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VALIDATION OF A VERTICAL PROGRESSION PORCINE BURN MODEL

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Abstract

Background—A major potential goal of burn therapy is to limit progression of partial to full-thickness) burns. To better test therapies, we developed and validated a vertical progression porcine burn model in which partial thickness burns treated with an occlusive dressing convert to full thickness burns that heal with scarring and wound contraction.

Methods—Forty contact burns were created on the backs and flanks of two young swine using a 150 gm aluminum bar preheated to 70°, 80°, or 90°Celsius for 20 or 30 seconds. The necrotic epidermis was removed and the burns were covered with a polyurethane occlusive dressing. Burns were photographed at 1, 24, and 48 hours as well as at 7, 14, 21, and 28 days post injury. Full thickness biopsies were obtained at 1, 4, 24, and 48 hours, as well as at 7 and 28 days. The primary outcomes were presence of deep contracted scars and wound area 28 days after injury. Secondary outcomes were depth of injury, reepithelialization, and depth of scars. Data were compared across burn conditions using ANOVA and χ^2 tests.

Results—Eight replicate burns were created with the aluminum bar using the following temperature/contact-time combinations: 70/20, 70/30, 80/20, 80/30, and 90/20. The percentage of burns healing with contracted scars were 70/20–0%, 70/30–25%, 80/20–50%, 80/30–75%, and 90/20–100% (P=0.05). Wound areas at 28 days by injury conditions were 70/20–8.1 cm^2, 70/30–7.8 cm^2, 80/20–6.6 cm^2, 80/30–4.9 cm^2, and 90/20–4.8 cm^2 (P=0.007). Depth of injury judged by depth of endothelial damage for the 80/20 and 80/30 burns at 1 hr was 36% and 60% of the dermal thickness respectively. The depth of injury to the endothelial cells 1 hour after injury was inversely correlated with the degree of scar area (Pearson's correlation r=−0.71, P<0.001).

Conclusions—Exposure of porcine skin to an aluminum bar preheated to 80°C for 20 or 30 seconds results initially in a partial thickness burn that when treated with an occlusive dressing progresses to a full thickness injury and heals with significant scarring and wound contracture.

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INTRODUCTION

Burns are fairly common and occasionally devastating injuries that may result in substantial morbidity. Partial thickness (2nd degree) burns can progress to full-thickness (3rd-degree) burns over the first few days after injury,[1] increasing the need for skin grafts, the risk of infection, and ultimately scarring and wound contracture.[2] Development of effective therapies to limit burn injury requires both a basic understanding of underlying pathophysiologic events that lead to burn injury progression, and validated animal burn models that mimic human burn injury progression. Due to the similarity of porcine to human skin,[1, 3] it is generally accepted that the pig is the preferred animal species in which to study burns.

A large number of porcine burn models have been reported in the literature. Burns have been created with scalding water, contact with heated metal or glass objects, or radiation of heat from a heat source to name but a few. We and others have used the comb model originally described by Regas and colleagues in rats.[4] We have utilized this model extensively in rats,[5, 6] and more recently in pigs.[7] In this model several full-thickness rectangular burns are created that are separated by thin strips of unburned skin that represent zones of stasis as described by Jackson.[8] With this model, when left untreated, most of the unburned interspaces undergo full thickness necrosis over the course of 2–3 days after injury.[4, 7] Using this model, a number of potential therapeutic agents aimed at minimizing burns injury progression have been investigated.[5, 6] While perhaps useful as a screening tool in the rat, this model is not representative of most clinical burns in humans, where burn injury progression occurs not only in the horizontal plane, but also in the vertical plane as partial-thickness burns convert to full-thickness burns requiring excision and grafting to minimize scarring and contractures.

The purpose of the current study is to develop and validate a porcine burn model of partial thickness burns that when treated with an accepted therapy (such as an occlusive dressing) will progress to full thickness injuries that result in significant scarring and wound contraction. It is our intention to then use this model to further evaluate several promising therapeutic agents that have been shown to be effective in reducing burn injury progression in both the rat and pig hot comb burn model. Furthermore, it is our hope that our colleagues in the medical and scientific communities will be able to use this model in their own investigations of treatments to prevent the conversion of partial to full thickness burn injuries.

METHODS

Study Design

A randomized controlled experiment was conducted to compare different depths of burn injury on injury progression, wound healing and scarring using various temperatures and durations of exposure. This project was approved by the Institutional Animal Care and Use Research Review Board.

Setting and Animals

This study was conducted in the Division of Laboratory Animal Research of a university-based academic medical center. Two young female Yorkshire pigs weighing 20–25 kg were used in this study. As with many studies of cutaneous injury and wound healing, domestic pigs were selected as the experimental animal since of all animals, the skin of pigs most closely resembles that of humans both structurally and functionally.[1, 3] Animals were given a standard diet ad lib several days prior to the investigation and were fasted overnight before any procedures. Housing and care for animals was in accordance with the National Research Council guidelines.[9]

Study Protocol and Interventions

Animals were sedated with a combination of acepromazine 0.1 mg/kg, atropine 0.02 mg/kg, ketamine 20 mg/kg, and xylazine 2 mg/kg by intramuscular injection. The pigs were then intubated endotracheally and maintained under a surgical plane of anesthesia with isoflurane 0.5–2.5% in room air. The flank and back hair was clipped with hair clippers and the skin was scrubbed with a povidine iodine solution.

Prior to creating the burn, a 2.5 cm by 2.5 cm black rectangular tattoo was created on the pig's flank to outline the placement of the hot bar. Burns were created on the animals' backs and flanks within the tattoos by applying a 2.5 cm by 2.5 cm, 150 gram aluminum bar preheated in hot water to 70°, 80°, or 90°C (Figure 1). The heated bar was wiped dry just prior to application to prevent water droplets from creating a steam burn on the skin. The bar was then placed at a vertical position perpendicular to the skin's surface and applied for a period of 20 or 30 seconds. The aluminum bar was applied using a spring-loaded device designed to control the amount of pressure applied to the skin (2 kg/6.25cm^2). A prior study has shown that the amount of force applied is a major determinant of burn depth.[10] Twenty burns were inflicted on each of 2 pigs (evenly distributed between both side of the pigs), for a total of 40 burns. Each of the five temperature-time combinations (70°C for 20s, 70°C for 30s, 80°C for 20s, 80°C for 30s, and 90°C for 20s) was replicated four times on each of the two animals. The location and order of burn infliction were randomized. In order to simulate burn blister debridement, the necrotic epidermis was gently removed by rubbing the burn with blunt side of tissue forceps (Figure 1). Removal of the necrotic epidermis results in delayed reepithelialization, and increased scar formation.[11] The wounds were covered with a polyurethane occlusive dressing (Tegaderm™, 3M, St Paul, MN) and wrapped with a gauze dressing and adhesive wrap. Dressing changes were performed daily for the first 5 days and at 7, 10, 14, and 21 days. Animals were observed frequently for signs of pain or discomfort and treated with IM buprenorphine 0.01 mg/kg as needed. All animals were euthanized by IV pentobarbital euthanasia 4 weeks after injury. Histopathological studies were done on formalin-fixed, alcohol-dehydrated, xylene-cleared, paraffin-embedded, hematoxylin and eosin stained 5 micron sections using conventional microscopy. Further analysis was performed using goat antibodies to porcine CD31 (R&D Systems, Minneapolis, MN), a marker of endothelial cells, and rabbit antibodies to human activated Caspace 3 (Cell Signaling Technoloy, Danvers, MA), a marker of apoptosis. All observations were performed by a board certified dermatopathologist (SAM) masked to burn conditions.

Measures and Outcomes

The primary outcomes were the presence or absence of an hourglass shaped contracted scar and the total surface area of the healed burns at 28 days after injury. Secondary outcomes were: gross reepithelialization, microscopic reepithelialization, level of initial injury as judged by morphometric analysis of dermal structures,[12] depth of apoptosis, the percentage of wounds that healed with a full thickness dermal scar and the depth of the scar on histomorphologic evaluation.

The burns were visualized at 1, 2, 5, 7, 10, 14, 21, and 28 days at which times digital images were obtained (Canon, PowerShot SD 1200IS, Lake Success, NY). Full thickness 4 mm punch biopsies (Miltex Instrument Company Inc., Lake Success, NY) were obtained from all burns on one of the animals at the following time points: 1, 4, 24, and 48 hours as well as after 7. Full thickness 8 mm biopsies were obtained from all burns on the other animal at 28 days.

Gross Scar Assessments

All scar assessments were made on the pig that did not have prior biopsies taken during the earlier phases of the experiment. The gross visual appearance of the scar was classified as none or minimal contraction vs. significant contraction as evidenced by an hour-glass shaped scar. We also calculated the total surface area of the healed burns at 28 days using the digital images and Image J software 1.43 (NIH.gov).

Gross Assessment of Reepithelialization by Visual Inspection

A gross estimation of wound reepithelialization was also performed by visualizing the burns in the pig that was not biopsied until day 28. Wounds were considered to be reepithelialized if the wound was covered by an opalescent white coating. Gross wound reepithelialization was classified as (0) none, (1): partial (1–50% reepithelialized), (2) nearly complete (51–95% reepithelialized), and (3) complete (>95% reepithelialized but red and without hair growth). Healed wounds that appeared normal in color and hair growth were classified as a 4. Inter-observer agreement using this classification was calculated by having two observers independently evaluate the wounds (Spearman's correlation=0.68).

Reepithelialization by Microscopic Evaluation

The percentage of microscopic reepithelialization at day seven was calculated by measuring the length of the neoepidermis in cross section and dividing it by the specimen's length multiplied by 100 (inter-observer agreement, r=0.99).[13]

Depth of Injury

The depth of injury at 1–48 hours was measured separately for each of the following dermal components: collagen, hair follicles, endothelial cells, and interstitial cells (fibroblasts, dermal dendritic cells, and macrophages).[12] The depth of injury was measured as the vertical distance from the most superficial surface of the burn to the lowest part of the specimen indicating injury using an ocular micrometer. Cellular necrosis was considered present if the cells appeared swollen or fragmented and/or the nuclei were pyknotic. The collagen was

considered injured when the fibers appeared thin, flattened, or completely absent and also by the presence of basophilic staining. The collagen fibers were viewed under normal as well as polarized light.[14] Apoptosis was identified from immunohistochemical staining, and apoptotic zones were defined as areas with more than 10 percent of cells staining positively for apoptosis. The depth of the beginning and end of these zones was recorded.

Scarring by histomorphologic analyses

The depth of the scar was measured as the vertical distance from the surface of the skin to the lowest level of the scar. The scar was identified using H&E stained specimens visualized under polarized light. The scar is characterized by thin collagen fibers running mostly in a horizontal direction.[14]

Data Analysis

Continuous data are presented as means and standard deviations (SD) and compared with analysis of variance (ANOVA). Binary data are presented as the percentage frequency of occurrence and compared with $\chi 2$ or the Fischer exact test as appropriate. Inter-observer agreement was measured with Pearson or Spearman correlations as appropriate. Linear regression and Pearson's correlation coefficients were used to assess the relationship between depth of initial injury and scar contracture expressed as surface area of the scar at 28 days. All data were analyzed using SPSS 18.0 for Windows (SPSS Inc., Chicago, IL) software. All histological assessments were performed by a board certified Dermatopathologist (SAM) masked to burn injury conditions.

RESULTS

We created 20 burns on each of the two pigs for a total of 40 burns, with 8 replicates corresponding to each of the five temperature duration conditions (70°C for 20s, 70°C for 30s, 80°C for 20s, 80°C for 30s, and 90°C for 20s). The 20 burns on the pig that were not biopsied until day 28 were used to assess the primary outcome of scar formation and the results are displayed in Figure 2. The percentage of burns healing with contracted, hour glass shaped scars by injury conditions were: 70/20–0%, 70/30–25%, 80/20–50%, 80/30–75%, and 90/20–100% (P=0.05; Figure 2). The mean (SD) areas of the tattoos on day 28 in cm^2 were: 8.10 (1.26), 7.83 (.85), 6.56 (1.20), 4.86 (2.01), 4.82 (1.10) (P=.007). This animal was also used to assess the secondary outcomes of depth of scar formation at day 28 and re-epithelialization as determined by gross inspection. The depth of scarring noted on microscopic evaluation increased with greater exposure times and durations (Figure 3). All 70/20 and 70/30 burns were re-epithelialized at 14 days, but only half of the 80/20 burns were, and no 80/30 and 90/20 were re-epithelialized at this time point (Table 1). At 21 days after injury, all burns were re-epithelialized based on gross inspection.

The pig that was biopsied during the early phases of the study was used for the secondary outcomes of burn depth and re-epithelialization by microscopic evaluation. The initial depths of injury at one hour after creating the burns are presented in Table 1. There was a significant increase in the depth of injury to collagen and the endothelial cells with increasing exposure times and temperatures (Table 1). At 1 hr 70/20, 70/30, 80/20 and 80/30

burns demonstrated a depth of injury limited to the upper half of the dermis while the 90/20 burns were deep dermal (Table 1). The effects of various exposure times and durations on the depth of injury at 4, 24, and 48 hours are also presented in Table 1. While the relationship between burn conditions and depth of collagen injury disappears at 48 hours, the trend for interstitial cells is stronger at the 24 and 48 hour time points. Endothelial depth of injury shows a significant increase with increasing temperatures and times at each of our time points in the first 48 hours (Figure 4). Also noted is plugging of the blood vessels with red blood cells. Differences in depth of injury to follicular cells were less discernable at different time points and burn conditions (Table 1). Photomicrographs of representative burns 4 hours after injury are presented in Figure 5. A zone of apoptosis was noted as early as 4 hours after injury in the more superficial (70/20 and 70/30) burns in the upper dermis, and did not progress to deeper levels at later time points (not shown). With the more severe burning conditions, apoptosis was not evident at any level at early time points, but zones of apoptosis appeared deep in the dermis at 24hrs, at the boundary of the area of necrosis (Figure 6). Only rare, isolated apoptotic cells were noted at later time points.

The mean (SD) percentage reepithelialization at day 7 on microscopy by burn conditions were 70/20, 55% (41%); 70/30, 18% (22%); 80/20, 25% (50%); 80/30, 0% (0%); and 90/20, 0% (0%); but were not significant (P=0.12). As seen in Figure 7, the depth of injury to the endothelial cells 1 hour after injury was inversely correlated with the degree of scar area (Pearson's correlation r=−0.71, P<0.001).

DISCUSSION

Burns are dynamic injuries that typically progress over the course of the first few days after injury,[15–17] possibly as a result of an inflammatory response, ischemia and/or ischemia-reperfusion.[18] Progression of partial thickness (2nd degree) burns to full thickness burns (3rd degree) is often referred to as burn conversion and is a critical event in the burn outcomes.[19] Partial thickness burns generally reepithelialize within 1–2 weeks and ultimately heal with minimal, if any, scarring. In contrast, full thickness burns often take longer than 2 weeks to reepithelialize and ultimately heal with significant scarring and wound contraction.[2] This leads to significant aesthetic and functional disability. Thus, burn wound management should include treatment to minimize burn wound conversion. Currently most burn wound therapies are designed to stabilize the patient, and prevent wound infection and desiccation.[19] However, there is no approved therapy that specifically limits burn injury progression.

One of the barriers for developing burn therapies to limit burn injury progression is a valid, reliable animal model that replicates burn wound conversion in humans. This study was specifically designed to identify a set of temperature and exposure times that reliably produce an initial mid-dermal, partial thickness burn, that when treated with a standard of care therapy (e.g., an occlusive dressing) progresses to a full thickness burn that ultimately heals with a contracted scar. In this study we found that exposure of porcine skin to a heated aluminum bar at 80°C for 20 or 30 seconds resulted in an initial mid-dermal partial thickness burn that converted to a full thickness burn. Supporting this conclusion are the facts that significant scar contraction occurred in both sets of burns (half of the 80/20 burns

and 3/4 of the 80/30 burns healed with contracted scars), and half of the 80/20 burns and all of the 80/30 burns required longer than 2 weeks to re-epithelialize. The results for the 80/20 burns confirm a prior study by our laboratory using an 80°C by 20 second burn that when treated with the standard of care resulted in significant scarring that was partially reversed when treated with an antagonist of the profibrotic cytokine TGF beta 1.[20] We also found evidence of apoptosis at the margin of the necrotic area suggesting a progression of injury with a potentially preventable apoptotic zone emerging at 24 hours in the more severe burn injuries. Furthermore, the delayed appearance of apoptosis in the more severe burns suggests the presence of a qualitatively different and unidentified pathophysiological mechanism from the superficial injuries.

The discovery that the depth of injury for the 80/20 and 80/30 burns is limited to the mid-dermis or reticular dermis at one hour further suggests the utility of our model in evaluating current and future burn therapies. Based on the current study, as well as the one above,[20] conversion of these burns from partial to full thickness may potentially be prevented by early interventions.

Of all of our histological measures of burn depth, we found endothelial injury to be the earliest and most consistent measure of depth of injury that distinguished between the various burn injury conditions. Since blood vessels contain fluid (blood) within their lumen they conduct heat much better than other dermal elements, such as collagen which functions as an insulator. As a result it is not surprising that the endothelial cells that surround the blood vessels are more susceptible to injury than other cell types. We also believe that plugging of the vessels with aggregated red blood cells, which is apparent early after injury, plays a role in burn injury progression. The importance of endothelial cell injury depth is further demonstrated by the inverse correlation between depth of endothelial injury at 1 hour and ultimate scar surface area, thus, the deeper the initial injury to the vessels, the greater the amount of scar contraction. While injury to endothelial cells did not appear to progress over the first 48 hours, evidence of injury progression to other elements of the dermis was notedThis suggests that burn injury progression may by driven in part by endothelial injury and resultant ischemia, and could potentially be limited by interventions to increase vascular supply to burned tissue.

Interstitial cells proved to be useful at 24 and 48 hours, but not at the 1 and 4 hours. Collagen denaturation was found to be a poor indicator of burn progression, as the measurements in each group were superficial and no progression was evident over the first 48 hours. This leads us to believe that collagen injury reflects initial heat denaturation rather than burn progression. Our measures of follicular injury were not significant despite the importance of hair follicle survival to burn healing in the literature.[6] We are currently developing an improved measure of depth of injury using an immuno-histochemical stain for the non-histone nuclear binding protein High Mobility Group Box 1, an early marker of necrosis.[21] This protein is released from nuclei before they become pyknotic and cytoplasmic swelling occurs, and should allow accurate determinations of interstitial depth of injury to be made at earlier time points.[22] In addition, we expect that the increased sensitivity of this measure will not only be an accurate measure of depth of follicular injury, but may also possibly be prognostic as well. Despite the limitations of the collagen and

interstitial measures of burn depth, they did concur with the endothelial data that depth of injury was not full thickness in either the 80/20 or 80/30 burns at 1 hour post burn.

Other investigators have also developed animal burn models in which healing was monitored over time. Cuttle et al developed a deep dermal partial thickness burn model using a glass bottle with the bottom replaced by a plastic wrap.[23] When using water at 92°C for 15 seconds, contact of the bottle with the pig's skin resulted in deep partial thickness burns that required 3–5 weeks to completely reepithelialize. Most such burns healed with contracted, purple, hypertrophic scars, similar to many of the burns in our current study. In contrast, our study evaluated a wider spectrum of injuries including more superficial as well as deeper burns. While deep partial thickness and full-thickness burns described by Cuttle and observed here with 90°C for 20 seconds may not be preventable, full-thickness burns that arise from conversion of mid-dermal partial thickness burns, such as those created with hot bars at 80°C for 20 seconds or 80°C for 30 seconds described here, are potentially preventable. In fact, we[5, 6] and others[24] have shown the ability of a number of therapeutic agents to reduce burn injury progression in the horizontal plane using the comb burn model. We are now planning to evaluate some of the same therapies in the vertical progression model in pigs that we believe more closely reflects what occurs in human burns.

Our study has several limitations. First, our sample size was relatively small. However, we attempted to minimize the number of animals euthanized by creating multiple burns on each animal. We recognize that use of a larger number of animals may have been preferred. Second, follow-up was limited to 28 days only. However, at this time point significant wound contracture and scarring was already evident with many of the burns. Thus it is unclear whether longer follow up would have altered our results. Third, it is possible that our results may be the result of delayed recognition of cell death rather than actual injury progression. However, our analysis of apoptosis indicating potentially preventable cell injury, further supports our contention that we are observing injury progression rather than delayed recognition. Furthermore, studies using other burn models have demonstrated the ability to limit injury progression after burns,[6,25] which could only be possible with injury progression and not delayed recognition. Fourth, as with all animal models, it is unclear how our results in the porcine model will generalize to human burns and other burn mechanisms. Finally, we required different animals for early biopsies and gross assessment, as the relatively large number of biopsies taken could have affected scar formation.

Conclusion

A vertical burn progression model in pigs was developed that creates initially partial thickness mid dermal burns that convert to full thickness burns with standard treatment consisting of an occlusive dressing. 80/20 and 80/30 burns made according to this model are appropriate for studying burn therapies that can be provided early after injury. We believe that moving beyond horizontal models of burn progression will facilitate the development of novel therapies that will reduce the morbidity and mortality resulting from burn injuries by preventing the conversion of partial to full thickness burns.

Acknowledgments

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Figure 1.

Interventions. Burns are made on tattooed areas of skin using a spring loaded aluminum bar (left). This bar was preheated in hot water and applied to the skin of the animal to create a 2.5 by 2.5 cm burn. Necrotic epidermis is scraped off to stimulate debridement (right). The burn is scraped with the handle of a scalpel that allows easy removal of the outermost layer which is the necrotic epidermis.

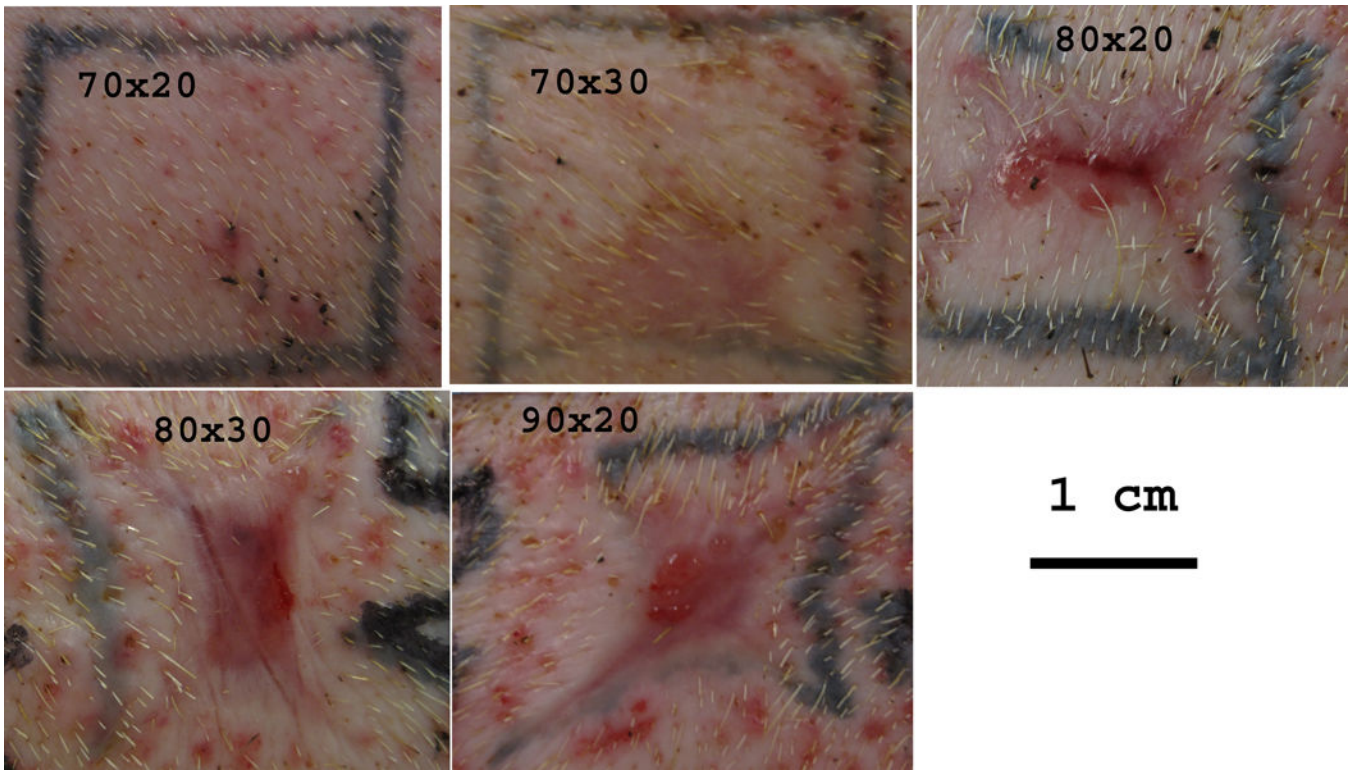

Figure 2.

Primary outcomes are illustrated by representative gross images of healed burns 28 days after injury and a histogram of the area of burn tattoos at day 28. The black line is the scale bar. Scarring and wound contracture is noted with the more extreme burns (80×20, 80×30, 90×20).

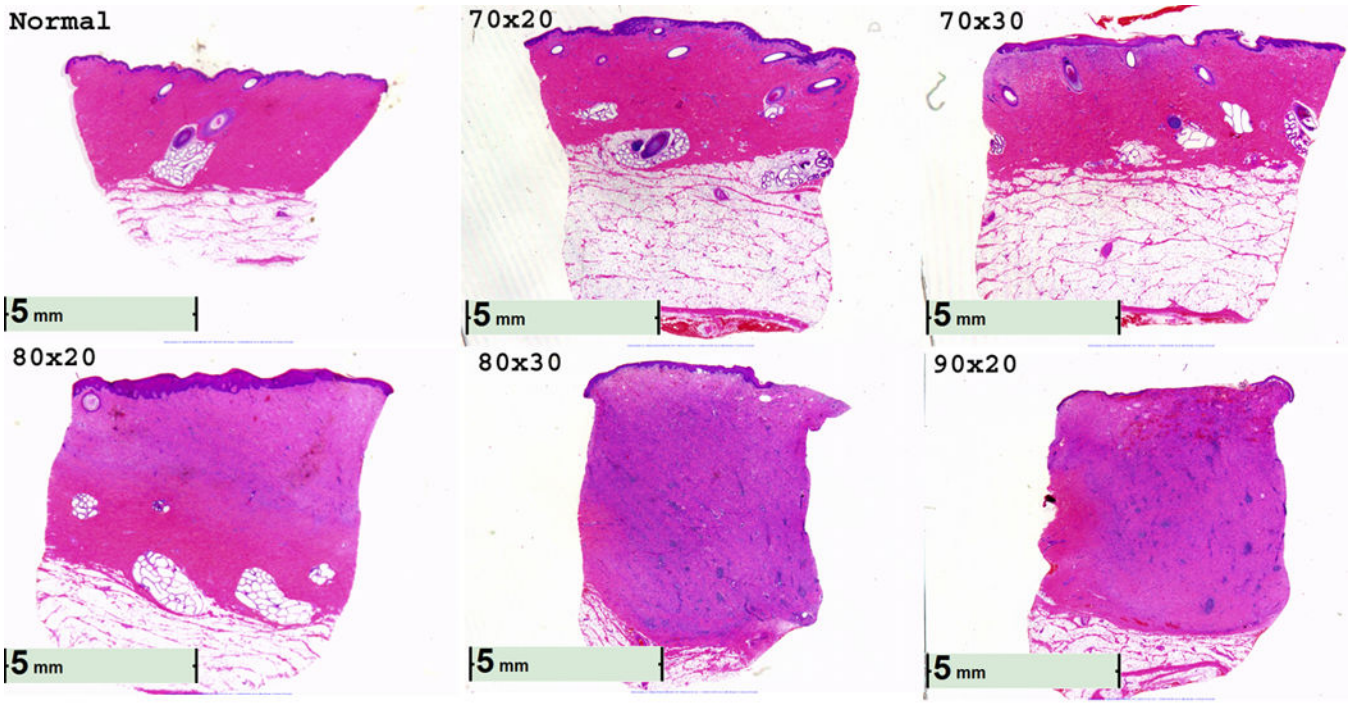

Figure 3.

Photomicrograph of representative normal uninjured skin and healed burns 28 days after injury (H&E). Progressively deeper scars (noted by the more dense magenta-purple colored tissue) are present with increasing temperatures and exposure times. The thickness of the scar with the 80×30 and 90×20 burns is approximately twice the thickness of normal uninjured skin.

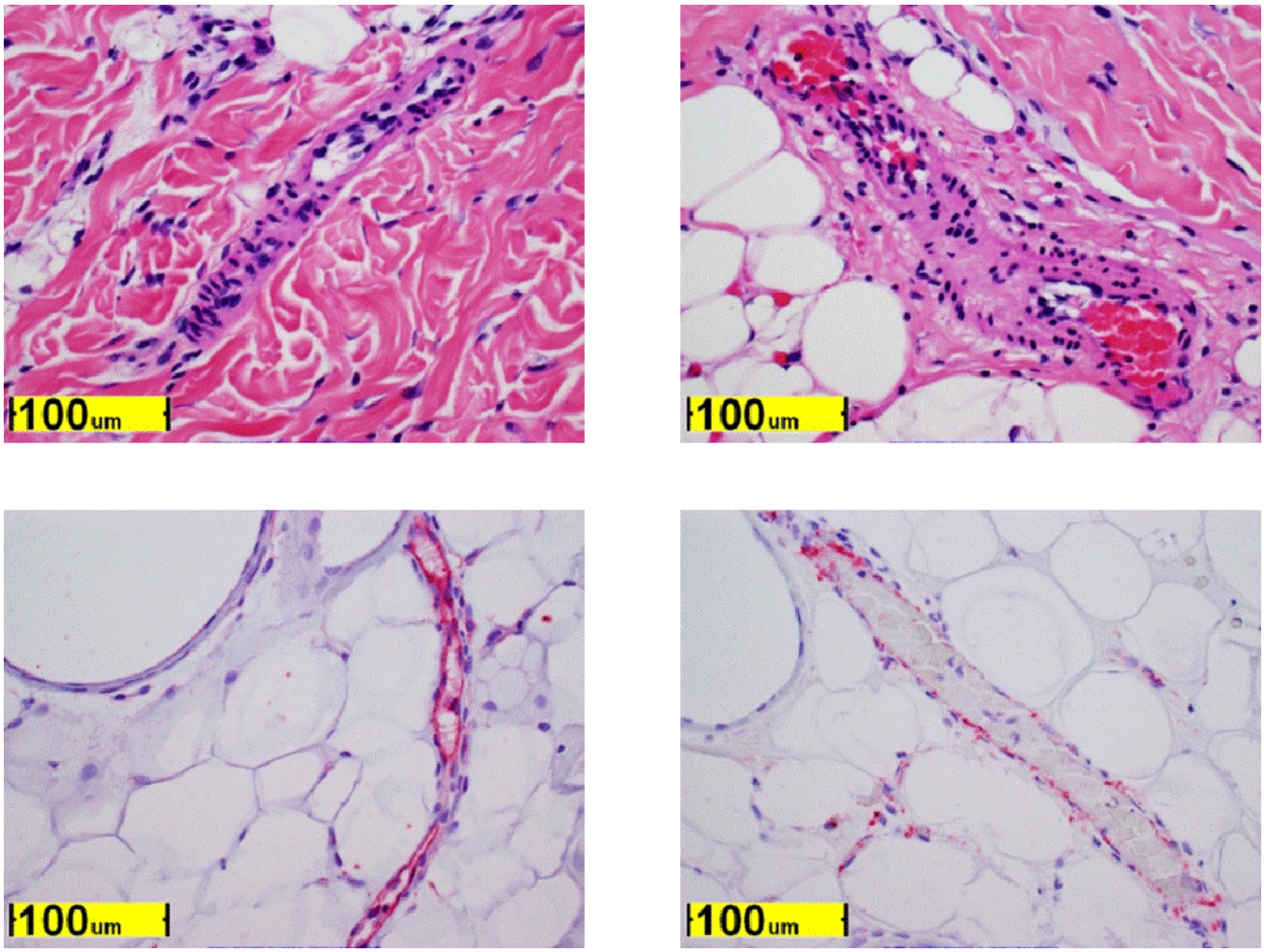

Figure 4.

Photomicrographs of normal uninjured blood vessels and necrotic blood vessels 4 hours after injury stained with Hematoxylin & Eosin (H&E) and CD31 antibodies. A. H&E stain of blood vessel from uninjured tissue. B. CD31 stained blood vessel from uninjured tissue. The red staining areas are the endothelial cell membranes. C. H&E stained injured blood vessel at a depth of 1.5 mm from the burn surface 4 hours after a 80°/30s burn. Note the presence karyopyknosis, cytoplasmic swelling, cytoplasmic vacuolization of endothelial and smooth muscle cells, and occlusion of the vessel lumen by red blood cells, all indicating endothelial cell injury. D. CD31 stained injured vessel from 90°/20s burn 4HRs post injury demonstrating similar features to the H&E and also spotty and discontinuous staining indicating endothelial cell damage.

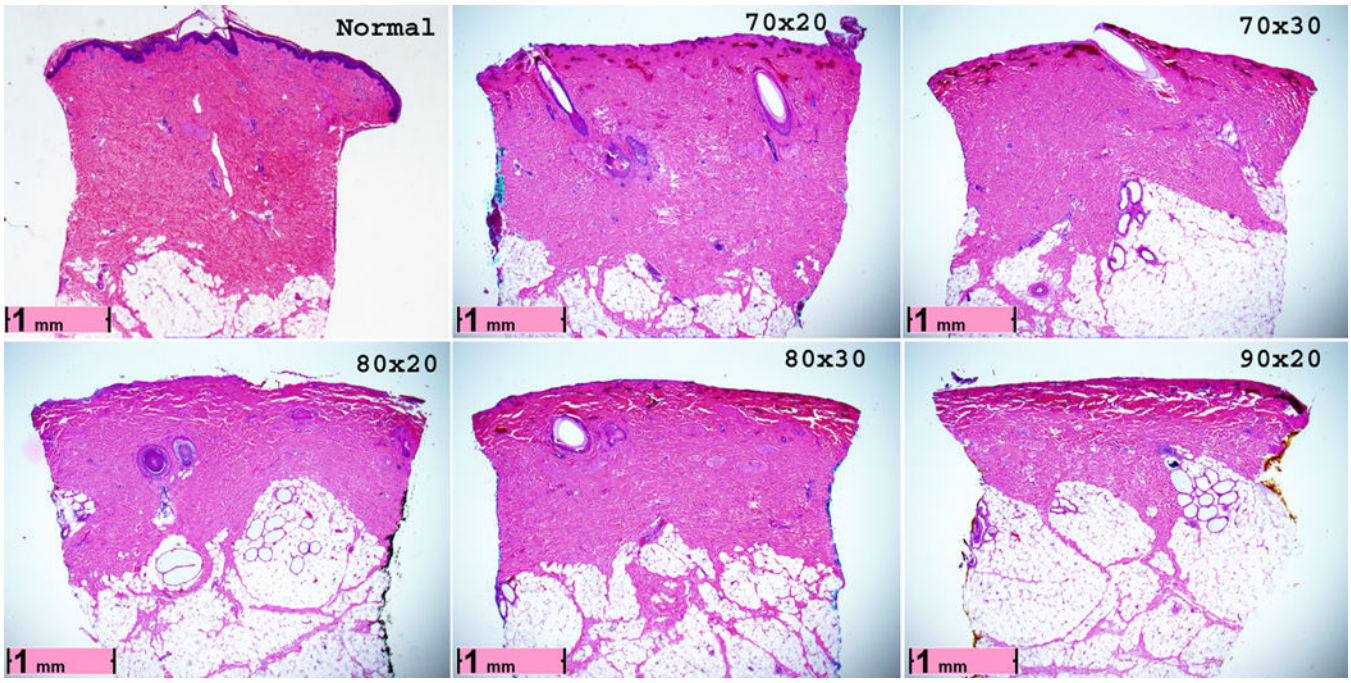

Figure 5.

Photomicrograph of normal uninjured skin and representative burns 4 hours after injury (H&E) with labeled identification of wound and burn conditions. With the more extreme conditions (80×20, 80×30, 90×20) multiple fissures are noted in the denatured collagen.

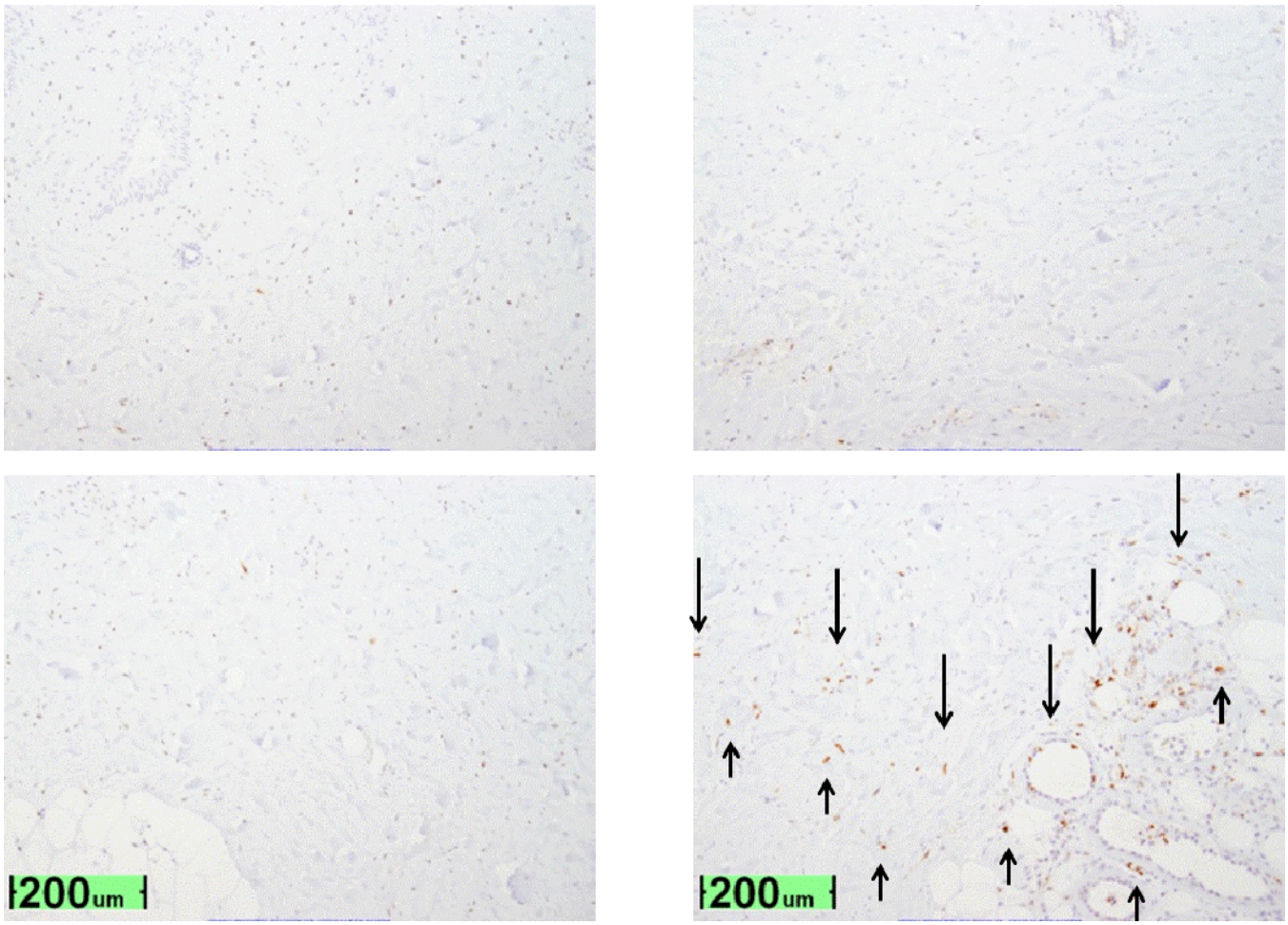

Figure 6.

Representative photomicrographs of Caspase 3a apoptosis stains. The pictures on the left indicate no staining for apoptosis in an 80°/20s burn at 4 hours after injury. On the right, apoptotic, brown staining cells are noted in the lower dermis in an 80°/20s burn 24 hours after injury. These pictures are representative of the 80°/20s, 80°/30s and 90°/20s burns, while the 70°/20s and 70°/30s burns have positive staining more superficially as early as 4 hrs.

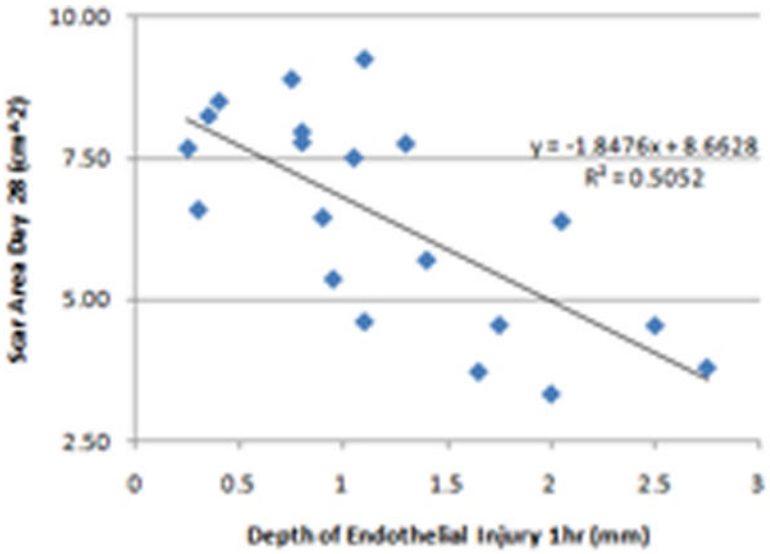

Figure 7.

The association between depth of endothelial cell injury at 1 hour after injury and the scar surface area at 28 days. As the depth of injury increases the scar size decreases indicating more contraction.

Table 1

Outcomes

	70×20	70×30	80×20	80×30	90×20	P Value
Scar Data						
Contracted scars, day 28, No. (%)	0 (0%)	1 (25%)	2 (50%)	3 (75%)	4 (100%)	.012
Mean (SD) scar area, day 28, cm^2	8.10 (1.26)	7.83 (.85)	6.56 (1.20)	4.86 (2.01)	4.82 (1.10)	**.007**
Mean (SD) scar depth, mm	0.5 (0.4)	0.8 (0.8)	1.8 (1.4)	4.8 (2.7)	4.8 (2.5)	**0.005**
Mean (SD) depth of injury, mm						
Collagen, 1 hr	0.25 (0.20)*	0.09 (0.10)	0.19 (0.09)	0.38 (0.19)	0.64 (0.14)	**0.001**
Endothelial, 1hr	0.89 (0.15)	0.46 (0.23)	0.91 (0.48)	1.51 (0.40)	2.26 (0.45)	**<.001**
Follicular, 1 hr	1.28 (0.10)	0.82 (0.66)	1.11 (0.30)	1.24 (0.11)	1.53 (0.12)	0.128
Interstitial, 1 hr	0.82 (0.44)	0.52 (0.32)	0.79 (0.10)	1.26 (0.28)	1.12 (0.46)	0.061
Collagen, 4 hr	0.20 (0.00)	0.33 (0.10)	0.34 (0.17)	0.41 (0.08)	0.51 (0.20)	**0.048**
Endothelial, 4 hr	0.89 (0.28)	1.31 (0.36)	1.21 (0.64)	1.84 (0.29)	1.85 (0.57)	**0.037**
Follicular, 4 hr	1.10 (0.33)	0.95 (0.43)	1.13 (0.25)	1.19 (0.41)	1.35 (0.33)	0.632
Interstitial, 4 hr	0.99 (0.37)	0.75 (0.32)	0.90 (0.25)	1.07 (0.23)	1.18 (0.15)	0.264
Collagen, 24 hr	0.16 (0.05)	0.22 (0.10)	0.26 (0.14)	0.25 (0.18)	0.38 (0.19)	0.351
Endothelial, 24 hr	0.86 (0.27)	0.98 (0.42)	1.48 (0.85)	2.08 (0.40)	1.84 (0.42)	**0.018**
Follicular, 24 hr	1.01 (0.42)	1.49 (0.29)	1.31 (0.88)	1.79 (0.20)	1.80 (0.13)	0.142
Interstitial, 24 hr	0.90 (0.34)	0.93 (0.49)	1.54 (0.93)	1.49 (0.31)	1.59 (0.45)	0.225
Collagen, 48 hr	0.38 (0.13)	0.32 (0.14)	0.39 (0.16)	0.42 (0.16)	0.33 (0.15)	0.053
Endothelial, 48 hr	0.78 (0.07)	1.12 (0.30)	1.21 (0.77)	2.01 (0.31)	2.10 (0.58)	**0.005**
Follicular, 48 hr	1.32 (0.54)	1.58 (0.29)	1.41 (1.00)	1.73 (0.16)	1.59 (0.17)	0.830
Interstitial, 48 hr	0.96 (0.08)	0.98 (0.14)	1.04 (0.76)	1.46 (0.21)	1.54 (0.30)	0.129
Reepithelialization						
Day 7 histology, Mean (SD)	55.0 (40.8)	17.5 (21.8)	25.0 (50.0)	0 (0)	0 (0)	0.116
Percent healed, day 10	100%	50%	50%	0%	0%	0.24
Percent healed, day 14	100	100	50	50	50	0.10
Percent healed, day 21	100	100	100	100	100	1.00

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* Total dermal thickness was 2.5±0.5 mm.