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Percutaneous Implants with Porous Titanium Dermal Barriers: An In Vivo Evaluation of Infection Risk

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Abstract

Osseointegrated percutaneous implants are a promising prosthetic alternative for a subset of amputees. However, as with all percutaneous implants, they have an increased risk of infection since they breach the skin barrier. Theoretically, host tissues could attach to the metal implant creating a barrier to infection. When compared with smooth surfaces, it is hypothesized that porous surfaces improve the attachment of the host tissues to the implant, and decrease the infection risk. In this study, 4 titanium implants, manufactured with a percutaneous post and a subcutaneous disk, were placed subcutaneously on the dorsum of eight New Zealand White rabbits. Beginning at four weeks post-op, the implants were inoculated weekly with 10^8 CFU *Staphylococcus aureus* until signs of clinical infection presented. While we were unable to detect a difference in the incidence of infection of the porous metal implants, smooth surface (no porous coating) percutaneous and subcutaneous components had a 7-fold increased risk of infection compared to the implants with a porous coating on one or both components. The porous coated implants displayed excellent tissue ingrowth into the porous structures; whereas, the smooth implants were surrounded with a thick, organized fibrotic capsule that was separated from the implant surface. This study suggests that porous coated metal percutaneous implants are at a significantly lower risk of infection when compared to smooth metal implants. The smooth surface percutaneous implants were inadequate in allowing a long-term seal to develop with the soft tissue, thus increasing vulnerability to the migration of infecting microorganisms.

Keywords

Surface texture; Titanium; *In vivo*; Bacteria; Percutaneous

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6. Conflict of Interest Statement

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1. Introduction

Currently, most amputees use a socket-type device to connect their prosthetic limbs to their bodies. These socket prostheses are designed to fit snugly around the residual limbs and are held in place mechanically through the use of belts, cuffs, or suction. Over the years, technological advancements with socket prostheses have greatly improved the lives of amputees, allowing them to be more mobile and to better engage in an active lifestyle. However, socket prostheses are not without limitations, including: overload and irritation of the adjacent soft tissues^{1–6}, disuse osteoporosis in the residual limb⁷, difficulty in ongoing socket fit due to weight fluctuations and muscular atrophy^{2, 5, 6}, and difficulty in fitting individuals with short residual limbs⁸.

To overcome these limitations, osseointegrated percutaneous implants are being developed as an alternative to socket-type devices^{9–15}. As with dental implants, osseointegrated percutaneous implants are anchored to the bone and pass through the skin, resulting in an abutment to which an artificial prosthetic attaches^{9, 11, 14, 16, 17}. In Europe, patients receiving these implants report improvements in mobility^{18, 19}, activity levels^{18, 19}, gait performance^{18, 19}, and “osseoperception,” a sensory feedback in the amputated limb from the surrounding environment^{9, 19, 20}. While osseointegrated percutaneous prostheses show great promise for the amputee population, they permanently disrupt the skin barrier and are at constant risk of infection^{14, 19}.

At a reported 18% infection rate, osseointegrated percutaneous prosthetics are similar to other clinically used percutaneous implants (i.e. bone-anchored hearing aids, catheters, and ventricular assist devices) with respect to increased infection rates^{14, 19}. Infection vulnerability in all percutaneous and subcutaneous implants relies upon, among other factors, the attachment and integration between the skin and the implant^{15, 21, 22}. It is assumed that poor skin integration with the percutaneous component is evident by a sinus tract formation between the skin and the implant. This creates opportunity for commensal and non-commensal bacteria to migrate into the sinus tract and colonize, resulting in infection, tissue morbidity, implant removal, and even mortality. The sinus tract formation can result from epidermal downgrowth, which is marked by the epithelial layer migrating down alongside the implant as an attempt to remove the implant with the ultimate goal of restoring the skin as the definitive barrier^{21, 23}.

There are many strategies targeted at improving the integration between the host soft tissue and the percutaneous device, including biological approaches (i.e. protein-coated devices^{24, 25}, drug-releasing devices²⁶, antimicrobial strategies, etc.) and engineering approaches (i.e. changes in device structure^{27, 28}, application of different materials^{13, 28–30}, surface topography alterations^{10, 31–33}, etc.). Previous work demonstrated that designing the percutaneous implant to have a subcutaneous disk increases the surface area for host tissue integration, thus improving implant-tissue attachment^{10, 27, 34, 35}. Likewise, altering the surface topography of the percutaneous device by creating micromachined grooves^{10, 33}, pits³³, or porous surfaces^{36, 37} increases the surface area on the implant allowing for increased cell attachment. While previous studies report skin attachment improvements at the skin/implant interface^{10, 13, 33, 36, 38–40}, it is not well understood if altering the surface topography on the percutaneous and/or the subcutaneous component of a percutaneous device is most important in the development of a barrier to infection when the implants are challenged with bacteria. Previous studies^{10, 32, 36, 37} aimed at preventing infection of percutaneous devices lack a strong infection signal, thus making it difficult to interpret the implant infection vulnerability.

This study aimed at improving the skin/implant interface of percutaneous implants with subcutaneous flanges while studying their infection vulnerability. Specifically, it was evaluated whether adding a porous coating to the percutaneous component, to the subcutaneous component, or to both components decreased the infection risk of the percutaneous implants. For the purposes of this study, the implants were not osseointegrated as our efforts were aimed at investigating infection risk of the percutaneous implants, and evaluating the interface integrity between the implant and the soft tissues. Our research objective was to study the infection susceptibility of metal percutaneous implants with smooth and porous coatings, investigating the hypothesis that *the incidence of infection of metal percutaneous devices will be lowest when both the percutaneous and the subcutaneous components have a porous coating; whereas, the incidence of infection will be highest when both the percutaneous and the subcutaneous components have a smooth surface.*

2. Materials and Methods

2.1.1 Implants—For this study, Ti6Al4V implants were fabricated at the School of Medicine Machine Shop (University of Utah, Salt Lake City, Utah USA). They consisted of two components – a cylindrical percutaneous post, measuring 10mm in diameter × 15mm in height, and a subcutaneous disk, measuring 30mm in diameter × 10mm in height (Figure 1). The percutaneous post was attached to the subcutaneous disk by a ¼"-20 threaded hole centrally located in the subcutaneous disk. The implant components had either a smooth polished surface or a porous surface coating (P^2 Thortex, Inc. Portland, OR). Scanning electron microscopy images (SEM 6100, JEOL, USA, Peabody, MA) of the porous coating on the implants determined the average pore size and porosity to be ~400µm and ~60%, respectively^{41–43}. A surface profilometer (Zygo NewView 5032, Natsume Optical Corp, Japan) was used to determine the surface microtopography of the smooth surface, with Ra (arithmetical mean roughness) and Rq (root-mean square roughness) values determined to be 0.64µm and 0.76µm, respectively.

Therefore, the following four implant combinations were investigated *in vivo*: (1) a smooth percutaneous post with a smooth subcutaneous disk (S/S), (2) smooth percutaneous post with a porous subcutaneous disk (S/P), (3) porous percutaneous post with a smooth subcutaneous disk (P/S), and (4) porous percutaneous post with a porous subcutaneous disk (P/P).

2.1.2 Passivation, Sterilization, and Endotoxin Testing—The titanium metal implants were passivated according to the ASTM F86 standard. Briefly, the implants were sonicated in distilled water for one hour, sonicated in acetone for one hour, sonicated in distilled water for one hour, and allowed to air dry. The implants were then autoclaved as routinely performed prior to surgical procedures for sterilization purposes. Following sterilization, a sampling of implants was tested using the LAL QCL-1000[®] Assay (Lonza, Walkersville, MD), according to manufacturer's directions, confirming endotoxin levels were below detection level (< 0.05 EU/ml).

2.2 Overview of Study Design

The study was conducted with an approved animal protocol from the Institutional Animal Care and Use Committee at the University of Utah. A total of eight New Zealand White Rabbits (age 5–30 months, sex indiscriminate, weight 6–9 kg) were randomly assigned to two groups. Each animal received the four implant combinations discussed above and these were surgically implanted on the animal's dorsum. The animals were allowed to heal and recover over a four-week period. Group 1 animals (n=6) then received weekly bacterial

inoculations directly to the skin/implant interface of the four implants. Group 2 (n=2) animals did not receive bacterial inoculations throughout the experiment, serving as a baseline control. The duration of bacterial inoculations was dependent on when signs of clinical infection were observed at any of the implant locations. When clinical infection was observed in one or more of the implants, the animal was euthanized and infection analysis was performed.

2.3 Animal Surgery

The day prior to surgery, a 25µg Duragesic (fentanyl) patch was placed on the animal dorsum. Pre-operatively on the day of surgery, animals were administered antibiotics (20mg/kg Cefazolin) to prevent development of infection due to the surgical procedures. The animals were then sedated (Xylazine 2mg/kg), and their backs were close-shaved. Animals were placed in ventral recumbence on a warm water blanket and secured. Anesthesia (Ketamine 20mg/kg) was administered, and throughout surgery animals received 1–5% Isoflurane in oxygen via facemask. The central portion of the dorsum was routinely scrubbed and draped for surgical procedures. Two 4-cm long incisions were made along the spine, the first, dorsal near the scapulae; the second incision was made more caudally near the iliac crest. For each incision, using blunt dissection, two subcutaneous pockets were created each lateral to the spine. The anatomical location of the implants on the animal's dorsum was randomized among animals. One subcutaneous disk was placed in each pocket. The incisions were then sutured closed (Ethicon vicryl 3-0). A small incision was made over the centrally located threaded hole in each subcutaneous disk. The percutaneous posts were then seated into the subcutaneous disks through the small incision. Animals were allowed to recover and freely mobilize following surgery. Post-operative antibiotics (20mg/kg Cefazolin) were administered twice a day for three days to prevent infection development due to the surgical procedures. No additional antibiotics were administered to the animals for the remainder of the study.

Overall animal health, implant condition, inflammation, and infection were inspected daily. Clinical infection was determined according to signs of Grade II clinical infection per Checketts et al⁴⁴. Symptoms of Grade II clinical infection included: redness of skin, swelling, discharge from implant site, pain and tenderness, temperature increase, and loss of animal appetite^{13, 44, 45}. When an implant was determined clinically infected, the animal was euthanized by initial administration of Xylazine (2mg/kg) followed by Beuthanasia (1ml/4.5kgs).

2.4 Staphylococcus aureus Inoculation

Producing a reliable infection signal of percutaneous implants can be difficult in laboratory animals due to their controlled environment and their superior ability to wound heal and fight infection^{10, 33, 40, 46–48}. Thus, an inoculation of bacteria was necessary to accurately study infection of these implants. Bacterial inoculations began after a four-week period following surgery to provide sufficient time for tissue integration and attachment to the implant. Therefore, the integrity of the dermal tissue barrier to infection could be challenged in each implant combination. Following four weeks post-op, Group 1 animals were started on a weekly inoculation of 10⁸ colony forming units (CFU) of *Staphylococcus aureus* (*S. aureus*) (ATCC# 49230, ATCC, Manassas, VA). The *S. aureus* was grown on Columbia Blood agar plates (Hardy Diagnostics, Santa Maria, CA) from a frozen stock with at least two passages prior to application⁴⁵. From colonies on the plate, a 0.5 McFarland standard was made in brain heart infusion broth with ~10% glycerol. Using a sterile pipette, a vial of the bacterial solution was applied to each implant site on each animal in Group 1 once per week. The weekly bacterial inoculations were given until signs of Grade II clinical infection manifested in any implant, at which point the animal was euthanized. The duration of the

experimental period came to completion when the last animal of Group 1 presented with infection.

2.5 Microbiology Analysis

Prior to bacterial inoculation, skin culture swabs (BBL™ CultureSwab™, Becton Dickinson, Sparks, MD) taken at each implant site recorded the baseline microbial flora on the skin. At sacrifice, skin culture swabs and soft tissue biopsies were obtained from each implant. The swabs from the skin cultures were then streaked onto Columbia blood agar plates (Hardy Diagnostics, Santa Maria, CA). For the soft tissue biopsy, a 2cm × 2cm area at the skin/post interface was scrubbed, as performed routinely prior to surgery, with alternating Povidone-iodine and 70% Ethanol scrubs. A 7-mm biopsy punch (Acu-Punch®, Acuderm Inc, Ft. Lauderdale, FL) of soft tissue was obtained from this scrubbed region. Using sterile forceps and a sterile scalpel, the specimen was placed in fastidious broth (Hardy Diagnostics, Santa Maria, CA). The specimens were sent for microbiological analysis (ARUP Laboratories, Salt Lake City, UT).

2.6 Histology

Each implant with attached soft tissue was carefully harvested from the animal after being euthanized. The specimens were fixed in 10% neutral buffered formalin, dehydrated (Tissue Tek Vacuum Infiltration Process, Miles Scientific), and embedded in methyl methacrylate according to routine laboratory procedures⁴⁹. Once polymerized, a ~4-mm thick transverse section, encompassing the entire implant with surrounding soft tissue was cut with a custom, water-cooled, high-speed, cut-off saw with a diamond-edged blade⁴⁹. These sections were then ground using a variable-speed grinding wheel (Buehler Incorporated, Lake Bluff, IL) to 50–70µm thick sections and polished to an optical finish⁴⁹.

The sections were stained with H&E. Briefly, the slides were deplasticized in xylene, then rehydrated in 100% ethanol, 95% ethanol, 80% ethanol, and distilled water. They were then placed in Mayer's Hematoxylin (Richard Allan Scientific) at 50–55°C for 10 minutes, in running distilled water for 10 minutes, and blotted dry with a Chem-Wipe. The sections were soaked in a solution of Eosin Y-Phloxine (Richard Allan Scientific) with Glacial Acetic Acid (Fisher Scientific) Solution (3:1) for 45 seconds, and then soaked in 100% ethanol for 45 seconds. The sections were evaluated under a light microscope (Nikon Macroscopic, SMZ800 1–50x, Nikon, Japan) and under a light microscope (Nikon Eclipse E600, Nikon, Japan). Digital photographs were taken (Optronics, Goleta, CA) and analyzed with commercially available imaging software (Optronics MagnaFire™ SP vs. 1.0×5, Optronics, Goleta, CA and Image-Pro® PLUS, Media Cybernetics, Inc, Bethesda, MD).

2.7 Histology Analysis

The following parameters were analyzed of the implant histological preparations: (a) extent of epidermal downgrowth as determined by the percentage of percutaneous post in contact with host tissue; (b) quantitative analysis of inflammatory cells – polymorphonuclear leukocytes (PMNs), lymphocytes, plasma cells, macrophages, foreign body giant cells (FBGCs); and (c) quantitative analysis of neovascularity (i.e. vessels measuring <100 µm in diameter).

To determine the degree of epidermal downgrowth, the length of post that was in contact with host tissue was measured and converted to a percentage based on the total length of the percutaneous post. The right and left side values were averaged and analyses were conducted.

Raw inflammatory cell count numbers were determined on both the right and left sides of the implant in the tissue adjacent to the post, tissue within the porous coating of the percutaneous post, tissue adjacent to the subcutaneous disk, and tissue within the porous coating of the subcutaneous disk. The left and right side raw cell count numbers were averaged for each section of analysis. To determine the amount of vasculature present, the blood vessels measuring less than 100 μm in diameter were counted and raw numbers were generated as described with the cell count numbers. The raw numbers for both the inflammatory cells and the blood vessels were assigned a grade from a histopathologic grading scale (Table 1), developed in accordance with a veterinary histopathologist and coauthor [Associated and Regional University Pathologists (ARUP) Laboratories, Animal Reference Pathology, Salt Lake City, UT]. The maximum grade of the four sections was used as the representative implant histopathologic grade, with which statistical analyses were performed.

2.8 Statistics

Infection data on the four implant types were graphically displayed with a Kaplan-Meier survival plot, and the infection risks of the four implant types were analyzed using the Cox Regression test (two-tailed, $p < 0.05$) (Stata/IC vs.10.1, Statacorp, College Station, TX). The histological data were analyzed with the Mann-Whitney U test (two-tailed, $p < 0.05$) (Stata/IC vs.10.1, Statacorp, College Station, TX).

3. Results

3.1 Implant Infection Time Course

During the four-week post-operative period, no clinical signs of infection of the percutaneous implants were observed in either Group 1, or Group 2 animals. Additionally, throughout the experimental period of 14 weeks, no clinical signs of infection were observed in the Group 2 animals. After 5 weeks of bacterial inoculations, Group 1 animals began to present with Grade II clinical signs of infection (Table 2). The first two infections were seen in the S/S implants, as confirmed by positive bacterial growth in the skin culture swabs, positive bacterial growth in the soft tissue biopsies, and histological evidence of granulation tissue and cellular debris. After 6 weeks of bacterial inoculations, the third infection was of a P/P implant. Following 9 weeks of bacterial inoculations, two more infections presented -- one P/S implant and the other a S/S implant. The last implant infection in the treated group developed in a S/S implant after 10 weeks of bacterial inoculations. There was no correlation found between site of implant placement and infection. Further, it was found that the wide age range and the animal gender did not have any role on infection vulnerability.

3.2 Infection Risk of the Four Percutaneous Implants with *S. aureus* Inoculations

The P/P implants had an 80% reduced risk of infection relative to the S/S implants (hazard ratio 0.19, 95% confidence, $p = 0.145$) (Figure 2). The P/S implants had a 77% reduced risk of infection relative to the S/S implants (hazard ratio 0.23, 95% confidence, $p = 0.192$). The S/P implants could not be statistically analyzed with the Cox Regression test as there were no failures or infections of these implants.

Evaluating the Kaplan-Meier plot, it was noted that the implants with a porous coating – the P/S, S/P, and P/P implants – seemed to have similar Kaplan-Meier curves with respect to the curve representing the S/S implants. A Kaplan-Meier Plot and a Cox Regression analysis was performed on the S/S implants and the implants with a porous coating (S/P, P/S, and P/P), demonstrating the S/S implants to have a significant, 7-fold increase in the risk to infection ($p = 0.022$) compared to the porous coated implants (Figure 2).

3.3 Implant Histology

Epidermal downgrowth and sinus tract formation was a common observation among all implants. Of the 26 implants histologically evaluated, 11 of the implants displayed epidermal downgrowth to the attachment point of the percutaneous post to the subcutaneous disk (Figure 3). The average percentage of post that was in contact with the tissue and the actual length of post that was in contact with the tissue was as follows for each implant: 5.81% (S/S; 0.87mm, SD 1.10), 5.06% (S/P; 0.76mm, SD 0.41), 5.56% (P/S; 0.83mm, SD 1.19), and 5.76% (P/P; 0.86mm, SD 1.00). In some cases among the S/S implants, the epidermis would migrate along and towards the perimeter of the subcutaneous disk (Figure 3). No statistical differences were found regarding epidermal downgrowth and implant type (i.e. S/S, S/P, P/S, or P/P). Accompanying the epidermal downgrowth, sinus tracts formed between the epidermis and the implant, being filled with keratin and degenerative neutrophils (Figure 3).

The porous coating on the subcutaneous disks was infiltrated with a fibrovascular tissue (Figure 4). The porous coated percutaneous posts did not display tissue infiltration to the extent of the subcutaneous disks. Rather the epidermis migrated downward yielding little to no infiltration of soft tissue into the pores of the post (Figure 4). The porous structures were filled with a mild to moderate presence of inflammatory cells, including polymorphonuclear leukocytes (histopathologic grade 2), lymphocytes (histopathologic grade 3), plasma cells (histopathologic grade 2), and macrophages (histopathologic grade 2). Multinucleated macrophages and foreign body giant cells were also observed to be present to a mild degree, corresponding to a histopathologic grade of 2 (Figure 5). Neovasculature was present to a moderate degree (histopathologic grade 3) in the pores and above the pores in the newly formed granulation tissue. Adipose tissue infiltrated the pores (histopathologic grade 3), above and below the thin fibrous capsule, and was significantly more abundant in the P/P and S/P implants than the S/S and P/S implants ($p < 0.05$) (Figure 6).

There was a fibrovascular tissue capsule surrounding the smooth subcutaneous disks with little attachment to the implant surface (Figure 7). This fibrous encapsulation was composed of organized extracellular matrix fibers that were aligned parallel to the implant surface, representative of an immature tissue that is commonly seen surrounding implanted materials⁵⁰. Similarly to the porous coated percutaneous posts, the epidermis migrated downward, and as a result, there was little epidermal and subcutaneous tissue attachment to the post (Figure 3). Evaluation of the cellular infiltrates surrounding the non-infected smooth surface implants was mainly composed of fibroblasts, lymphocytes, and PMNs. At the interface of the host tissue and the implant, there was a layer of inflammatory cells with a mild to moderate presence, including macrophages (histopathologic grade 3), FBGCs (histopathologic grade 2), PMNs (histopathologic grade 3), plasma cells (histopathologic grade 3), and lymphocytes (histopathologic grade 3). Adipose tissue infiltration or encapsulation of the smooth surface implants was minimal with a histopathologic grade of 1. Neovascularization was present to a mild degree (histopathologic grade 2).

Implants that were infected had cellular debris between the tissue and the implant, with granulation tissue surrounding the cellular debris (Figure 7).

4. Discussion

Preventing infection of osseointegrated percutaneous prostheses is absolutely necessary for clinical success. Our work investigated the infection susceptibility of metal percutaneous implants when a porous coating was added to the surface, testing the hypothesis that the *incidence of infection of metal percutaneous devices will be lowest when both the percutaneous and the subcutaneous components are porous coated on the implant; whereas,*

incidence of infection will be highest when both the percutaneous and the subcutaneous components have a smooth surface. Our aim was to determine whether or not the location of the porous coating on the percutaneous and/or subcutaneous components contributed to the incidence of infection. Our results show that when both the percutaneous and the subcutaneous components have a smooth surface they have a 7-fold increase in risk of infection compared to implants with a porous coating on one or both components. Incidence of infection is significantly reduced when the implant has a porous surface, and our results suggest that, at the least, a porous subcutaneous component is essential to decreasing this risk of infection, notably as the S/P implants did not develop infection during the study period.

Limitations to this study are twofold. First, due to the study design, we were not able to follow the infection risk of the remaining implants in an animal at the time one implant presented with infection. To do so would have required an antibiotic treatment, which would detract from the goals of the study. However, this study design allowed us to minimize any animal variation between the four implants. Ultimately, the presented results address the hypothesis regarding which implant was most vulnerable to infection and that was determined to be the S/S implants. Second, evaluations in a small animal model have inherent translational limitations as a result of skin physiological and anatomical differences between rabbits and humans. Most notably, rabbits have a panniculus carnosus, a muscle in the subcutaneous tissues, which humans do not possess. The presence of this underlying muscle in rabbits causes skin wound healing to occur more rapidly by contraction rather than by epithelialization, which is a slower healing process^{51, 52}. Despite this inherent limitation, this rabbit model provides important findings that can be applied to future studies when studying infections of percutaneous implants in animal models with skin more similar to humans.

We propose that the increased risk of infection with the smooth surface implants is due to the lack of attachment of the host tissue to the smooth surface. The result is an incomplete barrier to preventing microbial migration and subsequent infection. Smooth surface implants have less surface area for tissue attachment, and the fibrous capsule which develops around the surface allows the implants to move within the soft tissue making it difficult for any soft tissue seal to form⁵³. It is generally believed that any “mechanical irritation” produced by stress and movement at an implant interface yields a thicker fibrous capsule formation^{31, 53}, which was observed in this study. The absence of soft tissue attachment to the smooth surfaces was seen during the histological processing as the tissue would separate from the implant; whereas, this was not observed with the porous implants. It should be noted that the histological processing with PMMA embedment can cause the tissue to separate from the implant surface. However, this detachment was much less obvious, or not observed with the porous coated implants. The separation between the soft tissue and the smooth surface implants can be attributed to an absence of an initial attachment or integration formed *in vivo*.

The surface topography of implants greatly influences the tissue response and subsequent attachment to the implant, which in turn affects the implant’s vulnerability to infection. As seen in previous studies, tissue attachment to subcutaneous implants is increased when the implant is roughened or porous coated^{10, 31, 53–55}. In a study performed by Kim and colleagues, surfaces with varying roughness or grooves resulted in greater tissue attachment and less fibrous capsule formation compared to the smooth surfaces, which had a thick fibrous capsule formation, similar to the presented results. Tissue integration with a rough or porous surface is a barrier to infecting microorganisms. This has been shown in work by Merritt and colleagues²², who demonstrated that once subcutaneous porous implants were invaded with tissue, they were less susceptible to infection than an implant with a smooth

surface²². Unlike the implants we investigated, the implants in the work performed by Merritt and colleagues²² were subcutaneous with no percutaneous components. Our work found that, after four weeks post-op, infection vulnerability of porous implants was significantly decreased compared to smooth surface implants. This supports the results of Merritt et al²² in that smooth surface implants are more vulnerable to infection at later time points (i.e. at least 4 weeks post-op). The porous coated surfaces allow for tissue integration and attachment, providing a barrier to migrating microorganisms and a mechanical “lock” of the tissue with the device, thus limiting any movement or micromotion of the implant in the tissue that would weaken the attachment.

This study demonstrates that percutaneous implants with a smooth surface are at a significantly increased risk of infection compared to percutaneous implants with a porous coated surface. These results highlight the importance of soft tissue integration with the porous implant as being a barrier to invading microorganisms, and demonstrate the ineffectiveness of a smooth polished surface to allow for soft tissue attachment. Though the porous coating was effective in reducing the risk of infection, it was not completely effective in altogether eliminating the risk of infection. In light of the timing of clinical infections of percutaneous osseointegrated prosthetics, it is reported that these prosthetics present with infection on average three years after surgical implantation¹⁴. It is difficult to translate the timing of onset of infection with our results and with what is seen in the clinic as our study is an example of a more extreme bacteria-challenged environment, with the average of seven weeks before infection was observed. Thus our results further demonstrate that S/S implants in an excessive bacterial environment will develop infection more frequently than an implant with a porous coating. Further strategies evaluating implant designs and incorporating biologics into the implants and in the adjacent tissues will be necessary to initiate and successfully produce a healthy, permanent integration between the implant and the host tissue.

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Figure 1.

Porous coated and smooth surface percutaneous implants. (A and B) Percutaneous implants consisted of two components – a subcutaneous disk and a percutaneous post – that had a porous coating (A) or a smooth surface (B). The percutaneous post was attached to the subcutaneous disk through the central threaded hole. (C) Scanning electron microscope (SEM) image of the porous coating demonstrating the porosity to be ~60% and the pore size to be ~400μm (accelerating voltage: 20.0 kV; magnification: 65). (D) SEM image of smooth surface titanium (voltage: 20.0 kV; magnification: 65).

Figure 2. Kaplan-Meier survival estimates of the percutaneous implants over time. (A) Implants plotted independently to each other ($p > 0.05$). (B) S/S implants compared to implants with a porous coating (P/P, P/S, and S/P) ($p = 0.022^*$).

Figure 3.

Epidermal downgrowth with a sinus tract at the implant interface was observed in the majority of implant specimens. (A) Epidermis (black arrow) migrated down along the post (right black strip). (B) Epidermal downgrowth (black arrow) that continued to the point where the post (left side of photo) attached to the subcutaneous disk (black portion in bottom of photo). The sinus tract was filled with degenerative neutrophils (N) and keratin (K). (C) The epidermis migrated down the percutaneous post (black strip on right of photo) and continued to migrate adjacent to subcutaneous disk (black strip on bottom of photo). Note the absence of soft tissue attachment to smooth surface of implant. All 4× original magnification.

Figure 4. Tissue reaction to porous coated implants. (A) Vasculature, fibroblasts, and scattered inflammatory cells were observed within the porous structures and found lining the metal surface. 10× original magnification. (B) A macroscopic view of the implant demonstrates the downward migration of the epidermis (black arrow). The percutaneous post (P) with a porous coating (PC) is on the left and the subcutaneous disk (D) with a porous coating (PC) is at the bottom of the photo. 2× original magnification.

Figure 5. Cellular infiltrates and vascularity within and around the porous structures. Macrophages and foreign body giant cells lined the implant surface (black). During histological processing, some of the porous coating was removed (M), allowing a clear view of the cells lining the implant surface. 10× original magnification.

Figure 6. Adipose tissue infiltration into porous structures. Note the adipose tissue above and below the thin fibrous capsule. 4× original magnification.

Figure 7. Tissue reaction to smooth surface implants. (A) Fibrous capsule surrounding a smooth surface. (B) Cellular debris (D) and granulation tissue (G) in a clinically infected implant. All 4× original magnification.

Histopathologic criteria with corresponding grades for implant histology analysis. Polymorphonuclear leukocytes (PMN's), lymphocytes, plasma cells, macrophages, foreign body giant cells (FBGC's), percentage of adipose tissue coverage, and vasculature were counted per area on each histology section. The percentage of adipose tissue coverage refers to the percentage of area on the subcutaneous disk that was infiltrated with adipose tissue. Vasculature included blood vessels <100 µm in diameter.

Table 1

	PMN's	Lymphocytes	Plasma Cells	Macrophages	FBGC's	Adipose Tissue Coverage (%)	Vasculature
Histopathologic Grade	0	0	0	0	0	0	0
	1	1-10	1-30	1-10	1-3	1-20	1-20
	2	11-20	31-99	11-30	4-9	21-50	21-60
	3	21-40	91-270	31-90	10-27	51-80	61-180
	4	> 40	> 270	> 90	> 27	81-100	> 180

Summary of percutaneous implant infections. Treated and untreated animals are represented in rows with corresponding implants in columns. The number of weeks is the number of bacterial inoculation weeks until clinical infection presented.

Table 2

	S/S	S/P	P/S	P/P	Biopsy Bacterium of Infected Implant
Treated_A	5 weeks				<i>S. aureus</i> /SCN
Treated_B	5 weeks		NA		<i>S. aureus</i> /SCN
Treated_C			NA	6 weeks	<i>S. aureus</i>
Treated_D	9 weeks				<i>S. aureus</i>
Treated_E	NA		9 weeks		<i>S. aureus</i>
Treated_F	10 weeks				<i>S. aureus</i>
Total # of Infections of Treated Implants	4 of 5	0 of 6	1 of 4	1 of 6	
Untreated_G					No infections
Untreated_H					No infections
Total # of Infections of Untreated Implants	0 of 2	0 of 2	0 of 2	0 of 2	

CNS – Coagulase negative *Staphylococcus aureus*.
NA - implant removed from study due to percutaneous post missing during study duration