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Pig dorsum model for examining impaired wound healing at the skin-implant interface of percutaneous devices

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Abstract

Percutaneous medical devices are indispensable in contemporary clinical practice, but the associated incidence of low to moderate mortality infections represents a significant economic and personal cost to patients and healthcare providers. Percutaneous osseointegrated prosthetics also suffer from a similar risk of infection, limiting their clinical acceptance and usage in patients with limb loss. We hypothesized that transepidermal water loss management (TEWL) at the skin-implant interface may improve and maintain a stable skin-to-implant interface. In this study, skin reactions in a 3-month, pig dorsum model were assessed using standard histology, immunohistochemistry, and quantitative image analysis. Immunohistochemical analysis of peri-implant tissue explants showed evidence of: continuous healing (cytokeratin 6+), hypergranulation tissue (procollagen+), hyper-vascularity (Collagen 4+), and the presence of fibrocytes (CD45+ and procollagen type 1+). Importantly, the gross skin response was correlated to a previous load-bearing percutaneous osseointegrated prosthetic sheep study conducted in our lab. The skin responses of the two models indicated a potentially shared mechanism of wound healing behavior at the skin-implant interface. Although TEWL management did not reduce skin migration at the skin-implant interface, the correlation of qualitative and quantitative measures validated the pig dorsum model as a high-throughput platform for translational science based percutaneous interface investigations in the future.

Keywords

skin regression; wound healing; transepidermal water loss; implant interface

Introduction

Percutaneous medical devices are indispensable in contemporary clinical practice but the associated incidence of low to moderate mortality infections represent a significant economic and personal cost to patients in acute and long-term clinical care settings [1, 2, 3]. In 2002, there were an estimated 1.7 million healthcare-associated infections (HAIs) in the United States [4]. The majority of these HAIs were attributed to the placement and/or

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Conflict of Interest

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maintenance of bladder/urinary and central venous catheters [4]. Reductions in patient- and catheterization-related risk factors and the implementation of catheter management guidelines have improved outcomes, but do not address the inherent risk of infection associated with the skin-implant interface [5, 6, 7]. Unfortunately, none of these strategies supplant the protection provided by a stable, physiologic skin-implant interface barrier. Therefore, the most efficacious means of limiting the incidence of percutaneous device related HAIs remains the biomimetic and/or biomechanical optimization of the given device's design to foster an idealized, permanent skin seal at the skin-to-interface. For decades this rationale has motivated a significant number of translational and clinical, percutaneous device design related studies [8, 9, 10, 11, 12]. More recently, percutaneous osseointegrated (OI) prosthetic (POP) systems, a new class of upper- and lower-limb orthopaedic prosthetic percutaneous devices intended for civilian amputees, have begun to garner much of the field's research focus [13, 14].

There are approximately 185,000 amputations in the United States annually [15]. Independent of the upper- or lower-extremity amputations, many of these patients will suffer multiple dermatological and/or infection-related complications over the remainder of their post-amputation, prosthetic socket wearing life-time [16, 17, 18]. The clinical, biological, and socio-behavioral challenges presented by traditional prosthetic socket systems has fostered the development of an alternative upper- and lower-limb, POP docking systems in Europe [19, 20, 21, 22].

Retrospective European case studies would suggest POP systems provide improved proprioception and gait efficiency, but these systems possess their own inherent limitations [23, 24]. The chief limitations of POP systems are superficial (i.e. skin-implant interface) and deep-bone (i.e. osteomyelitis) infection rates of 18 and 30%, respectively, and continuous cutaneous regression (i.e. migration) at the skin-implant interface [23, 25]. Although the implantation procedure of POP systems varies due to differences in design specifications amongst the various investigating groups, the basis of all of the procedures requires the creation of a percutaneous access point, through which a percutaneous post will pass for exo-prosthetic attachment. The creation of this percutaneous access point creates an opening through which internal biological fluids may contact and interact with extracorporeal materials or antagonists. The rates of infection and soft-tissue adaptation observed at the skin-implant interface of POP systems are not too dissimilar in causality (e.g. inflammation mediators, soft-tissue stiffness discontinuities) and outcomes (e.g. wound bed cytokine concentrations, wound bed cellular constituencies, wound bed cellular densities) to the biomechanical and biochemical microenvironments reported in the literature of normal and pathological wound healing processes [26, 27, 28, 29]. Consequently, strategies intended to improve normal and/or pathological wound healing outcomes may be beneficial for maintaining the skin-implant interface of POP systems.

Wound care strategies focused on altering the gross and cellular-level behavior of acute and/or chronic wounds have motivated a significant amount of research [30]. The wound bed of acute and chronic wounds involves a well - orchestrated and finely balanced milieu of proteins, cytokines and chemokines that ensure a timely return to local and gross homeostasis. Dynamic reciprocity and wound bed redox signaling are essential components of a healthy, healing wound bed [31, 32, 33, 34]. Redox signaling, and in particular the roles of reactive oxygen species (ROS)/free radicals, free radical quenchers/antioxidants (e.g. nitric oxide [NO]), and O₂-dependent cellular processes, are believed to be critical interconnected processes in acute and chronic wound environments [32, 33, 34, 35]. The synergistic activity of each these processes have marked effects on intra-wound bed protein synthesis and degradation, as well as cytokine cascade up- or down-regulation [32, 33, 34]. Therefore, wound care strategies intended to alter or manage oxygen partial pressure [pO₂]

(a surrogate for exogenous oxygen infiltration and exchange in the wound bed, and redox signaling) at the wound site-extracorporeal interface provide a means of quickly improving wound healing outcomes. Specifically, transepidermal water loss (TEWL) management has been shown to cause advantageous phenotypic and morphological changes in acute and chronic wound beds, leading to accelerated, positive wound closure outcomes [36]. Similar to acute and chronic open wounds, redox signaling at the three(3) point junction (i.e. environment, skin, and implant) of the skin-implant interface of percutaneous devices may also play a critical role in wound healing outcomes. Therefore, the application of a TEWL or a similar set of techniques to a percutaneous system may be propitious in establishing or maintaining a stable, well-integrated skin-implant interface.

Despite the potential benefit of TEWL or similar approaches, current translational POP models are either too biomechanically aggressive, too dissimilar in physiological behavior, or cost-prohibitive to assess the efficacy of these approaches in a rigorous scientific model [37, 38, 39]. The ideal model would allow for multiple implantation sites to study various implant designs and treatment conditions simultaneously, while also reproducing the complex biochemical and biomechanical micro-environment of the percutaneous, skin-implant interfaces of load-bearing, human POP systems [40]. *In vivo* pig dorsum models address most of these parameters favorably, exhibiting significant biochemical and morphological similarities to human, cutaneous wound healing processes [41]. Steinstraesser *et al.* developed a pig dorsum-based, wound chamber model for the investigation of dermal wound-bed infections [42]. This type of model allowed in-phase motion with underlying skeletal structures during vertebral flexion and extension. Although differing in magnitude, the cyclic nature of the relative motion at the skin-implant interface mimicked the soft-tissue loading conditions/modes observed in human POP systems and load-bearing, bone-anchored, percutaneous implant models [39]. Therefore, the development of a similar percutaneous implant, pig dorsum model may be particularly advantageous for the translational study of percutaneous device designs intended to: 1) improve skin-implant interface longevity through the creation of a stable skin-implant interface, and 2) examine percutaneous interface wound-site management techniques (e.g. TEWL).

Established clinical wound management techniques (e.g. moist/wet-dressings or occlusive dressings) may be beneficial in the resolution of the challenges presented by skin-implant interfaces of percutaneous medical devices, and explicitly POP systems [36, 43, 44]. The introduction of those techniques into percutaneous device related translational studies may be particularly advantageous for furthering the clinical understanding of the mechanisms, which underlie adaptive wound healing processes at the skin-implant interface. Furthermore, one would expect that due to the similarities in human and pig skin and wound behavior, the regenerative and restorative capacity of these wound care techniques would facilitate similar positive wound closure outcomes in translational studies. Therefore, in the following pilot study, we hypothesized that TEWL management of the skin-implant interface of a simulated percutaneous osseointegrated prosthetic would help to improve and maintain a stable skin seal in an adapted pig dorsum model. The objectives of the analyses were two-fold. First, the qualitative and quantitative results of this study were compared to the results of a previously conducted load-bearing, POP sheep model for the evaluation and validation of a pig dorsum, percutaneous device model as a high throughput analogy for the study of the skin-implant interface behavior in POP systems. Second, the analyses and measurements were used to assess the influence of TEWL management in fostering improved wound closure outcomes, lengthening skin-implant interface survival, activating differing wound bed cell types, and reducing cutaneous regression.

Materials and Methods

Implant Fabrication and Coating

Two porous titanium coated sub-dermal fixation surfaces were fabricated; solid (Figure 1a, left) and perforated surfaces (Figure 1a, right), respectively. Percutaneous implants included two major design components: 1) percutaneous post, and 2) sub-dermal fixation surface (Figure 1b, top to bottom). The sub-dermal fixation surfaces and lower portions of the percutaneous posts of each device type were coated with 1mm thick, commercially pure titanium (Ti) porous coating (donated by Thortex Corp., Portland, OR). The perforated surfaces were machine drilled with approximately 50, 1.5 mm diameter through-holes prior to Ti porous coating. In accordance with ASTM standard B600-91, all implants were cleaned, passivated, and sterilized prior to surgical implantation.

Animal Model

Animals—In accordance with a University of Utah Institutional Animal Care and Use Committee (IACUC)-approved protocol, two 3-month old, Yucatan micro-pigs were obtained for inclusion in an adapted pig dorsum model [42]. Following a concurrent quarantine and acclimatization period, the animals were housed in a 12:12hr light cycle room with segregated, climate controlled (20-25 °C, 30% humidity) pens in accordance with AALAS standards. Animals were fed a standard porcine feed for the entirety of the study. At the conclusion of the study, the animals were euthanized accordance to American Veterinary Medical Association standards by intravenous injection of Beuthanasia D® (including pentobarbital and phenytoin derivatives) at 1 mL/4.5 kg (10 lbs) body weight dosage.

Surgical Procedure—In accordance with a University of Utah IACUC-approved protocol, the dorsum of each animal was shaved and aseptically prepared for surgery. After the aseptic surgical scrub, the cranial (superior) and caudal (inferior) boundaries of the surgical field were marked to create an implantation grid. The first percutaneous exit-site was marked 30 mm inferior to the cranial boundary and 30 mm lateral to the mid-line (Figure 1c and 1d). Ipsi-lateral implantation sites were staggered along the same sagittal line, 40 mm apart. Contralateral implantation sites were created in a similar fashion, beginning 50 mm from the cranial boundary. Two bi-lateral, semicircular subcutaneous pockets were incised by blunt dissection from a single dorsal midline incision. Percutaneous implant exit-sites were created using a six-millimeter biopsy punch. Sub-dermal fixation surface type and implantation site pairs were decided at random (selected by a member of the surgical staff not connected to the study). Following closure, the entire surgical field was dressed with sterile gauze and covered with stockinette. The animals were allowed to ambulate freely for the entirety of the 3 months. The animals were cleaned with a mild detergent and their dressings changed weekly for the two weeks immediately following implantation, after which the animals were cleaned weekly and the stockinette changed.

Tran-Epidermal Water Loss (TEWL) Management—Following weekly cleaning, the treatment animal (G1) received one dollop (approximately the size of a finger-tip) of Vaseline® per implant site, spread gently by gloved finger-tip. A new, gloved finger-tip was used to administer the Vaseline® to each site. The untreated animal (G2) received no Vaseline®. Administration of Vaseline® began one week post-implantation and continued for the entirety of the 12 week study. The degrees of inflammation, redness, discharge/exudate, and infection were graded using a modified 5-tier, Holgers grading metric [45]. Skin-implant interface sites graded 3 or higher were referred to the husbandry staff for veterinary consultation. Per protocol, Grade 5 skin-implant interface sites would require immediate veterinary consultation, implant removal, and antibiotic treatment or

euthanization. At necropsy, or when infection was noted, culture swabs were collected from a site distal to the implant to characterize normal skin flora and at the skin–implant interface to identify the potential pathogens for periprosthetic infection. As described in our previous publication [46], a semi-quantitative analysis was undertaken to determine the degree of bacterial colonization.

Qualitative Measurements

Histological Sample Preparation—Excised soft tissue-implant interface samples were fixed in 1:10 buffered formalin, then infiltrated (Tissue Tek Vacuum Infiltration Processor, Miles Scientific, Princeton, MN) and embedded in poly(methyl)methacrylate (PMMA) using an established technique [47]. After embedment, sections were cut using a diamond-blade precision saw (Buehler Isomet® 100, Lake Bluff, IL). Sections were then ground and polished to an optical finish (i.e. ~50 µm thickness sections), stained with Hematoxylin and Eosin (H&E), and examined microscopically. Photomicrographs were quantitatively analyzed using ImageJ (version 1.44p, National Institutes of Health, Washington DC). Photoshop® (Adobe Systems Inc., San Jose, CA) was used to create publication quality figures.

Immunohistochemical Staining—Primary antibodies purchased for this study included: CD45 (rabbit, 1:200, Abcam (ab10559) Cambridge, MA), cytokeratin 6 (K6) (mouse, 1:200, Abcam (ab18586) Cambridge, MA), procollagen type 1 (rat, 1:100, Abcam (ab64409) Cambridge, MA), and collagen IV (rabbit, 1:200, Abcam (ab6586) Cambridge, MA). Secondary antibodies used in this study included: donkey anti-rabbit [FITC] (CD45 and collagen IV) (1:200, Thermo Scientific (Pierce) Rockford, IL); goat anti-mouse (K6) (1:1000, Abcam (ab98640) Cambridge, MA); and goat anti-rat (procoll) (1:500, Abcam (ab98387), Cambridge, MA). Co-localization of CD45+ and pro-collagen+ labeling was used to identify fibrocytes.

Sagittal tissue samples, including the skin-implant interface, were OCT-embedded and flash frozen in liquid nitrogen for –80°C storage and subsequent immunohistochemical analysis. In general, sections were thawed at room temperature for 30 minutes, encircled with liquid blocker (Electron Microscopy Services, Hatfield, PA), and then placed in –20°C acetone (Sigma-Aldrich, St. Louis, MO) for 7 minutes. The acetone was allowed to evaporate under ambient conditions and the sections were then rehydrated in two, 10-minute Tris-buffered saline (Thermo Scientific, Rockford, IL) washes. Samples were incubated with Tris-diluted primary antibodies and then washed in an additional Tris-buffered saline treatment. Primary antibody incubation temperatures and times varied for each label. Secondary antibodies were applied to the sections for 30 minutes and then washed by immersing in buffered saline for an additional 20 minutes. Following secondary labeling, the sections were dried and 100 µL of DAPI-Fluoromount-G (Southern Biotech, Birmingham, AL) applied to each prepared section before being covered for visualization. Stained sections were imaged using a Nikon A1, confocal microscope (Nikon Instruments Inc., Melville, NY). Confocal images were quantitatively analyzed using ImageJ (version 1.44p, National Institutes of Health, Washington DC). Photoshop® (Adobe Systems Inc., San Jose, CA) was used to create publication quality figures.

Quantitative Measurements

Wound Margin Length and Migration Kinetic—Four-times (4×) magnification photomicrographic composite images of animals from groups G1 (n=6 implants) and G2 (n=6 implants) were examined to locate the epithelial junction using a software based image analysis program (Image-Pro Plus, Media Cybernetics Inc., Silver Springs, MD). Pixel-based measurement traces of the wound margin began at the point of epidermal inversion/

downturn and continued to the epidermal junction identified previously (Figures 2a and 2b, *downward arc*). A mean epidermal wound margin was calculated for each implant using the “right” and “left” margins of the relevant H&E cross-section. The mean 3-month wound margin length and wound margin migration kinetic from groups G1 and G2 were then compared for analysis. Measurements were made three times each, for each implant by blinded members of the research group to limit observer bias.

Fixation Surface Coverage and Gross Cutaneous Regression—The percent of dermal tissue attachment to the sub-dermal fixation surface and the gross cutaneous regression rate were calculated using a computer-based measuring tool (ImageJ, version 1.44p, National Institutes of Health, Washington DC). The amount of dermal tissue attachment to the sub-dermal fixation surface in G1 and G2 animals was expressed as the ratio of soft-tissue integrated into the fixation surface to the total length of the fixation surface exposed to soft-tissue immediately post-implantation (i.e. Time “0”). Mean percentages of covered fixation surfaces were calculated from groups G1 and G2 and compared. In addition, the millimeter per month rate of gross cutaneous regression was calculated utilizing the length measures captured above. Measurements were made three times each, for each implant by blinded members of the research group to limit observer bias.

Vascular Quantification—The density of vascularity was used as a surrogate measure for wound healing activity. The average fluorescent signal intensities were calculated for the pre-determined fixed areas (5 × 5 mm) within the granulation bed and healthy cutaneous tissue. The data are expressed as area fractions.

Statistical Analysis—To determine whether the researchers applied the same criteria for scoring/measuring the wound margin length and fixation surface coverage, an inter-rater reliability coefficient was calculated. Determining the degree of inter-rater reliability would validate the use of their measurements for statistical analysis. The inter-rater reliability coefficient for the wound margin length and fixation surface coverage was $r = 0.96$. The high degree of reliability justified the use of the average of the three raters’ scores as the outcome variables for subsequent statistical analysis.

Post hoc statistical comparison analyses were conducted for a sample size of one (1) animal per study group. Within each animal there were six positions on the dorsum of each pig. Therefore, the implant position was ‘nested’ within each animal. To correctly model the lack of independence of position on the dorsum of the animal, a generalized estimating equation, linear regression model was fit to the data. The regression model specified an autoregressive correlation structure, where caudal positions were less correlated than cranial positions. Comparative differences were assumed statistically significant for p -values < 0.05. All numerical values and graphic presentations of data are presented as mean ± standard error of the mean (SEM). Where appropriate, 95% confidence intervals are also presented.

Results

Gross Skin Morphology, Adaptation, and Microbiology

Photomicrographs of the skin-implant interfaces of treated (G1) and untreated (G2) animals showed clear evidence of a soft-tissue adaptation to the *in situ* porous titanium coated substrates (Figure 2). Three distinct, concentric regions of epidermal-dermal response were identified (Figure 2) within the peri-implant soft-tissues (proximal to distal): 1) an interface region (*); 2) a transition region (**); and 3) a stress-absorbance region (***). The

“interface” region possessed a thin (~1-3 cells), migratory epidermis (K6+), and a wound bed of hyper-cellular (DAPI density), hypervascular (Coll-4+) granulation tissue. The granulation tissue obstructed epidermal contact with the papillary dermis in this region, consistent with an actively healing wound bed (Figures 2a and 2b). In addition, the migratory epithelial edge/wound margin was much longer in the treated group ($2813 \pm 219 \mu\text{m}$), than in the untreated group ($1400 \pm 108 \mu\text{m}$), suggesting TEWL management induces a more active, migratory keratinocyte phenotype (Figure 4). The transition region possessed a comparatively thicker epidermis than that of the interface region, although the increase in stratum thickness did not translate into terminal differentiation (i.e. denucleation and cornification) of the keratinocytes of this region. Similar to the sub-epithelial tissue of the interface region, the granulation tissue underlying the transition region was also hyper-cellular and hypervascular. The comparative difference in area fractions of Coll-4+ staining between skin proximal (i.e. granulation tissue region) and distal (i.e. unwounded tissue) was $19 \pm 10\%$ and $3 \pm 2\%$, respectively; again indicative of an actively healing wound bed (Figures 2a and 2b, demarcated by *dashed* line and basal epidermal surface). Finally, the outermost region, the stress-absorbance region was characterized by epidermal hyperplasticity and the development of irregular rete ridge-like structures (Figure 2a and 2b). Superficial keratinocytes of this region were denucleated and cornified, indicating keratinocyte differentiation. Independent of treatment condition, there was no discernible granulation tissue underlying this region. On the distal edge of the stress-absorbance region, skin super-structure normalized to a characteristic, homeostatic/unwounded archetype.

The deleterious effects of infectious pathogens/biofilms on the wound healing processes at the skin-implant interface were also considered. During the course of the study, no implant wound site received a Holgers Grade of 4 or greater. The skin responses described by the Holgers grades observed during this study are consistent with other percutaneous implant related studies [39, 45]. All skin-implant interfaces (100%) were colonized with normal skin flora (at both, post-surgery and post-euthanasia). Those environmental organisms included *coagulase negative Staphylococcus*, and *Staphylococcus aureus*, *enterococcus*, *bacillus* sp. and *diphtheroid*. However, none of the sites showed clinical indications of infection at the time of the euthanasia. Therefore, at the 3-month time-point it was difficult to determine the opportunistic role of these species in inducing infection-mediated edema or inflammation at the skin-implant interface. More specifically, it was difficult to determine the degree to which infection-mediated edema or inflammation did or did not negatively affect the gross wound healing behavior at the skin-implant interface.

Wound Margin Length and Skin Regression Kinetics

At 12 weeks post-implantation, the skin nearest the percutaneous interface showed evidence of on-going proximal regression in both the treated and untreated groups (Figures 3a and 3b, respectively). The mean wound margin length and rate of wound margin growth/regression were $6.02 \pm 0.96 \text{ mm}$ and $2.01 \pm 0.32 \text{ mm/month}$ (Figure 3c) for the treated group (G1), and $1.74 \pm 0.22 \text{ mm}$ and $0.57 \pm 0.07 \text{ mm/month}$ for the untreated group (G2) (Figure 3c), respectively. Comparatively, the treated group elicited a wound margin growth/epidermal regression rate nearly four (4) times the response seen in the untreated group.

Fixation Surface Coverage and Gross Cutaneous Regression

Percent sub-epithelial, and/or dermal, attachment to sub-dermal fixation surfaces, measured as the ratio of sub-dermal fixation surface coverage, was reduced in the treatment group (Figure 3d). At 3-months post-implantation, approximately $36.5 \pm 3.3\%$ of the G1 (treated) surfaces were covered by sub-epithelial soft-tissue. Over the same time-period, the percentage of G2 (untreated) surface coverage was approximately $77.8 \pm 1.8\%$. Comparatively, G2 (untreated) implants showed 41% more surface coverage than G1

(treated) implant surfaces (95% CI: 35, 48; $p < 0.001$). The rates of gross cutaneous regression for G1 and G2 implants were 2.76 ± 0.29 mm/month and 0.85 ± 0.10 mm/month, respectively. The comparative difference in surface coverage after 3 months implantation between groups G2 and G1 suggests Vaseline® based TEWL management may be contraindicated for the maintenance of the skin-implant interface of percutaneous devices.

Skin-Implant Interface Wound Healing Response

Immunohistochemical analysis of the skin-implant interface of the model POP sub-dermal fixation surface showed the development of a hypergranulated, hypervascular peri-implant soft tissue region and the presence of fibrocyte/fibrocyte-like cells (Figure 4). When comparing histological samples from the G1 and G2 animals, qualitative increases in collagen 3 (Coll-3+) (i.e. collagen matrix deposited early in the wound healing process) and collagen 4 (Coll-4+) (i.e. deposited during basement membrane maturation) expressions were observed. Although not quantified, these comparative increases suggested treatment-dependent increases in cellular density, and potentially increases in activity within the peri-implant granulation tissue proximal to the skin implant interface (Figure 4, area denoted by (#) and (*)). In addition, a qualitative increase in keratin 6 (K6+) expressions, between treated and untreated implants, indicated a highly active biochemical and bioactive epithelial response indicative of a migratory epithelium and migratory keratinocyte phenotype common to the cutaneous wound healing response [48]. The histological juxtaposition of G1 implants to G2 implants suggested the G1 skin-implant interfaces and peri-implant granulation tissue regions were markedly more active, 3-months post-implantation. In addition, co-localization of CD45+ and pro-collagen+ cells suggested the presence of fibrocytes in the peri-implant soft-tissues of both G1 and G2 implants (Figure 5).

Discussion

Development of Pig Dorsum Model for Skin-Implant Interface Characterization

The inherent clinical and translational effectiveness of a given *in vivo* model rests on the ability of that model to reproduce the physiology and pathophysiology observed in the corresponding human conditions. The cutaneous behavior of porcine models is known to be ideal for wound healing related studies, but to-date, no load-bearing, POP studies have been conducted in pigs due in large part to skeletal limitations. A single-stage, amputation-implantation load-bearing POP sheep model developed by our group has provided critical information about bone attachment to the porous coated, press-fit titanium endo-implants [39, 49]. The sheep model was limited in its capacity to describe the mechanisms of soft-tissue adaptation and healing responses, because of the lack of intra-animal controls (i.e. one implant per animal). When comparing the previous sheep and the current pig dorsum models, there are distinct similarities in soft-tissue morphology and regression kinetics over a 3-month implantation period despite the absence of direct skeletal load-bearing in the pig model (Figures 6a and 6b) [49]. Skin regression kinetics for the untreated pig dorsum percutaneous device and sheep model implants were 0.84 ± 0.11 mm/month and 0.67 ± 0.11 mm/month, respectively. This data correlates with sub-epithelial soft-tissue/dermal attachment data from the untreated pig dorsum and sheep models, $77.8 \pm 1.8\%$ and $86.2 \pm 2.6\%$, respectively. The comparison of quantitative measures would suggest that the sheep and untreated pig dorsum percutaneous device models shared a similar mechanism of cutaneous regression, further indicating a potentially analogous physiological condition. Consequently, the investigators believe the soft-tissue behavior exhibited in both models may share a common cause and effect modality, a belief further supported by the qualitative comparison of the two models.

As in the sheep study, the global soft-tissue reaction suggested a coordinated, epidermal-dermal response. Further data alignment can be seen in the development of the three contiguous, cutaneous response regions (the interface, transition, and stress absorbance regions, Figure 2). Similarities in time-dependent skin response support the potentiality of a shared underlying mechanism of soft-tissue behavior that transcends purely host-cell/tissue-biomaterial interactions. The investigators believe that one of the chief antagonists of the aberrant wound healing observed in both studies was the relative motion at the skin-implant interface and the host-tissue's attempts to resolve those complex loading conditions. The added qualitative and quantitative measures availed the pig dorsum percutaneous implant model allowed for further, in-depth investigation of the biochemical and biomechanical participants of this extremely complex, macro- and micro-wound healing environment. Ultimately, the similarities between the load-bearing, POP sheep model and the pig dorsum model appeared to validate the development and use of this approach as a more economical, high-throughput method for evaluating percutaneous interface design features. On-going pig dorsum models with biomimetic surfaces may help to better characterize and describe the soft-tissue responses seen here and in other percutaneous systems.

Effect of Transepidermal Water Loss (TEWL) Management on Peri-Implant Tissue Behavior

The influence of TEWL management and mechanical microenvironmental stability on positive wound closure outcomes is well-established. However, the utility of similar approaches for the percutaneous device skin-implant interface is not well-defined. Although the pO_2 and redox signaling products of the skin-implant interfaces of this study were not measured explicitly, the global effects of TEWL management on the skin-implant interface of percutaneous devices in this pig dorsum model showed potentially deleterious gross tissue responses (i.e. inflammation and peri-implant hyperplasticity) for percutaneous implant survival, disproving the hypothesis.

Gallant-Behm *et al.* found that the inclusion of occlusive dressings for TEWL management effected pro- and anti-fibrotic epidermal signaling through a change in keratinocyte proliferation and migration in an *in vivo*, rabbit hypertrophic scar (HS) model [44]. The cumulative effect of the concomitant responses was a reduction in dermal fibrosis and HS formation. In the study presented here, alteration of the air-liquid boundary led to a similar amplification of keratinocyte proliferation and migration; as evidenced by epidermal hyperplasia and quantitatively by the increased length of the migrating wound margin (i.e. migration rate of the skin-implant interface) when compared to untreated implants. Both results were indicative of continuous wound healing. Furthermore, when combined with sub-epithelial tissue attachment data, the resultant trend would suggest gross cutaneous regression. The investigators believe coordinated epithelial and sub-epithelial fixation may be critical for long-term percutaneous device survival in patients.

Understanding the perceived roles of the epithelium and the cells of the epithelium in proximal skin migration around percutaneous implants is of critical importance for the promotion of *in situ* percutaneous device longevity. The effect of Vaseline® based TEWL management on the epithelium, and specifically keratinocytes, caused qualitative shifts in keratinocyte phenotype (K6+), wound-site hypergranulation and fibroplasia (procoll-1+), hypervascularization (Coll-4+), and wound bed/interface cellular population densities (DAPI density). These accelerated wound healing effects would be expected following TEWL management. The evidence of accelerated wound healing processes supports the role of continuous, proximal skin migration (an attempt to reestablish a homeostatic/uninterrupted epithelium) as the chief mediator of percutaneous device failure.

Many of these proteins, specifically K6, have been characterized in epidermal-driven normal and/or pathologic wound healing situations [28, 50, 51, 52]. These TEWL-mediated,

histomorphometric responses would suggest a predominant effector role for keratinocyte and fibroblast/fibroblast-like cell derived wound healing modalities [27, 36, 51, 53, 54, 55]. Consequently, identifying cell types and populations with evolved mechanotransductive and mechanoactive structures may be essential for further characterization of the peri-implant interface wound healing micro-environment.

Identification of Fibrocytes in Peri-implant Soft-tissues

Fibroblasts and myofibroblasts are the most common cell types associated with normal and/or pathological wound healing scenarios, but recent literature would suggest fibrocytes may also play a role in fibrotic pathologies [56, 57, 58, 59]. Fibrocytes are described as a circulating peripheral blood-derived progenitor cell population that exhibit characteristics of both fibroblasts and macrophages via the expression of extracellular matrix proteins and pro-inflammatory and pro-angiogenic cytokines [57]. Despite accounting for approximately 0.5% of circulating non-erythrocytes, fibrocytes have been suggested to influence the behavior of homeostatic, pro-inflammatory, pro-fibrotic, pathophysiological processes via the expression of numerous cytokines and chemokines [59]. To date no group has identified fibrocytes in peri-implant tissues at the skin-implant interface of percutaneous devices, but as evidence here, fibrocytes may play a role in wound healing around percutaneous devices (Figure 5). The investigators do not believe that TEWL management alone fostered the appearance of fibrocytes, but further studies may better elucidate the activities of fibrocyte cell populations in peri-implant tissues.

Conclusion

Despite the preliminary nature of the findings discussed here, the effect of TEWL or “air-liquid interface” management in the preservation of the skin-implant interface of percutaneous devices has been shown to be insufficient to ensure the desired skin-implant integration outcome. Traditional TEWL management is employed to remedy intransigent/chronic wounds. Data from this study would suggest that any protocol intended to manage or control the TEWL around a percutaneous exit-site may, in fact, amplify one of the failure modalities (i.e. epidermal regression) traditionally associated with percutaneous device failure by accelerating wound margin migration. The comparative similarities between the cutaneous response of a previously conducted skeletal, load bearing sheep POP model and the pig dorsum model discussed here demonstrates the utility and economics of the pig dorsum model for the investigation of skin-implant interface behavior. Although promising from a translational science perspective, the metabolic, cardiovascular, and neurologic comorbidities, as well as the inherent surgical challenges of traumatic and non-traumatic limb salvage, must be considered before extrapolating the results of a pig-dorsum model to human amputees. That said, however, the model’s ability to serve as a high-throughput (i.e. multiple implantation sites) assessment tool for examining load-bearing POP system design features may in the future prove invaluable to the clinical advancement and implementation of POP technology for patients with limb loss.

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References

1. Darouiche RO. Device-Associated Infections: A Macroproblem that Starts with Microadherence. *Clin Infect Dis*. 2001; 33:1567–72. [PubMed: 11577378]
2. Scott RD, Center for Disease Control and Prevention. The Direct Medical Costs of Healthcare-Associated Infections in U.S. Hospitals and the Benefits of Prevention, 2009. Division of Healthcare Quality Promotion, National Center for Preparedness, Detection, and Control of Infectious Diseases, Coordinating Center for Infectious Diseases. Feb.2009
3. Crnich CJ, Drinka P. Medical Device-Associated Infections in the Long-Term Care Setting. *Infect Dis Clin N Am*. 2012; 26:143–64.
4. Klevens RM, Edwards JR, Richards CL, Horan TC, Gaynes RP, Pollock DA, Cardo DM. Estimating Health Care-Associated Infection and Deaths in U.S. Hospitals, 2002. *Public Health Rep*. 2007; 122:160–6. [PubMed: 17357358]
5. Trautner BW, Darouiche RO. Catheter-Associated Infections: Pathogenesis Affects Prevention. *Arch Intern Med*. 2004; 164:842–50. [PubMed: 15111369]
6. McCann MT, Gilmore BF, Gorman SP. Staphylococcus epidermidis device-related infections: pathogenesis and clinical management. *J Pharm Pharmacol*. 2008; 60:1551–71. [PubMed: 19000360]
7. Francolini I, Donelli G. Prevention and control of biofilm-based medical-device-related infections. *FEMS Immunol Med Microbiol*. 2010; 59:227–38. [PubMed: 20412300]
8. Gerritsen M, Paquay YG, Jansen JA. Evaluation of the tissue reaction to a percutaneous access device using titanium fibre mesh anchorage in goats. *Journal of Materials Science: Materials in Medicine*. 1998; 9:523–528. [PubMed: 15348850]
9. Jansen JA, Paquay YG, van der Waerden JP. Tissue reaction to soft-tissue anchored percutaneous implants in rabbits. *J Biomed Mater Res*. 1994; 28:1047–1054. [PubMed: 7814432]
10. Loesberg WA, te Riet J, van Delft FC, Schon P, Figdor CG, Speller S, van Loon JJ, Walboomers XF, Jansen JA. The threshold at which substrate nanogroove dimensions may influence fibroblast alignment and adhesion. *Biomaterials*. 2007; 28:3944–3951. [PubMed: 17576010]
11. Paquay YC, de Ruijter JE, van der Waerden JP, Jansen JA. Tissue reaction to Dacron velour and titanium fibre mesh used for anchorage of percutaneous devices. *Biomaterials*. 1996; 17:1251–1256. [PubMed: 8799510]
12. Walboomers XF, Croes HJE, Ginsel LA, Jansen JA. Contact guidance of rat fibroblasts on various implant materials. *J. Biomed. Mater. Res*. 1999; 47:204–212. [PubMed: 10449631]
13. Pendegrass CJ, Goodship AE, Blunn GW. Development of a soft tissue seal around bone-anchored transcutaneous amputation prostheses. *Biomaterials*. 2006a; 27:4183–4191. [PubMed: 16618500]
14. Pendegrass CJ, Goodship AE, Price JS, Blunn GW. Nature's answer to breaching the skin barrier: an innovative development for amputees. *J Anat*. 2006b; 209:59–67. [PubMed: 16822270]
15. Owings M, Kozak L. Ambulatory and inpatient procedures in the United States, 1996. *Vital Health Stat 13*. 1998; 139:1–119. [PubMed: 9866429]
16. Lyon CC, Kulkarni J, Zimerson E, Van Ross E, Beck MH. Skin disorders in amputees. *J Am Acad Dermatol*. 2000; 42:501–7. [PubMed: 10688725]
17. 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; 89:422–9. [PubMed: 18295618]
18. Meulenbelt HE, Geertzen JH, Jonkman MF, Dijkstra PU. Determinants of Skin Problems of the Stump in Lower-Limb Amputees. *Arch Phys Med Rehabil*. 2009; 90:74–81. [PubMed: 19154832]
19. Aschoff HH, Kennon RE, Keggi JM, Rubin LE. Transcutaneous, distal femoral, intramedullary attachment for above-the-knee prostheses: an endo-exo device. *J Bone Joint Surg Am*. 2010; 92:180–186. [PubMed: 21123601]
20. Hagberg K, Branemark R. One hundred patients treated with osseointegrated transfemoral amputation prostheses--rehabilitation perspective. *J Rehabil Res Dev*. 2009; 46:331–344. [PubMed: 19675986]
21. Hagberg, K.; Haggstrom, E.; Jonsson, S.; Rydevik, B.; Branemark, R. Osseoperception and osseointegrated prosthetic limbs, *Psychoprosthetics*. Springer; London: 2008. p. 131-140.

22. Kang NV, Pendegrass C, Marks L, Blunn G. Osseocutaneous integration of an intraosseous transcutaneous amputation prosthesis implant used for reconstruction of a transhumeral amputee: Case report. *J Hand Surg Am.* 2010; 35:1130–1134. [PubMed: 20541327]
23. 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; 27:114–20. [PubMed: 14571941]
24. Hagberg K, Brånemark R, Gunterberg B, Rydevik B. Osseointegrated trans-femoral amputation prostheses: prospective results of general and condition-specific quality of life in 18 patients at 2-year follow-up. *Prosthet Orthot Int.* 2008; 32:29–41. [PubMed: 18330803]
25. Tillander J, Hagberg K, Hagberg L, Brånemark R. Osseointegrated Titanium Implants for Limb Prostheses Attachments. *Clin Orthop Relat Res.* 2010; 468:2781–88. [PubMed: 20473597]
26. Mustoe TA, Cooter RD, Gold MH, Hobbs R, Ramelet AA, Shakespeare PG, Stella M, Téot L, Wood FM, Ziegler UE. International Clinical Recommendations on Scar Management. *Plast Reconstr Surg.* 2002; 110:560–71. [PubMed: 12142678]
27. Werner S, Grose R. Regulation of Wound Healing by Growth Factors and Cytokines. *Physiol Rev.* 2003; 83:835–70. [PubMed: 12843410]
28. Profyris C, Tziotzios C, Do Vale I. Cutaneous scarring: Pathophysiology, molecular mechanisms, and scar reduction therapeutics - Part I. The molecular basis of scar formation. *J Am Acad Dermatol.* 2012; 66:1–10. [PubMed: 22177631]
29. Tziotzios C, Profyris C, Sterling J. Cutaneous scarring: Pathophysiology, molecular mechanisms, and scar reduction therapeutics - Part II. Strategies to reduce scar formation after dermatologic procedures. *J Am Acad Dermatol.* 2012; 66:13–24. [PubMed: 22177632]
30. Murphy PS, Evans GRD. Advances in Wound Healing: A Review of Current Wound Healing Products. *Plast Surg Int.* 2012; 190436:1–8.
31. Eisenbud DE. Oxygen in Wound Healing – Nutrient, Antibiotic, Signaling Molecule, and Therapeutic Agent. *Clin Plast Surg.* 2012; 39(3):293–310. [PubMed: 22732377]
32. Schreml S, Szeimies RM, Prantl L, Karrer S, Landthaler M, Babilas P. Oxygen in acute and chronic wound healing. *Br J Dermatol.* 2010; 163:257–268. [PubMed: 20394633]
33. Schultz GS, Davidson JM, Kirsner RS, Bornstein P, Herman IM. Dynamic Reciprocity in the Wound Microenvironment. *Wound Repair Regen.* 2011; 19(2):134–148. [PubMed: 21362080]
34. Sen CK, Sashwati R. Redox Signals in Wound Healing. *Biochim Biophys Acta.* 2008; 1780(11):1348–1361. [PubMed: 18249195]
35. Martin P, Leibovich SJ. Inflammatory cells during wound repair: the good, the bad and the ugly. *Trends Cell Biol.* 2005; 15(11):599–607. [PubMed: 16202600]
36. Mustoe TA, Gurjala A. The role of the epidermis and the mechanism of action of occlusive dressings in scarring. *Wound Rep Regen.* 2011; 19:S16–21.
37. Perry EL, Beck JP, Williams DL, Bloebaum RD. Assessing peri-implant tissue infection prevention in a percutaneous model. *J Biomed Mater Res B Appl Biomater.* 2010; 92:397–408. [PubMed: 19927331]
38. Isackson D, McGill LD, Bachus KN. Percutaneous implants with porous titanium dermal barriers: an in vivo evaluation of infection risk. *Med Eng Phys.* 2011; 33:418–26. [PubMed: 21145778]
39. Jeyapalina S, Beck JP, Bachus KN, Williams DL, Bloebaum RD. Efficacy of a porous-structured titanium subdermal barrier for preventing infection in percutaneous osseointegrated prostheses. *J Orthop Res.* 2012; 30(8):1304–11. [PubMed: 22294380]
40. Middelkoop E, van den Bogaerdt AJ, Lamme EN, Hoekstra MJ, Brandsma K, Ulrich MMW. Porcine wound models for skin substitution and burn treatment. *Biomaterials.* 2004; 25:1559–67. [PubMed: 14697858]
41. Sullivan TP, Eaglstein WH, Davis SC, Mertz BA. The pig as a model for human wound healing. *Wound Rep Reg.* 2001; 9:66–76.
42. Steinstraesser L, Vranckx JJ, Mohammadi-Tabrisi A, Jacobsen F, Mittler D, Lehnhardt M, Langer S, Kuhn C, Gattermann S, Steinau HU, Eriksson E. A Novel Titanium Wound Chamber for the Study of Wound Infections in Pigs. *Comp Med.* 2006; 56(4):279–85. [PubMed: 16941955]
43. Wiechula R. The use of moist wound-healing dressings in the management of split-thickness skin graft donor sites: a systematic review. *Int J Nurs Pract.* 2003; 9:S9–17. [PubMed: 12694482]

44. Gallant-Behm CL, Mustoe TA. Occlusion regulates epidermal cytokine production and inhibits scar formation. *Wound Rep Reg.* 2010; 18:235–44.
45. Holgers KM, Tjellström A, Bjursten LM, Erlandsson BE. Soft Tissue Reactions Around Percutaneous Implants: A Clinical Study of Soft Tissue Conditions Around Skin-Penetrating Titanium Implants for Bone-Anchored Hearing Aids. *Am J Otol.* 1988; 9:56–9. [PubMed: 3364537]
46. Chou TG, Petti CA, Szakacs J. Evaluating antimicrobials and implant materials for infection prevention around transcutaneous osseointegrated implants in a rabbit model. *J Biomed Mater Res A.* 2010; 92:942–952. [PubMed: 19291687]
47. Emmanuel J, Hornbeck C, Bloebaum RD. A Polymethyl Methacrylate Method for Large Specimens of Mineralized Bone with Implants. *Stain Technol.* 1987; 62:401–10. [PubMed: 3433310]
48. Wong P, Coulombe PA. Loss of keratin 6 (K6) proteins reveals a function for intermediate filaments during wound repair. *J Cell Biol.* 2003; 163(2):327–337. [PubMed: 14568992]
49. Holt BM, Bachus KN, Beck JP, Bloebaum RD, Jeyapalina S. Immediate post-implantation skin immobilization decreases skin regression around percutaneous osseointegrated prosthetic (POP) implant systems. *J Biomed Mater Res A.* 2012 In press.
50. Wong P, Coulombe PA. Loss of keratin 6 (K6) proteins reveals a function for intermediate filaments during wound repair. *J Cell Biol.* 2003; 163:327–37. [PubMed: 14568992]
51. Mahdavian Delavary B, van der Veer WM, van Egmond M, Niessen FB, Beelen RH. Macrophages in skin injury and repair. *Immunobiology.* 2011; 216:753–62. [PubMed: 21281986]
52. Abreu-Velez AM, Howard MS. Collagen IV in Normal Skin and in Pathological Processes. *N Am J Med Sci.* 2012; 4:1–8. [PubMed: 22393540]
53. Sarrazy V, Billet F, Micallef L, Coulomb B, Desmoulière A. Mechanisms of pathological scarring: Role of myofibroblasts and current developments. *Wound Rep Reg.* 2011; 19:S10–15.
54. Ogawa R. Mechanobiology of scarring. *Wound Rep Regen.* 2011; 19:S2–S9.
55. Widgerow AD. Cellular/extracellular matrix cross-talk in scar evolution and control. *Wound Rep Reg.* 2011; 19:117–33.
56. Abe R, Donnelly SC, Peng T, Bucala R, Metz CN. Peripheral Blood Fibrocytes: Differentiation Pathway and Migration to Wound Sites. *J Immunol.* 2001; 166:7556–62. [PubMed: 11390511]
57. Herzog EL, Bucala R. Fibrocytes in health and disease. *Exp Hematol.* 2010; 38:548–56. [PubMed: 20303382]
58. Grieb G, Steffens G, Pallua N, Bernhagen J, Bucala R. Circulating Fibrocytes – Biology and Mechanisms in Wound Healing and Scar Formation. *Int Rev Cell Mol Biol.* 2011; 291:1–19. [PubMed: 22017972]
59. Reilkoff RA, Bucala R, Herzog EL. Fibrocytes: emerging effector cells in chronic inflammation. *Nat Rev Immun.* 2011; 6:427–35.

Fig 1.

Six (6) percutaneous, porous coated, titanium implants [a and b] were implanted into the dorsum [c and d] of two pigs for a 3-month (12-week) implantation period. Implants with or without 1.5 mm diameter perforations were staggered longitudinally (40 mm apart), either side of the dorsal midline. The skin-implant interfaces of one pig were treated (Group 1 – G1) with a petroleum-based gel to mimic transepidermal water loss (TEWL) techniques. The skin-implant interfaces of the remaining animal were untreated (Group 2 - G2). The implants and surrounding tissues of all twelve (12) implants were collected after 3 months for qualitative and quantitative analyses.

Fig 2.

Skin-implant interfaces of percutaneous implants showing the wound healing responses. A representative micrograph (4×) of the treated implants (G1) showed TEWL management at the skin-implant interface of percutaneous devices elicited the development of a substantially larger area of peri-implant granulation tissue region [*a; demarcated by the region below the dashed line*] when compared to untreated (G2) implants [*b; demarcated by the region below the dashed line*]. The differences in granulation tissue expression lead to differences in cutaneous regression/downgrowth. Independent of TEWL management, the cutaneous tissue at the skin-implant interface formed three distinct regions in response to percutaneous insult: 1) an interface region (*); 2) a transition region (**); and 3) a stress absorbance region (***). These three regions were identified in a percutaneous osseointegrated prosthetic sheep model conducted previously.

Fig 3.

Transepidermal water loss (TEWL) management increased cutaneous regression at the skin-implant interface and decreased total percutaneous implant surface coverage. Qualitatively, treated implants possessed longer wound margins and hyperplastic epidermal layers when compared to untreated implants [a and b, respectively]. When compared quantitatively, the application of Vaseline® to the skin-implant interface for TEWL management (treated group) led to increases in epidermal regression and decreases in percutaneous implant surface coverage and global soft-tissue integration [c and d, respectively]. Arrows denote representative positions of the treated and untreated 3 phase junctions/skin-implant interfaces.

Fig 4.

Immunohistochemical analysis of treated (G1) and untreated (G2) implants showed increased expression of proteins associated with active, and/or continuous, cutaneous wound healing. Collagen 4 (Coll-4+) [*green*] expression in the skin is associated with the endothelial and epithelial basement membrane. Within the granulation tissue of the G1 implant group (#) the qualitative increase in Coll-4 expression would suggest an increase in peri-implant vascularity, and a persistently active wound bed [*a*]. Comparatively, the granulation tissue of the G2 implant group (*) showed far less coll-4 expression [*b*]. In addition to Coll-4, relative increases in keratin 6 (K6+) [*red*] expression in the epidermis of G1 implants, compared to G2 implants, indicated a more actively migrating wound margin [*c*]. Increased wound margin mobility would suggest a less stable and mature wound, decreasing the likelihood of sustainable skin-implant integration.

Fig 5.

Identification of fibrocytes within the skin-implant interface region of percutaneous devices at 3 months post-implantation would suggest a persistent pro-inflammatory and pro-fibrotic wound environment. A localized staining technique (CD45+ and pro-collagen+) was used to identify the potential location of fibrocytes (*white dashed circles*) [C], a specialized 'fibroblast-like', pro-fibrotic cell line, in the granulation tissue (the orange dashed line region bounded by the red (epidermal-dermal basement membrane), and the yellow (distal perimeter) dashed lines) of G1 implants [B and C]. Fibrocytes are suspected to play a role in numerous pathophysiological, fibrotic conditions and may play a role in the foreign body reaction and unique wound healing process present at the skin-implant interface of percutaneous devices.

Fig 6. Photomicrographs from the peri-implant region of an *in vivo* load bearing percutaneous osseointegrated prosthetic sheep model and the *in vivo* percutaneous post implant pig dorsum model showed qualitative histological similarities. The skin within the peri-implant regions of both the ovine model [a] and the pig-dorsum model [b] showed evidence of gross soft-tissue adaptation, described by the development of an interface region (*), a transition region (**), and a stress absorbance region (***) [not shown at this magnification].