions to these common problems. In addition, I will learn proper tissue analysis techniques alongside the necessary sterile practices involved in all medical research oriented labs. It is my goal to dedicate my professional career to the understanding and improvement of percutaneous devices, in an overarching effort to increase quality of life for those who undergo the loss of a limb.

References

1. Jeyapalina S. Fluorhydroxyapatite coatings for facilitating epithelial cells adhesion to the implant surface for preventing infection in percutaneous implant systems. IACUC Protocol #15-07012.

2. Ziegler-Graham K, MacKenzie EJ, Ephraim PL, Travison TG, Brookmeyer R. Estimating the Prevalence of Limb Loss in the United States: 2005 to 2050. Arch Phys Med Rehabil 2008; 89: 422-429.

3. Sullivan J, Uden M, Robinson KP, Sooriakumaran S. Rehabilitation of the trans-femoral amputee with an osseointegrated prosthesis: the United Kingdom experience. *Prosthet Orthot Int* 2003; 27(2):114-20.

4. Hagberg K, Brånemark R. One hundred patients treated with osseointegrated transfemoral amputation prostheses—rehabilitation perspective. *J Rehabil Res Dev* 2009; 46(3): 331-44.

5. Berlin Ö, Brånemark R, Hagberg K, et al. Osseointegrated prosthesis for the rehabilitation of amputees: two-year results of the prospective OPRA study. In: *2012 Orthopaedic Surgical Osseointegration Society 4th International Advances in Orthopaedic Osseointegration Conference Syllabus Abstracts*. Orthopaedic Surgical Osseointegration Society: San Francisco, CA; 2012: 23.

6. Theveneau E, Mayor R. Collective cell migration of epithelial and mesenchymal cells. Cell. Mol. Life Sci. 2013; 70: 3481-3492.