Vital, Porcine, Gal-Knockout Skin Transplants Provide Efficacious Temporary Closure of Full-Thickness Wounds: Good Laboratory Practice-Compliant Studies in Nonhuman Primates

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Vital, genetically engineered porcine skin transplants have long been regarded as a promising treatment option for severe burn wounds. The objective of this two-part, preclinical study was to evaluate the ability of vital, split-thickness skin xenotransplants derived from designated pathogen-free, alpha 1,3 galactosyltransferase knockout miniature swine to provide temporary wound closure of full-thickness wound defects intended to model severe and extensive, deep partial- and full-thickness burn wounds. In part 1 of the study, four full-thickness wound defects were introduced in four cynomolgus macaques recipients and, then engrafted with two xenografts and two allografts to achieve temporary wound closure. On POD-15, autografts were used to achieve definitive wound closure and were observed until POD-22. In part 2 of the study, four additional subjects each received two full-thickness wound defects, followed by two xenografts to achieve temporary wound closure, and were observed postoperatively for 30 days without further intervention. All grafts were assessed for signs of adherence to the wound bed, vascularity, and signs of immune rejection via gross clinical and histological methods. Xenograft and allograft comparators were equivalent in part 1, and later autografts were otherwise indistinguishable. In part 2, all xenotransplants demonstrated adherence, vascularity, and survival until POD-30. These were unexpected results that exceed previously published findings in similar models. Furthermore, the ensuing GLP-study report directly supported regulatory clearance, permitting a phase I clinical trial. This solution holds great promise as an alternative to human cadaver allograft, the current standard of care for the treatment of severe burns.

Severe deep partial- (second-degree) and full-thickness (third-degree) burns are responsible for the majority of burn-related deaths 1, which affect civilians, first responders [2], and military personnel [3] numerous etiologies [4]. Although advancements in materials, protective equipment, and precautionary measures have reduced burn incidence over the past decades, these ruinous injuries remain all too common today [5–7]. Autograft material obtained from the patient's own body is the ideal treatment modality [8, 9] as it can restore barrier function while minimizing infectious disease and availability issues, and lacks immunological compatibility complications. However, in burns that injure 20% or more total body surface area (TBSA), autografting can be clinically contraindicated and, therefore, availability is limited [10–12]. Without adequate, immediate wound closure [8, 13], these victims are at great risk of mortality as a result of fluid loss, pH imbalances, refractory organ failure [1, 14], and preventable infection [15–18]. Thus, additional, equivalent therapy options are essential.

One commonly used substitute is human cadaver allograft (HCA) [9, 19–23]. While allograft skin will inevitably be rejected [5, 24, 25], its temporary use can be lifesaving and act as an interim barrier replacement while autograft sites and the patient's body overall heals. The use of HCA has been shown to offer many advantages, including the reduction of pain, wound sepsis, as well as water, electrolyte, and protein losses [9, 23, 26–31]. Although HCA can reliably be stored and banked, shortages often arise due to a plethora of problems, including persistent infectious disease concerns [8, 9, 30, 32–36] and a limited source of qualifying donors [22, 29, 37, 38], both of which are difficult to address and seemingly impossible to correct. These culminate into a fundamental supply and demand imbalance, representing a persistent unmet clinical need. While there are many other alternatives on the market today, "no skin substitute produced to date is able to approximate the biologic properties of viable human skin" [20] and "there is no satisfactory replacement for human allograft skin" [30].

HCA reduces life-threatening, preventable infections largely because of the inclusion of metabolically active cells. HCA remains viable and can achieve "wound closure" as opposed to "wound coverage" [19, 39, 40]. As a result of a fibrin(ogen) seal that is formed between a viable graft and the wound, an effective barrier is created which blocks bacteria and other infectious

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agents [17, 19, 41, 42]. Following excision and debridement of the wound [10, 43, 44], an initial step in the creation of this barrier involves dissolved oxygen in the wound bed, which allows the grafts to avoid immediate, ischemic death. Termed "plasmatic imbibition" in 1888 [45], this process allows the graft to survive until perfusion is established. As a result, blood flow can subsequently be restored via contact between exposed vasculature in the recipient wound bed and the vacated vessels in the graft. This phenomenon results in an observable return of the natural, perfused hue of the skin graft. Over time, original graft vasculature degenerates and is replaced with new vessels that originate from the recipient wound bed. Gradually, this process will either permanently restore circulation [46–52] or persist until immunological incompatibilities catalyze humoral or later cellular-mediated rejection of the graft [5, 24, 25, 53]. Early on in this wound healing continuum, the total number of fibrocytes decreases, fibroblast-like cells emerge, and two discrete layers of fibrin(ogen) are formed [15, 21, 42, 50, 54, 55]. This process, referred to as graft "take" or "adherence," is central to the ability of the graft to prevent infectious agents from entering the wound [13, 29, 56] and provide the antibacterial barrier exhibited uniquely by viable grafts [17, 19, 42, 57].

This life-saving process necessitates that vital grafts have adequate perfusion and metabolically active cells [9, 12, 23, 29, 58]. Unfortunately, alternatives to date have lacked the fundamental viability [58] that makes HCA effective. The lack of such suitable alternatives [13, 41, 59], coupled with the severely limited supply of HCA [31, 60–62], makes transplantation of vital animal skin grafts a promising treatment for burns [63–67]. Porcine grafts are especially promising, especially given that they share many physiologic characteristics with human skin [31, 39], including similar rete ridges, papillary dermis, and hair coverage [68–73] and can be genetically altered to make them better suited for human patients [74]. Grafts sourced from swine could minimize mortality and morbidity via the same mechanism of action as HCA and, thus, serve as a plausible alternative therapeutic for severe and extensive, deep partial- and full-thickness burns when autograft is not clinically advisable.

Immunological incompatibilities between porcine donors and human recipients have limited the effectiveness of live-cell, wild-type grafts in the past. Although these products contained viable cells, wild-type xenogeneic tissues undergo hyperacute rejection via a rapid (minutes to hours), antibody-mediated immunological rejection, which leads to premature loss of the graft due to ischemia [53, 75, 76]. To address this incompatibility, a genetically engineered galactosyltransferase knockout (GalT-KO) herd of miniature swine has been developed [63, 77–81], which lacks the dominate offending alpha (1,3)-galactose epitope and can be used as a source of vital GalT-KO grafts [60, 63, 64, 82]. A considerable body of preclinical, published data demonstrate that graft material from these source animals has been shown to delay graft rejection [67, 74, 83, 84], permitting prolonged survival (weeks to months), characterized as "acute" rejection and an associated time course similar to the cell-mediated immune mechanism demonstrated by donor recipients of human allografts [60, 63–65, 82].

Previous studies in the available literature, involving skin grafts from genetically modified swine in similar nonhuman primate models predominantly reported graft survival times of 11 to 14 days [63–65, 82, 85]. In this study under good laboratory practices (GLP), we specifically examine the safety, tolerability, survivability, and other clinical efficacy metrics of sterilely processed xenografts sourced from designated pathogen-free (DPF) donor animals, in non-human primates (NHP), in order to model the treatment of severe burn wounds in human patients.

METHODS

Ethics Statement

This study was conducted in accordance with the U.S. Department of Agriculture's (USDA) Animal Welfare Act (9 CFR parts 1, 2, and 3), the Guide for the Care and Use of Laboratory Animals, and all state, local laws and regulations. A standing Institutional Animal Care and Use Committee (IACUC) independently reviewed and monitored all guidelines, study protocols, surgical procedures, and animal care.

Animals

All xenografts used in both part 1 and part 2 of this study were obtained from animal donors sourced from a closed colony of specialized, genetically engineered DPF alpha 1,3 GalT-KO miniature swine. The original colony of miniature swine were developed by Sachs et al at the Massachusetts General Hospital [77, 81, 86] with funding support from the National Institute of Health and the Department of Defense. Source animals were housed separately from noncolony members, under prescribed isolation (barrier) conditions within a Biological Safety Level (BSL)-2, positive pressure, biocontainment establishment (XenoTherapeutics, Boston, MA).

In part 1, one male cynomolgus monkey served as the common-source donor for allograft tissue to ensure comparability of allograft material across each of the four allograft recipients and minimize unintended variability when compared with the xenograft test articles.

Graft recipients selected for part 1 included four (4) male, nonnaïve cynomolgus monkeys (*Macaca fascicularis*) of Chinese origin (cohort 1, $n = 4$; Figure 1). In part 2, two male, nonnaïve and two female, naïve cynomolgus monkeys of Chinese origin were assigned as graft recipients (cohort 2, $n = 2, 2$; Figure 2). This subject choice was necessary, as only humans and nonhuman primates possess preformed antibodies to the alpha-Gal epitope and, thus, reject wild-type porcine tissues in a similar manner [87, 88]. Furthermore, cynomolgus monkeys are well established in previous literature as scientifically appropriate subjects for such studies [89, 90]. Lastly, the inclusion of nonnaïve animals in this study was based on overall limited subject availability and to most prudently and ethically steward the use of research animals. Subjects were selectively chosen based on previous participation in studies that were determined to have no known interference with this series of experiments.

Animal health, including clinical observations, body weight and body condition, food consumption, electrocardiogram (ECG) tracings, respiratory rate, body temperature, neurological examinations, and clinical pathology were monitored at predetermined regular intervals through both experimental parts 1 and 2 under veterinary supervision.

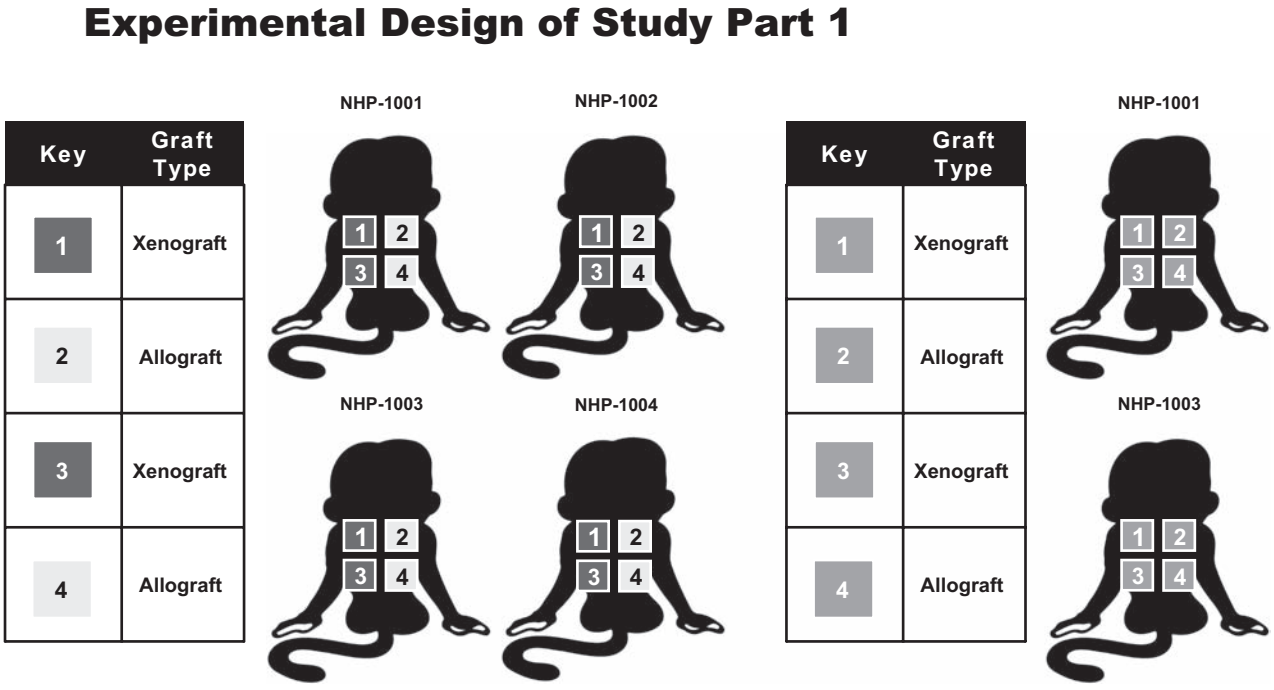

Figure 1. Experimental design for study part 1, which compared the ability of vital xenografts versus allografts from a common-source donor as a means of temporary wound closure in treatment of full-thickness wound defects, prior to definitive closure with autografts.

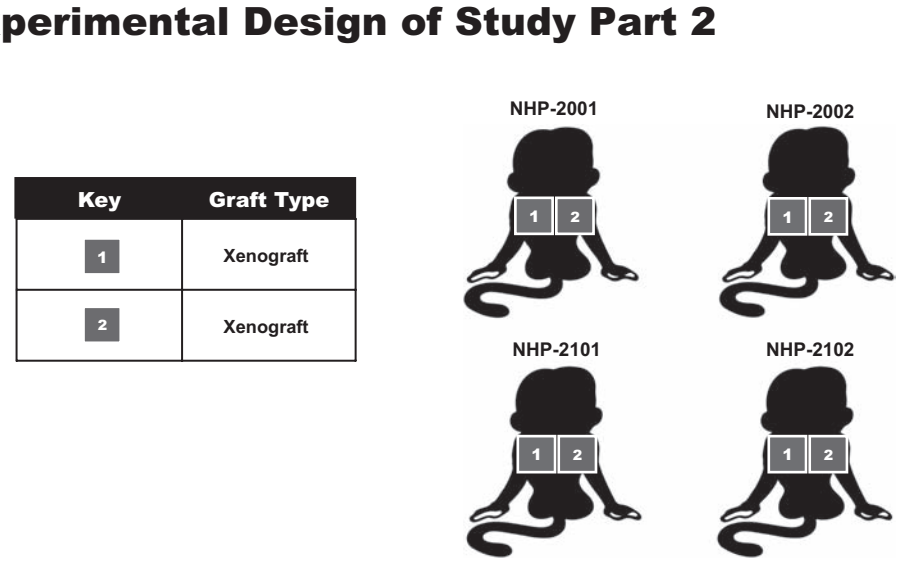

Figure 2. Experimental design for study part 2, which observed the ability of vital porcine xenografts to provide temporary wound closure in treatment of full-thickness wound defects. Study part 2 evaluated xenografts only, over an extended period of observation. Number of wound beds surgically introduced, anesthetic episodes, and overall "design" impact variables were reduced, when compared with Part 1.

Procurement and Preparation of Porcine Skin Xenografts

One genetically engineered, DPF, GalT-KO miniature swine served as the single donor for all xenografts used in this study. This donor was euthanized using a captive bolt and transported in isolation to a sterilized operating room. Skin surfaces were disinfected with chlorhexidine acetate (NolvasanR Surgical Scrub, Fort Dodge Animal Health, Fort Dodge, IA) and 70% isopropyl alcohol, hair was removed, and the donor was draped. Split-thickness skin grafts were harvested between the scapula and inferior margin of the lowermost rib using an air-driven Zimmer dermatome (Medfix Solution, Inc., Tucson, AZ). Grafts were inspected to verify thickness of 0.056 cm and trimmed so that they were approximately 25 cm^2 each.

After the xenografts were trimmed, they were placed in antibiotics and then packaged in cryovials (Simport, T310-1A, Beloeil, QC). Approximately 5 ml of cryoprotective media (CryoStor CS5 media, BioLife Solutions, Bothwell, WA) was added to each vial before it was sealed and frozen using a controlled rate freezer.

The xenografts were stored at –80°C until use. Before surgery, they were prepared for use by placing the sealed vials in a 37°C water bath for approximately 1 minute, followed by three 1-minute serial washes in normal saline with gentle agitation.

Procurement and Preparation of NHP Allografts

One cynomolgus monkey (*Macaca fascicularis*) served as the common allograft donor for all allografts. Split-thickness skin grafts were obtained using an air-driven Zimmer dermatome set to 0.056 cm, measuring approximately 9 cm^2. Following harvest, the subject was euthanized per standard protocol and under the direction of veterinarian staff. Finally, each allograft harvested was preserved in an identical manner as the xenografts per standard protocol.

Procurement and Preparation of NHP Autologous Skin Grafts

Four ($n = 4$) split-thickness skin grafts were created from each animal using an electric Zimmer dermatome set to approximately 0.056 cm and measuring approximately 9 cm^2. Each split-thickness graft that was removed was placed separately into a 50-ml conical tube or sterile specimen cup and then, transferred for cryopreservation per protocol as described above for later use as autograft for definitive wound closure in Post Operative Day (POD)-15 in part 1 of the study.

Surgical Procedure and Experimental Design

In part 1, four recipients underwent xenograft transplantation via four sequential, independent surgical procedures. Subjects were positioned on the operating table in sternal recumbency. Heart rate, respiratory rate, blood pressure, end-tidal carbon dioxide (ETCO2), and body temperature was continually monitored throughout the procedure.

Following autograft procurement, the four ($n = 4$) partial thickness wound beds were surgically converted into full-thickness wound beds on the dorsal aspect of each subject (Figure 3). The four resulting wound beds were independent but equivalent with regards to overall size, depth, and anatomical location. Photograph(s) were taken of each wound site.

Centered medially along the spine, two xenografts were placed on the anatomic left (wound site 1, superior and wound site 3, inferior) and two comparator allografts from the common NHP donor were placed on the anatomic right (wound site 2, superior and wound site 4, inferior). A total of 16 ($n = 16$) xenograft and allograft test articles were fenestrated and uniformly sutured in place using simple interrupted, 3-0 nylon sutures evenly distributed across the four recipients (Figure 4). By POD-15, all xenograft and allografts were to be replaced with autografts, mimicking standard clinical definitive wound closure. Autografts would be engrafted in identical fashion to their predecessors as previously described. Residual xenograft or allograft tissue would be preserved for later histological or microbiological analysis.

In part 2, the total number of wound sites and skin grafts were reduced from four to two per subject. Similar to part 1, two equivalently spaced, full-thickness wound beds were centered on the dorsal aspect of each subject between the inferior aspect of the scapulae and superior to the iliac crests. Two xenografts (wound site 1 and wound site 2) were placed on the anatomic left and right, respectively (Figure 5). In total, this schema provided a total of eight ($n = 8$) xenografts for observation across the four subjects ($n = 4$).

Postoperative Surgical Care

Postoperative care was standardized across all eight recipients. Bacitracin or a triple antibiotic ointment was applied to overlying pressure dressings consisting of Xeroform petrolatum gauze (Medtronic), Telfa™ nonadhesive dressing (Covidien, Minneapolis, MN), and sterile dry gauze maintained in place with Tegaderm™ (3M, St. Paul, MN). SoftRoll, VetRap™, and Elastikon were added to secure the dressing onto the wounds/grafts. Primate jackets were fitted to avoid the animal scratching the wounds for all subjects. Animals received Buprenorphine (0.03 mg/kg, IM) immediately post-surgery (POD-0) and every other day thereon as required. Clinical observations were performed at least twice daily. Complete blood count (CBC) and serum chemistries (Chem17 and Lyte4) were taken concomitant with dressing changes. Subjects were monitored for signs of illness or distress and provided supportive therapy or pain management as needed by veterinary staff.

Postoperative Assessment of Graft Survivability

In part 1, xenografts and allografts were evaluated for duration of survivability, including time to immune-mediated rejection, beginning on POD-5 and subsequently inspected every other day on POD 7, 9, 11, 13, and 15. Assessment of graft viability, judged by color, texture, and warmth to the touch were

Figure 3. Study part 1, animal 1002, POD-0. Representative image, illustrating the four partial thickness wound beds, created initially via dermatome, were surgically converted into full-thickness wound defects via scalpel. This was performed across all four subjects to model significant injuries as a result of severe burns or trauma.

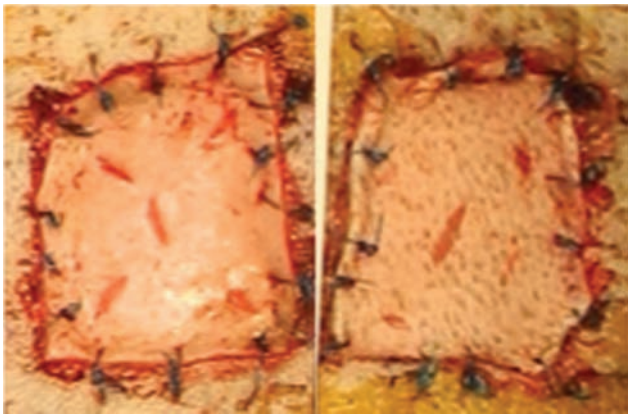

Figure 4. Study part 1, animal 1002, POD-0. Representative image, comparing porcine split-thickness xenografts versus allografts from a common-source donor, as a means of temporary wound closure in treatment of full-thickness wound defects in a nonhuman primate recipient. Left: xenograft at wound site 1. Right: allograft at wound site 2.

performed, and photographs were obtained. Additionally, at each observation, three 3-mm punch biopsies were obtained for later histological analysis.

In part 2, xenografts were evaluated for duration of survivability on POD 7, 14, 21, and at the end of study, POD-30. In identical fashion to part 1, graft viability was judged by color, texture, and warmth to the touch, and photographs were taken of the wound sites and skin grafts at each assessment.

Subjects were euthanized per study design by veterinary staff on POD-22 and POD-30 (part 1 and part 2, respectively). Following a necropsy of each subject was performed by a veterinary pathologist. Animals underwent a comprehensive examination of the external surface of the body.

For all subjects, frozen or formalin-fixed tissues, designated for staining with hematoxylin and eosin were trimmed, embedded, and sectioned. Slides were prepared by a third-party laboratory and were later read and interpreted by a blinded, independent, board-certified veterinary pathologist.

Statistical Analysis

Simple mean and standard deviation were used exclusively as comparative statistics were not required.

RESULTS

Gross Clinical Assessment

In part 1 of the study, all xenograft and allograft comparators were clinically indistinguishable over the course of the study with respect to graft adherence, evidence of immune-mediated rejection, and overall presentation (Figure 6). Upon first inspection at POD-5, all 16 grafts showed signs of perfusion, adherence, and vascularity. At final evaluations, all xenografts and allografts remained well vascularized and were fully adherent to the wound bed (Table 1), exhibiting minimal to moderate epidermolysis. At POD-15, graft adherence remained so significant that surgical removal (Figure 7) was required to facilitate the definitive wound closure as dictated by the original study protocol. All eight autografts

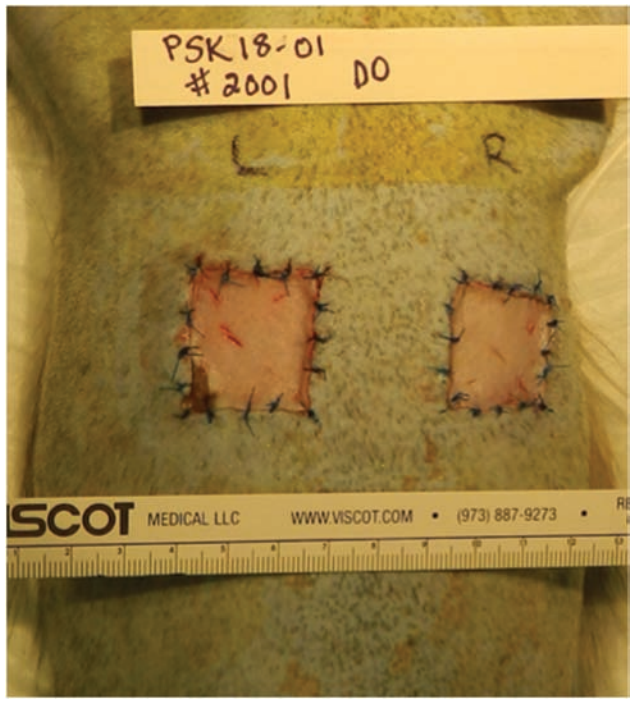

Figure 5. Study part 2, animal 2001, POD-0. Representative image, illustrating the reduction of total number of wound sites and grafts placed in all subjects from four in part 1 to two in part 2. Two xenografts (wound site 1 and wound site 2) were placed on the anatomic left and right, respectively. In total, this schema provided a total of eight (n = 8) xenografts for observation across the four subjects (n = 4).

subsequently engrafted on POD-15 healed fully by POD-22. Furthermore, no clinical difference in autograft survival or overall appearance was observed between those wounds previously closed with xenograft or allograft (Figure 8).

In part 2 of the study, all eight xenografts across the four skin graft recipients survived until the end of study on POD-30 (Table 2). Xenografts uniformly presented with moderate epidermolysis by POD-14, progressing to complete epidermolysis by POD-21. By POD-30, xenograft sites displayed partial or full re-epithelialization in all four subjects (Figure 9).

Histological Assessment

In part 1, microscopic evaluation of the serial biopsies demonstrated no evidence of acute immune rejection in any of the xenografts or allografts by POD-15. Histological evaluation showed the xenograft test articles to be slightly better than the allograft test articles. Specifically, grafts were comparable for their lack of acute rejection, local inflammation, and maintenance of wound coverage. However, a limited but observable decrease in epithelial coverage was observed in the allograft comparators, and necrosis scores were marginally better in favor of the xenograft. Autografts subsequently placed on POD-15 appeared histologically indistinguishable regardless of the preceding graft type. Epithelization of the autografts in the two subjects (n = 2), following either xenograft or allograft, scored identically, and necrosis and inflammation in

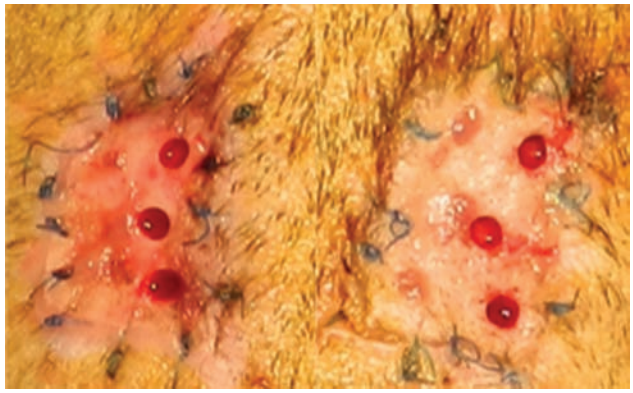

Figure 6. Study part 1, animal 1002, POD-15. End of study observations of porcine split-thickness skin grafts versus allograft from a common-source donor, as a temporary wound closure in treatment of full-thickness wound defects in a nonhuman primate recipient. Left: xenograft at wound site 1. Right: allograft at wound site 2. All grafts were fully vascularized and remained adherent, requiring mechanical (surgical) removal to permit subsequent, definitive closure via autograft placement. Note: Incidental, 3-mm punch biopsy artifacts are visible in each photograph, and were performed per protocol to obtain tissue samples for later analysis.

response to the graft and fibrosis of the autograft were likewise similar, regardless of pretreatment (Figures 10–12).

During part 2, in order to reduce the impact to subjects attributed to the study design of part 1, a blinded pathologist performed histological assessment of tissues and samples obtained at the end of the study (Figure 13). Microscopic evaluation of the wound beds on POD-30 demonstrated good filling of the wound defect. A mature dermal collagen network had formed, surrounded by a variable layer of new collagen that was distinct in appearance from the host dermis bordering the wound site, which was interpreted to be that of the xenograft dermis. Epithelial ulceration was present in four out of eight of the wound sites and ranged from mild (~25% of wound surface) to marked (>75% of wound surface). New granulation tissue was most prominent between the wound surface and the xenograft, assessed to be in response to ulceration and previous loss of xenograft epithelium. Edema was minimal and considered within normal range. A general inflammatory reaction surrounding the xenograft was observed, characterized by variable (minimal to moderate) infiltrates, generally dominated by macrophages, lymphocytes, polymorphonuclear cells, and plasma cells. Multinucleated giant cells constituted a minimal portion of the inflammatory component. These inflammatory infiltrates were distinct from those observed as a response associated with fragments of hair, suture material, or other foreign material and were distinct from the inflammatory response directed at areas of epithelial ulceration.

Mortality

During part 1, subjects 1002 and 1003 survived to the scheduled end of study in fair health and underwent necropsy on POD-22. Two animals were euthanized early due to moribundity based on veterinarian assessment. Subject 1004 was euthanized on POD-12, as per veterinarian instruction, for inappetence, lethargy, pale color, dehydration, and hypothermia. Similarly, subject 1001 was euthanized on POD-15, following veterinarian's direction, after a continued period of inappetence and weight loss. Body weight for subject 1004 was consistent until POD-5 and a 14% loss was detected by POD-11; the subject was euthanized the following day. Subject 1001's body weight showed a 12% loss by POD-11, which further decreased to a total 16% loss by POD-13. By POD-15, there were no signs of improvement and, at that time, euthanasia was directed.

There were no changes in the hematology, serum chemistry, or coagulation parameters in either subject. Gross necropsies were performed on all NHP recipients and were consistently found to be unremarkable with the exception of irritation around the back of each animal. Later histopathological assessment of skin samples collected at gross necropsy exhibited superficial dermal chronic inflammation with mild epithelial hyperplasia in samples taken from skin surrounding the wound sites. Additional evaluation of other tissues obtained from these necropsies indicated no histopathological changes in the kidney, lung, liver, or spleen that would suggest any test article-related toxicity nor was there any histopathological evidence of an adverse systemic response in any graft recipient.

In part 2, all subjects ($n = 4$) tolerated the initial surgical procedure and placement of bilateral xenografts (only) without significant clinical issues and survived to the scheduled end of study point on POD-30. All four subjects maintained a body condition score ranging between 2.5 (lean) and 3 (optimum) during the study, though each of the subjects lost weight following the surgical procedure. Total body weight losses, from pre-surgery to the end of study (POD-30) were: –3.8% (subject 2001), –8.2% (subject 2002), –10.0% (subject 2101), and –9.4% (subject 2102). Necropsy and evaluation methods, identical to those performed on subjects in Part 1, were unremarkable.

DISCUSSION

Xenografts Performed Equivalently to Allografts and Survived Beyond Previous Research Findings

In part 1, both clinical and histological evaluations demonstrated indistinguishable overall performance and duration of graft survival between the xenograft test articles and the allograft comparators. Moreover, the absolute duration of survival of 15 days postoperatively in three subjects—without immunesuppression—was remarkable. Based on our previous experience in this model, sloughing and necrosis was expected by this time. Thus, POD-15 was chosen as a reasonable study transition point at which to replace the temporary dressings with autografts to achieve definitive wound closure. However, instead of presenting as exposed wound beds needing intervention, all 16 grafts were fully adherent, and graft to wound perfusion was clearly evident at each site on POD-15. This unexpected status necessitated surgical removal of the overlying, vascular tissues in order to proceed with the remainder of study part 1. It is unknown how long the allografts and xenografts would have survived without surgical intervention, given the healthy appearance of the grafts and lack of evidence suggesting acute rejection.

Building on the results in part 1, the study design of part 2 was fashioned to reduce the deleterious impact to the subjects

Table 1. Duration of postoperative graft survival (in days) of xenograft and allograft comparators, as determined by independent, gross clinical assessment for each of the four subjects in study part 1

Results of clinical assessments of xenografts and allografts in study part 1				
Wound site no.	**1**	**2**	**3**	**4**
Graft type	**Xenograft**	**Allograft**	**Xenograft**	**Allograft**
Subject no.	**Graft postoperative survival (in days)**			
NHP 1001	15	15	15	15
NHP 1002	15	15	15	15
NHP 1003	15	15	15	15
NHP 1004	12	12	12	12

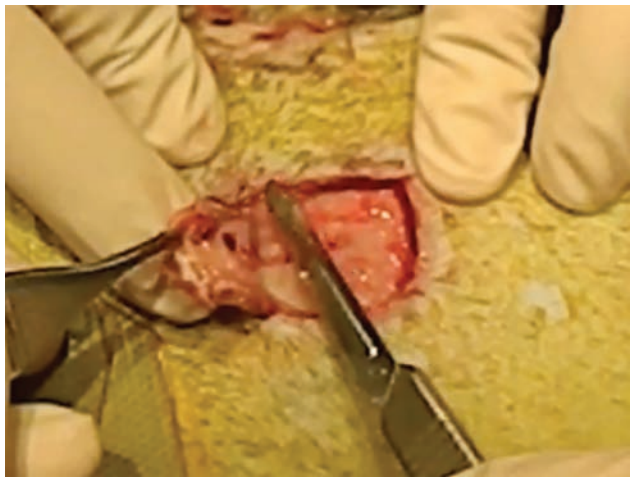

Figure 7. Study part 1, animal 1002, POD-15. Representative image from one subject, illustrating the significance of graft adherence at POD-15, which required surgical removal of the overlying graft to allow for the preplanned definitive closure, via autograft, as dictated in the original GLP-study design. Based on previous experience, grafts should have "sloughed" between 10 and 14 days postoperatively such that the underlying wound bed would be exposed. This prolonged survival and significant adherence was consistent across all four subjects.

associated with the combination of a significant wound size to TBSA ratio, the total number of grafts introduced, the duration of initial surgery, and the frequency of postoperative sedations required to perform the every-other-day biopsying. Furthermore, allografts were eliminated to isolate outcomes due solely to the xenograft test articles. Lastly, the total period of observation was extended from 15 to 30 days postoperatively, intending to circumscribe the presentation of signs of rejection.

Similar to part 1, the results of this experiment also exceeded hypothesized expectations. In part 2, graft survival at every wound site, in all four subjects, was observed at POD-30. Moreover, while early signs of rejection were observed on POD-21, complete immunological acute rejection was still not yet observed on POD-30 as anticipated.

In sum, the robust survival observed in 16 xenografts (n = 16) in all eight subjects represents an unprecedented outcome, especially given the lack of any immunosuppressive regimens to promote graft survival. It is also worth emphasizing that the findings were reproduced independently, with each experiment performed under regimented, highly monitored good laboratory practices, later audited by a thirdparty. Yamamoto et al in their 2018 review article note that "to the best of our knowledge" in all previous studies reported to date, graft survival times were 2 weeks or less [65], in 44 subjects in similar, genetically modified porcine donor-NHP recipient models, with only two exceptions. According to Fujita et al [85], one subject experienced graft survival duration of 21 days; in addition, a secondary subject experienced a graft survival duration of 31 days. Both cases, however, utilized immunosuppression regimens (tacrolimus) to prolong survival and involved transgenic modifications, instead of a single genetic knockout.

Causative factors for such unexpected and unprecedented graft survival will require further investigation and characterization. If the underlying mechanisms responsible for these outcomes were better understood, optimization of current practices in transplantation would be possible, which could catalyze discovery of other novel processes that could enhance the field in general. The authors postulate that the success demonstrated here is due in part to the use of DPF source animals rigorously and continuously maintained in a positive pressure biocontainment facility. Furthermore, achievement of United States Pharmacopeia (USP) <71> sterility standards, possible only by procuring the skin under stringent aseptic conditions and later processing under sterile conditions to selectively remove commensal skin flora, likely played a significant role. Lastly, optimized cryopreservation procedures and use of specific reagents retained maximum residual cellular viability. It is our hypothesis that the convergence of several optimized factors and practices in tandem yielded this unprecedented outcome.

Definitive Closure via Autografts not Impaired by Xenografts

In part 1, definitive closure achieved via autografting of wounds previously treated with xenograft and allograft demonstrated only marginal differences, assessed independently by both histological and clinical means. Gross outcomes of the four (n = 4), xeno–allo pairs were indistinguishable. Microscopic evaluation revealed negligible differences in inflammation at autograft sites, and fibrosis and tissue ingrowth were only marginally higher for the autografts treated previously with xenografts. Otherwise, morphology of the comparators was comparable, and there were no substantial differences in the epithelialization

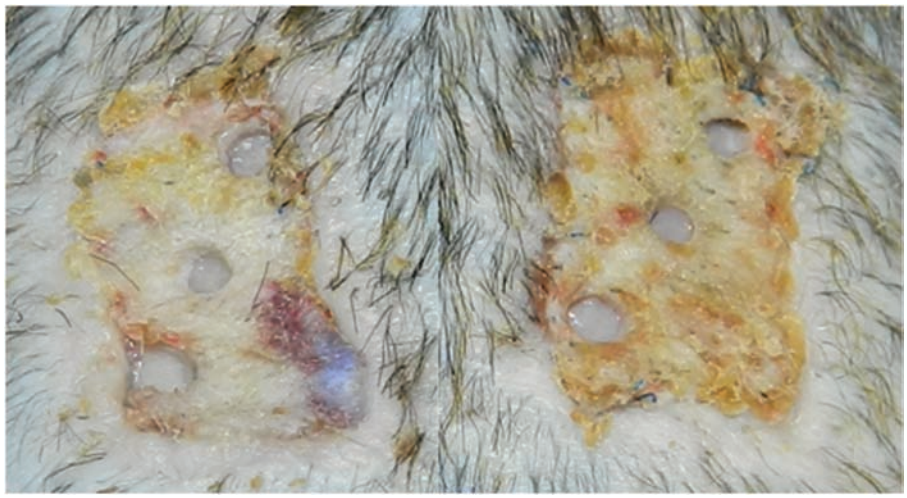

Figure 8. Study part 1, animal 1002, POD-22. Comparison of autografts used for definitive wound closure, following treatment with either porcine split-thickness xenografts or allograft from a common-source donor, used as a temporary wound closure in treatment of full-thickness wound defects in a nonhuman primate recipient. Left: autograft at wound site 1, originally treated with xenograft. Right: autograft at wound site 2, originally treated with allograft. Note: Incidental, 3-mm punch biopsy artifacts are visible in each photograph.

Table 2. Duration of postoperative graft survival (in days) of xenografts (only) as determined by independent, gross clinical assessment for each of the four subjects in study part 2

Results of clinical assessments of xenografts in study part 2		
Wound site no.	**1**	**2**
Graft type	**Xenograft**	**Xenograft**
Clinical assessment	**Postoperative graft survival days**	
NHP 2001	30	30
NHP 2002	30	30
NHP 2101	30	30
NHP 2102	30	30

Allograft comparators were eliminated to isolate outcomes due solely to the xenograft test articles. Additionally, the experimental design of Study Part 2 was fashioned to reduce the deleterious impact to the subjects in Study Part 1, which resulted from a combination of significant wound size to TBSA ratio, the total number of grafts introduced, the duration of initial surgery, and the frequency of postoperative sedations required to obtained every-other day biopsies.

of subsequent autografts. The lack of appreciable impact on autograft adherence and survival between the two groups is an important finding if vital porcine skin grafts are to be considered a potential alternative to HCA, envisioned to be employed as an interim treatment prior to definitive closure with autografts.

In part 2, residual, viable, xenograft dermal tissue was observed at POD-30. Future studies are needed to evaluate and characterize the ultimate wound granulation and contracture, but this suggests improved scarring outcomes may also be possible.

Adverse Events and Subject Mortality

Two subjects in part 1 (1001 and 1004) were euthanized prior to the end of the study based on observations of postoperative lethargy and weight loss. There were no other changes in the hematology, serum chemistry, or coagulation parameters that indicated an underlying issue with animal health status or provided explanation for the change. Independent gross necropsy of both subjects indicated no abnormalities, except irritation of the skin in regions in contact with the bandage adhesive. This finding is hypothesized to be refractory to the prolonged and frequent replacement of adhesive bandaging (TegaDerm) necessary to protect the surgical sites. Histological examination of skin samples taken from skin surrounding the wound sites collected at gross necropsy exhibited superficial dermal chronic inflammation with mild epithelial hyperplasia, but microscopic findings were considered to be incidental and comparable to the allograft comparator sites and considered otherwise incidental and unrelated to the xenograft test article. Combined, these findings suggest that the premature loss of subjects 1004 and 1001 was idiopathic and is not directly attributable to the xenograft test article.

It is posited that the cachectic presentation of the two subjects was a result of the study design itself. The initial surgically introduced trauma, involving wound site creation and subsequent engraftment, was significant. In each subject, four large-sized, full-thickness wounds of relatively large size when compared with the subjects TBSA were

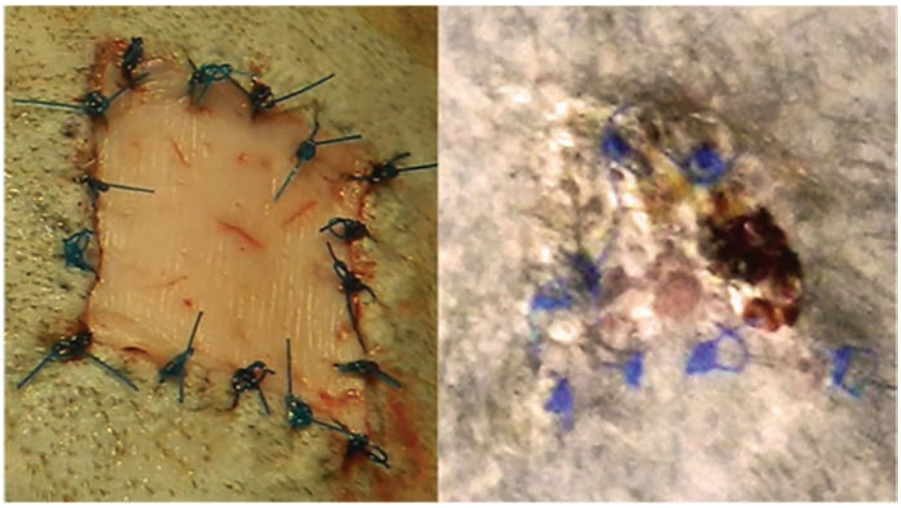

Figure 9. Study part 2, animal 2102, wound site 2. Progression of porcine split-thickness skin grafts over time, used as a temporary wound closure in treatment of full-thickness wound defects, in a nonhuman primate recipient. Left: POD-0, xenograft at wound site 2. Right: POD-30, same xenograft at wound site 2. By POD-30, xenografts displayed either partial or complete re-epithelialization in all four subjects. Xenografts uniformly presented with moderate epidermolysis by POD-14, progressing to complete epidermolysis by POD-21. By POD-30, xenograft sites displayed partial or full re-epithelialization in all four subjects.

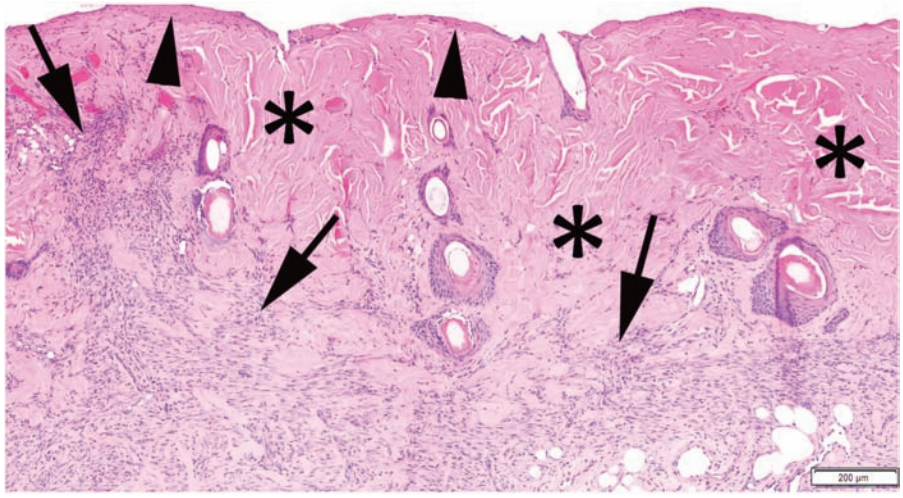

Figure 10. Study part 1, animal 1002, xenograft, POD-15. H&E, low-power image depicting good overall tissue viability with some surface and follicular epithelial necrosis. Mild fibrosis with infiltration into residual xenograft can be observed with overall mild inflammation noted.

introduced. This required initial forceful use of a dermatome, later deepened with scalpel. As a result, the duration of each surgery was several hours. The cumulative impact associated with the numerous and frequent sedations required to obtain the longitudinal biopsies and clinical graft assessments would be considerable. A clinical hallmark of patients experiencing extensive trauma or considerable surgery is the increased need for resuscitative fluids and caloric support [91]. As a result, the study design in part 2 intentionally reduced this perceived surgical burden. Conversely, all four subjects of similar size tolerated the surgery and survived until the end of study without significant issues. Moreover, each of the four subjects in part 2 also presented with postoperative weight loss, consistent with subjects in part 1, but to a lesser degree that remained within acceptable parameters. The combination of negative postmortem findings and improved outcomes correlated with reduced surgical burden support the notion that the outcomes of subject 1001 and 1004 were not correlated to an effect caused by the xenograft test article.

CONCLUSION

Given the shortages in supplies of HCA—the clinical "gold standard" in the treatment of patients with severe burns [6, 15, 26, 28, 29, 58, 61]—the outcomes demonstrated in this study are encouraging. Great care was taken to closely replicate characteristics of current clinical treatment methods, including route of administration, severity, depth, and cumulative wound size, clinically relevant subject, absence of immunosuppressive drugs to prolong graft survival, and other critical variables needed to yield results that would be

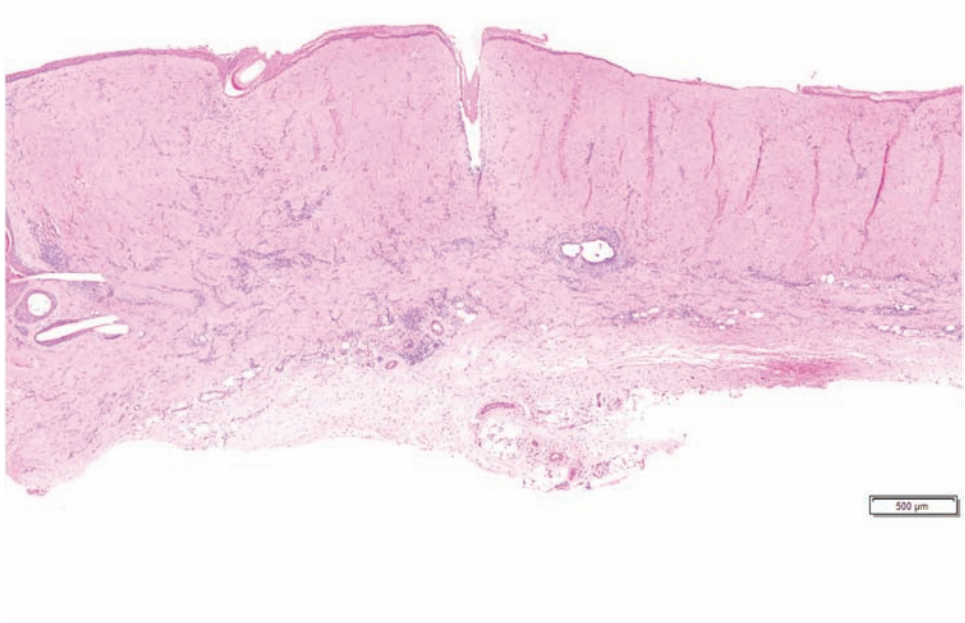

Figure 11. Study part 1, animal 1002, xenograft, POD-15. H&E, high-power image depicts tissue viability with surface and follicular epithelial necrosis. Mild fibrosis is seen, with infiltration into residual xenograft tissues and mild inflammation overall.

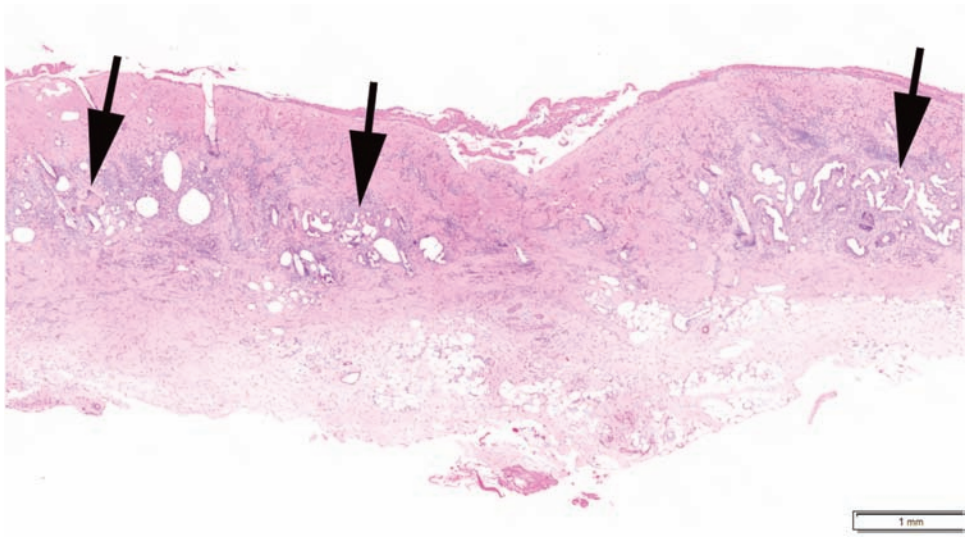

Figure 12. Study part 1, animal 1002, autograft, POD-22. H&E, high-power image demonstrating residual autograft (asterisks) with good overall viability. No surface epithelium and some surface necrosis noted, along with extensive fibrosis with infiltration into the autograft (arrows).

highly translatable to human patients. While the precise in vivo performance will be dependent on the recipients' specific condition and clinical status, preclinical studies suggest comparability of porcine xenografts to allograft and, as a consequence, will afford patients similar therapeutic benefits as those provided with HCA.

In parallel with these experiments, in vitro evaluations (reported separately) confirmed the preservation of viable cells following cryopreservation and quantified this residual metabolic activity. Chiu et al [61] emphasizes the need for the use of accurate nomenclature as, in this context, these data indicate that the materials described herein are better termed "skin xenotransplants" rather than xenografts in order to differentiate the present material from commonly available devitalized, lyophilized, or terminally sterilized porcine "collagen prostheses" that are unable to form an "organic union" [61]. To this point, Yamamato and Cooper et al also refer to such novel, vital, porcine-derived therapeutics as skin xenotransplants [65]. As such, we too have adopted this convention.

These studies strongly suggest that cryopreserved, porcine skin xenotransplants represent a therapeutic with potential significant advantages to the current standard of care. Therefore, the use of xenografts as an external, temporary treatment, holds tremendous promise and is being evaluated in a phase I clinical trial in 2019. Patients with severe, extensive, and deep partial- and full-thickness burn could benefit immensely from such a safe and efficacious innovation.

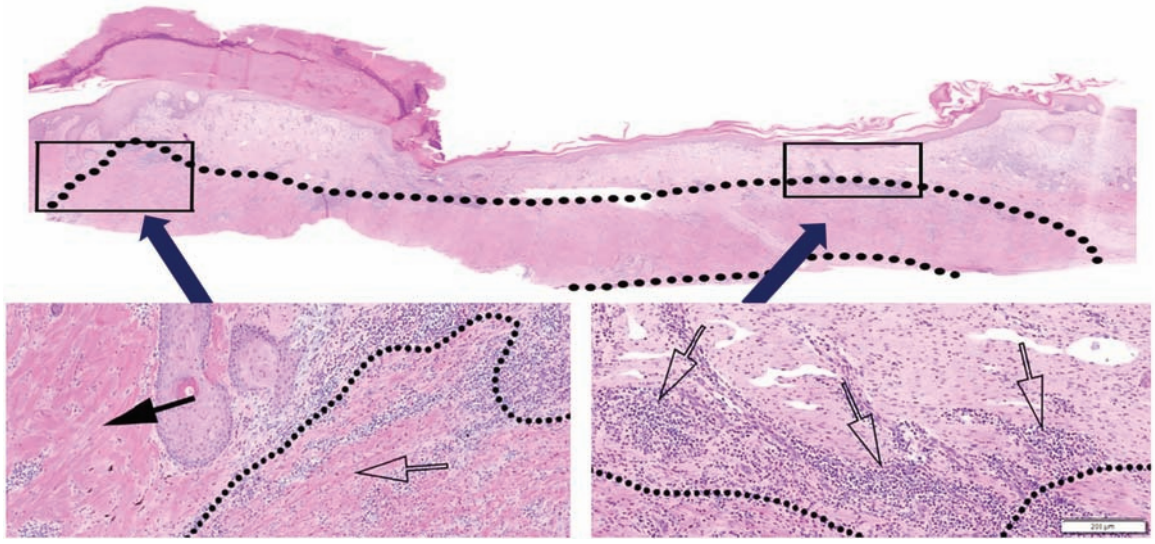

Figure 13. Study part 2, animal 2102, wound site 2, xenograft, POD-30. Top, center: H&E, low-power image of wound site depicts complete epithelial coverage. Dotted line surrounds the residual xenograft tissue. Bottom, left: H&E, higher power image of the large inset box. To the right and below the dotted line is the dermal component of the xenograft, with the xenograft dermal matrix indicated by an open arrow. To the left of the dotted line is the host dermis (black arrow) and the host dermal matrix. Mild inflammation is present and interpreted to be in response to the xenograft test article. Bottom, Right: H&E, higher power image of the small inset box. The dotted line roughly demonstrates the junction between the xenograft test article (below dotted line) and new collagen tissue (above dotted line), with intact epithelium at the top of the image. Mild inflammation in response to the xenograft (open arrows) is observed.

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REFERENCES

1. Kallinen O, Maisniemi K, Böhling T, Tukiainen E, Koljonen V. Multiple organ failure as a cause of death in patients with severe burns. J Burn Care Res 2012;33:206–11.
2. Kahn SA, Patel JH, Lentz CW, Bell DE. Firefighter burn injuries: predictable patterns influenced by turnout gear. J Burn Care Res 2012;33:152–6.
3. Atiyeh BS, Gunn SW, Hayek SN. Military and civilian burn injuries during armed conflicts. Ann Burns Fire Disasters 2007;20:203–15.
4. Lee RC, Astumian RD. The physicochemical basis for thermal and non-thermal 'burn' injuries. Burns 1996;22:509–19.
5. Benichou G, Yamada Y, Yun SH, Lin C, Fray M, Tocco G. Immune recognition and rejection of allogeneic skin grafts. Immunotherapy 2011;3:757–70.
6. Pruitt BA, Wolf SE, Mason AD. Epidemiological, demographic, and outcome characteristics of burn injury. In: Herndon DN, editor. Total burn care. 4th ed. Edinburgh: Elsevier (U.S.); 2012. p. 14–45.
7. American Burn Association. 2017. National Burn Repository Annual Report. available from http://ameriburn.org/wp-content/uploads/2018/04/2017_aba_nbr_annual_report_summary.pdf; accessed 31 May. 2019.
8. Kobayashi H, Kobayashi M, McCauley RL, Herndon DN, Pollard RB, Suzuki F. Cadaveric skin allograft-associated cytomegalovirus transmission in a mouse model of thermal injury. Clin Immunol 1999;92:181–7.
9. Cleland H, Wasiak J, Dobson H et al. Clinical application and viability of cryopreserved cadaveric skin allografts in severe burn: a retrospective analysis. Burns 2014;40:61–6.
10. Ismail Aly ME. Chapter 12. Operative Wound Management. In: Herndon DN, editor. Total burn care. 4th ed. Edinburgh: Elsevier (U.S.); 2018. p. 114–30.
11. Barret JP, Wolf SE, Desai MH, Herndon DN. Cost-efficacy of cultured epidermal autografts in massive pediatric burns. Ann Surg 2000;231:869–76.
12. Kua EH, Goh CQ, Ting Y, Chua A, Song C. Comparing the use of glycerol preserved and cryopreserved allogenic skin for the treatment of severe burns: differences in clinical outcomes and *in vitro* tissue viability. Cell Tissue Bank 2012;13:269–79.
13. Middelkoop E, Sheridan RL. Chapter 15 - Skin substitutes and 'the next level'. In: Herndon DN, editor. Total burn care 2nd ed. Philadelphia: Elsevier (U.S.); 2018. p. 167–73.
14. Culnan DM, Wolf SE. Chapter 32 - Critical care in the severely burned: organ support and management of complications. In: Herndon DN, editor. Total burn care, 5th ed. Edinburgh: Elsevier (U.S.); 2018. p. 328–54.
15. Burd A, Chiu T. Allogenic skin in the treatment of burns. Clin Dermatol 2005;23:376–87.
16. Lisiecki J, Levi B, Klein GL. (2018) Chapter 25 - importance of mineral and bone metabolism after burn. In: Herndon DN, editor. Total burn care, 5th ed. Edinburgh: Elsevier (U.S.); 2018. p. 268–75.
17. Church D, Elsayed S, Reid O, Winston B, Lindsay R. Burn wound infections. Clin Microbiol Rev 2006;19:403–34.
18. Culnan DM. Chapter 11 - treatment of infection in burn patients. In: Herndon DN, editor. Total burn care, 5th ed. Edinburgh: Elsevier (U.S.); 2018. p. 93–113.
19. Thornton J, Gosman A. Skin grafts and skin substitutes and principles of flaps. Selected Readings in Plastic Surgery 2004;10:16–9.
20. Castagnoli C, Alotto D, Cambieri I et al. Evaluation of donor skin viability: fresh and cryopreserved skin using tetrazolioum salt assay. Burns 2003;29:759–67.
21. Sheridan RL, Tompkins RG. Skin substitutes in burns. Burns 1999;25:97–103.
22. Branski LK. Chapter 14 - the skin bank. In: Herndon DN, editor. Total burn care, 5th ed. Edinburgh: Elsevier (U.S.); 2018. p. 159–61.
23. Bravo D, Rigley TH, Gibran N, Strong DM, Newman-Gage H. Effect of storage and preservation methods on viability in transplantable human skin allografts. Burns 2000;26:367–78.
24. Medawar PB. A second study of the behaviour and fate of skin homografts in rabbits: a report to the War Wounds Committee of the Medical Research Council. J Anat 1945;79(Pt 4):157–76.4.
25. Burke JF, Quinby WC, Bondoc CC, Cosimi AB, Russell PS, Szyfelbein SK. Immunosuppression and temporary skin transplantation in the treatment of massive third degree burns. Ann Surg 1975;182:183–97.
26. Ben-Bassat H, Chaouat M, Segal N, Zumai E, Wexler MR, Eldad A. How long can cryopreserved skin be stored to maintain adequate graft performance? Burns 2001;27:425–31.
27. Desai MH, Herndon DN, Broemeling L, Barrow RE, Nichols RJ Jr, Rutan RL. Early burn wound excision significantly reduces blood loss. Ann Surg 1990;211:753–9; discussion 759.
28. Zuo H, Song G, Shi W, Jia J, Zhang Y. Observation of viable alloskin vs xenoskin grafted onto subcutaneous tissue wounds after tangential excision in massive burns. Burns Trauma 2016;4:23.

29. Wachtel TL, Ninnemann J, Fisher JC, Frank HA, Inancsi W. Viability of frozen allografts. Am J Surg 1979;138:783–7.
30. Pirnay JP, Vandenvelde C, Duinslaeger L, Reper P, Vanderkelen A. HIV transmission by transplantation of allograft skin: a review of the literature. Burns 1997;23:1–5.
31. Hermans MHE. Porcine xenografts vs. (cryopreserved) allografts in the management of partial thickness burns: is there a clinical difference? Burns 2014;40:408–15.
32. Fishman JA, Greenwald MA, Grossi PA. Transmission of infection with human allografts: essential considerations in donor screening. Clin Infect Dis 2012;55:720–27.
33. Nellore A, Fishman JA. Donor-derived infections and infectious risk in xenotransplantation and allotransplantation. Xenotransplantation 2018;25:e12423.
34. Denner J. Reduction of the survival time of pig xenotransplants by porcine cytomegalovirus. Virol J 2018;15:171.
35. Gala JL, Vandenbroucke AT, Vandercam B, Pirnay JP, Delferrière N, Burtonboy G. HIV-1 detection by nested PCR and viral culture in fresh or cryopreserved postmortem skin: potential implications for skin handling and allografting. J Clin Pathol 1997;50:481–84.
36. Pianigiani E, Pierce JL. Skin: specific recovery and processing issues. In: Fehily D, editor. Tissue and cell processing. Oxford (U.K.): Blackwell Publishing; 2012. p. 217–28. Available from https://optn.transplant.hrsa.gov/; accessed 31 May. 2019.
37. OPTN/SRTR. 2017 Annual Data Report. American Journal of Transplantation 2019;s2:1–516.
38. OPTN: Organ Procurement and Transplantation Network. Available from https://optn.transplant.hrsa.gov/; accessed 31 May. 2019.
39. Hosseini SN, Mousavinasab SN, Fallahnezhat M. Xenoderm dressing in the treatment of second degree burns. Burns 2007;33:776–81.
40. Pegg DE. Viability assays for preserved cells, tissues, and organs. Cryobiology 1989;26:212–31.
41. Pruitt BA, Levine NS. Characteristics and uses of biologic dressings and skin substitutes. Arch Surg 1984;119:312–22.
42. Teh BT. Why do skin grafts fail? Plast Reconstr Surg 1979;63:323–32.
43. Rosenberg L, Shoham Y, Krieger Y et al. Minimally invasive burn care: a review of seven clinical studies of rapid and selective debridement using a bromelain-based debriding enzyme (Nexobrid®). Ann Burns Fire Disasters 2015;28:264–74.
44. Branski LK, Herndon DN, Barrow RE. Chapter 1 - a brief history of acute burn care management. In:Herndon DN, editor. Total burn care. 4th ed. Edinburgh: Elsevier (U.S.); 2012. p. 1–7.
45. Greenwood J, Amjadi M, Dearman B, Mackie I. Real-time demonstration of split skin graft inosculation and integra dermal matrix neovascularization using confocal laser scanning microscopy. Eplasty 2009;9:309–18.
46. Converse JM, Uhlschmid GK, Ballantyne DL Jr. "Plasmatic circulation" in skin grafts. The phase of serum imbibition. Plast Reconstr Surg 1969;43:495–99.
47. Converse JM, Smahel J, Ballantyne DL Jr, Harper AD. Inosculation of vessels of skin graft and host bed: a fortuitous encounter. Br J Plast Surg 1975;28:274–82.
48. Birch J, Brånemark PI. The vascularization of a free full thickness skin graft. I. A vital microscopic study. Scand J Plast Reconstr Surg 1969;3:1–10.
49. Birch J, Brånemark PI, Lundskog J. The vascularization of a free full thickness skin graft. II. A microangiographic study. Scand J Plast Reconstr Surg 1969;3:11–7.
50. Rudolph R, Klein L. Healing processes in skin grafts. Surg Gynecol Obstet 1973;136:641–54.
51. Smahel J. The healing of skin grafts. Clin Plast Surg 1977;4:409–24.
52. Clemmesen T. The early circulation in split skin grafts. Acta Chir Scand 1962;124:11–8.
53. Abbas AK, Lichtman AHH, Pillai S. Cellular and molecular immunology. Edinburgh: Elsevier; 2017.
54. Laurens N, Koolwijk P, de Maat MP. Fibrin structure and wound healing. J Thromb Haemost 2006;4:932–9.
55. Branski LK, Mittermayr R, Herndon DN et al. Fibrin sealant improves graft adherence in a porcine full-thickness burn wound model. Burns 2011;37:1360–66.
56. Tavis MJ, Thornton JW, Harney JH, Woodroof EA, Bartlett RH. Graft adherence to de-epithelialized surfaces: a comparative study. Ann Surg 1976;184:594–600.
57. Lim K, Yoon C, Lee J. Antimicrobial effect of skin for allograft and management in burn wound. Open Journal of Organ Transplant Surgery 2017; 7:1–11.
58. Ge L, Sun L, Chen J et al. The viability change of pigskin *in vitro*. Burns 2010;36:533–38.
59. Sheridan R. Closure of the excised burn wound: autografts, semipermanent skin substitutes, and permanent skin substitutes. Clin Plast Surg 2009;36:643–51.
60. Leto Barone AA, Mastroianni M, Farkash EA et al. Genetically modified porcine split-thickness skin grafts as an alternative to allograft for provision of temporary wound coverage: preliminary characterization. Burns 2015;41:565–74.
61. Chiu T, Burd A. "Xenograft" dressing in the treatment of burns. Clin Dermatol 2005;23:419–23.
62. Wood M, Hale HW Jr. The use of pigskin in the treatment of thermal burns. Am J Surg 1972;124:720–3.
63. Weiner J, Yamada K, Ishikawa Y et al. Prolonged survival of GalT-KO swine skin on baboons. Xenotransplantation 2010;17:147–52.
64. Leonard DA, Mallard C, Albritton A et al. Skin grafts from genetically modified α-1,3-galactosyltransferase knockout miniature swine: a functional equivalent to allografts. Burns 2017;43:1717–24.
65. Yamamoto T, Iwase H, King TW, Hara H, Cooper DKC. Skin xenotransplantation: historical review and clinical potential. Burns 2018;44:1738–49.
66. Platt JL, Lin SS. The future promises of xenotransplantation. Ann N Y Acad Sci 1998;862:5–18.
67. Cooper DKC, Ekser B, Tector AJ. A brief history of clinical xenotransplantation. Int J Surg 2015;23(Pt B):205–10.
68. Schook LB, Collares TV, Darfour-Oduro KA et al. Unraveling the swine genome: implications for human health. Annu Rev Anim Biosci 2015;3:219–44.
69. Sullivan TP, Eaglstein WH, Davis SC, Mertz P. The pig as a model for human wound healing. Wound Repair and Regeneration: Official Publication of the Wound Healing Society [and] the European Tissue Repair Society 2001;9:66–76.
70. Denner J. Paving the path toward porcine organs for transplantation. N Engl J Med 2017;377:1891–93.
71. Vodicka P, Smetana K Jr, Dvoránková B et al. The miniature pig as an animal model in biomedical research. Ann N Y Acad Sci 2005;1049:161–71.
72. Lineen E, Namias N. Biologic dressing in burns. J Craniofac Surg 2008;19:923–8.
73. Elliott R, Hoehn J. Commercial porcine skin for wound dressings. Plastic and Reconstructive Surgery 1973;52:401–5.
74. Cooper DKC, Gaston R, Eckhoff D et al. Xenotransplantation—the current status and prospects. British Medical Bulletin 2018;125:5–14.
75. Robson SC, Schulte am Esch J 2nd, Bach FH. Factors in xenograft rejection. Ann N Y Acad Sci 1999;875:261–76.
76. Wang ZY, Martens GR, Blankenship RL et al. Eliminating Xenoantigen Expression on Swine RBC. Transplantation 2017;101:517–23.
77. Mezrich JD, Haller GW, Arn JS, Houser SL, Madsen JC, Sachs DH. Histocompatible miniature swine: an inbred large-animal model. Transplantation 2003;75:904–7.
78. Kolber-Simonds D, Lai L, Watt SR et al. Production of -1,3-galactosyltransferase null pigs by means of nuclear transfer with fibroblasts bearing loss of heterozygosity mutations. Proceedings of the National Academy of Sciences 2004;101:7335–40.
79. Lai L, Kolber-Simonds D, Park KW et al. Production of alpha-1,3-galactosyltransferase knockout pigs by nuclear transfer cloning. Science 2002;295:1089–92.
80. Hawley RJ. Genetic modification of pigs by nuclear transfer. Xenotransplantation 2002;9:159–60.
81. Sachs DH, Leight G, Cone J, Schwarz S, Stuart L, Rosenberg S. Transplantation in miniature swine. I. Fixation of the major histocompatibility complex. Transplantation 1976;22:559–67.
82. Albritton A, Leonard DA, Leto Barone A et al. Lack of cross-sensitization between α-1,3-galactosyltransferase knockout porcine and allogeneic skin grafts permits serial grafting. Transplantation 2014;97:1209–215.
83. Gao H, Zhao C, Xiang X et al. Production of α1,3-galactosyltransferase and cytidine monophosphate-N-acetylneuraminic acid hydroxylase gene double-deficient pigs by CRISPR/Cas9 and handmade cloning. J Reprod Dev 2017;63:17–26.
84. Burlak C, Paris LL, Lutz AJ et al. Reduced binding of human antibodies to cells from GGTA1/CMAH KO pigs. Am J Transplant 2014;14:1895–900.
85. Fujita T, Miyagawa S, Ezoe K et al. Skin graft of double transgenic pigs of N-acetylglucosaminyltransferase III (GnT-III) and DAF (CD55) genes survived in cynomolgus monkey for 31 days. Transpl Immunol 2004;13:259–64.
86. Leonard DA, Kurtz JM, Mallard C et al. Vascularized composite allograft tolerance across MHC barriers in a large animal model: VCA tolerance in miniature swine. American Journal of Transplantation 2014;14:343–55.
87. Galili U, Shohet SB, Kobrin E, Stults CL, Macher BA. Man, apes, and Old World monkeys differ from other mammals in the expression of alpha-galactosyl epitopes on nucleated cells. J Biol Chem 1988;263:17755–62.
88. Holmes BJ, Richards AC, Awwad M et al. Anti-pig antibody levels in naïve baboons and cynomolgus monkeys. Xenotransplantation 2002;9:135–47.
89. Rosso MC, Badino P, Ferrero G, Costa R, Cordero F, Steidler S. Biologic data of cynomolgus monkeys maintained under laboratory conditions. PLoS One 2016;11:e0157003.
90. Lee SJ, Kim JS, Chee HK et al. Seven years of experiences of preclinical experiments of xeno-heart transplantation of pig to non-human primate (cynomolgus monkey). Transplant Proc 2018;50:1167–171.
91. Carson JS, Herndon DN. Chapter 28. Nutritional needs and support for the burned patient. In: Herndon DN, editor. Total burn care, 5th ed. Edinburgh: Elsevier (U.S.); 2018. p. 287–300.