IMMEDIATE TANGENTIAL EXCISION ACCELERATES WOUND CLOSURE BUT DOES NOT REDUCE SCARRING OF MID-DERMAL PORCINE BURNS

L’EXCISION TANGENTIELLE IMMÉDIATE ACCÈLÈRE LA CICATRISATION MAIS NE RÉDUIT PAS LES CICATRICES DES BRÛLURES INTERMÉDIAIRES CHEZ LE PORC

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SUMMARY. Current evidence supports the use of excision to remove eschar from deep dermal and full-thickness burns. However, the role of excision of mid-dermal burns remains unclear. This study aimed to develop a porcine model that could produce reproducible mid-dermal thermal burns that undergo tangential excision; and investigate the effects of immediate tangential excision (30 minutes post-burn) on healing and scarring. An aluminum bar preheated in hot water (70°C) was applied for 20 or 30 s to produce a total of sixteen mid-dermal burns per pig on each of six pigs. Thirty minutes after burn creation, half of the burns were tangentially excised. Four partial-thickness wounds per pig were created as controls. Depth of burn injury (1 and 24 h), reepithelialization (7 and 10 d) and scar depth (28 d) were assessed microscopically. Total scar surface area was grossly evaluated on day 28. Exposure of porcine skin to a preheated aluminum bar at 70 °C for 20 or 30 sec resulted in reproducible mid-dermal burns, where immediate excision enhanced complete wound closure as judged by complete re-epithelialization, but did not reduce initial depth of injury, scar contraction and scar depth. Immediate surgical intervention is sufficient to enhance wound closure, but not to mitigate mid-dermal burn scar formation. This work provides a suitable animal model to evaluate novel therapies that may be used to inhibit burn progression, accelerate wound closure and decrease scarring, especially those therapies unable to penetrate burn eschar.

Keywords: tangential early excision, thermal injury, burn progression, wound healing, porcine model

RÉSUMÉ. Les données actuelles des connaissances sont en faveur de l’excision des brûlures des 2ème degré profond et 3ème degré. L’intérêt de l’excision des brûlures intermédiaires reste mal précisé. Cette étude se penche sur un modèle porcin destiné à la réalisation de brûlures intermédiaires reproductibles et à l’évaluation de l’effet l’excision ultra précoce (30 mn après la brûlure) sur l’épidermisation et la cicatrisation de ces brûlures. Six porcs ont subi chacun un total de 16 brûlures intermédiaires infligées au moyen d’une barre d’aluminium chauffée à 70°C et appliquée pendant 20 à 30 s. La moitié des zones brûlées étaient excisées à la trentième minute. Quatre brûlures superficielles servaient de contrôle. La profondeur de la brûlure (à h1 et h24), la réépithélialisation (à J7 et J10) et l’épaississement de la cicatrice (à J28), étaient étudiées microscopiquement. La surface cicatricielle totale était évaluée à J28. L’exposition pendant 20 à 30s de la peau d’un porc à de l’aluminium préalablement chauffée à 70°C entraîne une brûlure intermédiaire reproductible. L’excision immédiate en favorise la guérison lorsqu’elle est jugée sur la réépithélialisation mais n’en réduit ni la profondeur, ni la rétraction cicatricielle, pas plus que l’épaississement de la cicatrice. L’excision immédiate favorise la fermeture de la plaie mais pas son évolution vers des séquelles. Ce travail permet de décrire un modèle animal fiable dans le but d’évaluer de nouvelles thérapeutiques destinées à limiter le progression des lésions, accélérer la fermeture et diminuer la survenue de séquelles, en particulier celles incapables de pénétrer dans une lésion constituée.

Mots-clés: excision tangentielle ultra précoce, brûlure, évolution, cicatrisation, modèle porcin

Introduction

Burns are very common injuries, causing approximately 450,000 people per year to seek medical attention in the U.S.1 Thermal burns are characterized as having three concentric zones of intensity - a central zone of coagulation, transitional zone of stasis and outer zone of hyperemia - that radiate outward in the degree of intensity from the initial site of injury.2 These complex wounds often undergo burn injury progression,3,4 converting the zone of stasis into coagulative necrosis, and thereby advancing partial-thickness burns into slower healing, full-thickness burns. In other words, a burn that appears superficial 1 hour post-injury may become deeper within 24 hours.5 Since burn eschar found in the zone of coagulation is comprised of denatured proteins, toxins and microorganisms,6 it is believed that early excision reduces the release of these

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Manuscript: submitted 08/09/2015, accepted 10/10/2015.
toxins and inflammatory mediators into the circulation, and thus decreases the risk of bacterial colonization, sepsis and multi-organ failure. In addition, it is believed that excision eliminates the physical impedance of the eschar, thereby promoting faster reepithelialization.

The concept of early excision of burn eschar followed by grafting was first introduced in the early 1940s. Later, tangential excision was reintroduced around 1970 by Janzekovic and Jackson as a method to shave off the burn eschar in a sequential manner until a viable wound bed is exposed. Tangential excision followed by immediate grafting has rapidly gained worldwide acceptance as part of the standard treatment of burns. Clinical studies suggest that early excision (anywhere from 1-7 days after injury) accelerates spontaneous wound closure, reduces infection, and shortens hospital length of stay, promotes better graft uptake if grafting is required, and improves healing and outcome. It is clear from the literature that the field supports the use of excision to remove eschar from major deep dermal and full-thickness burns. However, it is unknown if excision without grafting of mid-dermal burns would also accelerate cell migration and reepithelialization. Therefore, we hypothesized that if the presence of necrotic eschar contributes to burn injury progression and impedes cell migration, then early eschar removal of mid-dermal burns may limit this progression, accelerate reepithelialization, and reduce scarring.

Therefore the goals of this study were several-fold: 1) to develop a reliable, porcine model with standardized and reproducible mid-dermal thermal burns that undergo tangential excision without grafting; and 2) investigate the effects of immediate tangential excision of mid-dermal burns on healing and scarring. In this study, we used excision 30 minutes post-burn to immediately remove the necrotic tissue from the wound. We intentionally covered the burns with non-biological dressings to determine the effectiveness of surgical debridement alone without enhancement by a biological dressing. We believe that such a model would be useful for the development and evaluation of topical therapies otherwise unable to penetrate burn eschar.

Materials and methods

Setting and animals

This study was conducted in the Division of Laboratory Animal Research at Stony Brook University and approved by the Institutional Animal Care and Use Committee. Six female domestic Yorkshire pigs (20-25 kg) were used in the study. Pigs were chosen for this in vivo experiment because it has been demonstrated that pig skin closely mimics that of the human. For example, both human and pig skin contain developed epidermal rete ridges, papillary bodies in the dermis, and an abundance of subcutaneous fatty tissue, as well as have similarly sized and distributed blood vessels. Animals were given a standard diet ad lib several days prior to the investigation and were fasted overnight before any procedures. Handling, housing and care of the animals were in accordance with the National Research Council guidelines.

Experimental protocol

A randomized experiment was conducted to determine the effect of immediate burn excision on healing using a previously established contact thermal injury model. 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 0-5.0% in O2 USP. The hair on the backs and flanks of each pig was clipped.

While under general anesthesia, 16 burns were created on each pig’s back and flanks using a 150-gram aluminum bar (Small Part Inc., Miami Lakes, FL) cleaned with 70% isopropyl alcohol and then preheated in hot water (70 °C) for 5 minutes. The aluminum bar (2.5 cm × 2.5 cm × 7.5 cm) was wiped dry to remove water droplets to avoid the creation of a steam burn on the skin. The bar was then applied perpendicularly to the skin for 20 or 30 seconds with 2 kg of pressure (Fig. 1a). Such application resulted in 2.5 cm × 2.5 cm mid-dermal burns (Figs. 1b and 1c).

Immediately after burn creation, the necrotic epidermal layer of tissue was gently removed by scraping the burn with the blunt handle of a scalpel. Half of the burns (8 of 16 per pig) were tangentially excised 30 minutes post-burn down to punctate bleeding (Figs. 1d and 1e). The depth of excision was set at the maximum depth of 0.75 mm using a dermatome with one-pass (Padgett Model S Slimline Electric Dermatome, Integra LifeSciences Corporation, Atlanta, GA). Depth of excision was determined by histological examination of the thickness of the excised skin to be 0.73 mm ± 0.1 mm. The dermatome was also used to create 4 partial-thickness wounds (one pass, 0.73 mm depth) per pig as controls (Fig. 1f). These controls were performed to determine if damage to the vasculature during excision contributes to injury progression. Note that the surface area of the excised tissue was slightly larger than the surface area of the burn to ensure complete tangential excision of the burn. The total body surface area of the burns in each pig was less than 5%.

A summary of the conditions is shown in Table 1. Animals were treated with Buprenorphine and a Fentanyl trans-dermal patch for post surgical pain on day 0. They were monitored for signs of pain and/or discomfort throughout the study period and treated with Buprenorphine as needed.

Wounds were covered with dry non-adherent gauze (Telfa, Kendall Healthcare Products Company, Mansfield, MA), polyurethane film (Tegaderm, 3M Health Care, St. Paul, MN), a gauze bandage roll (Sof-Form, Medline Industries, Inc., Mandeville, LA) and an adhesive elastic bandage (Tensoplast, BSN
Medical S.A.S., Vibraje, France), according to our previously published protocol. Wounds were photographed and dressings were changed at least twice a week.

Full thickness 4 mm punch biopsies were taken at 1 hour, 24 hours, 7 and 10 days from the upper left corner of all wounds, lower right corner, upper right corner, and lower left corner respectively, leaving a perimeter of 5 mm from the burn edge to eliminate edge effect or reepithelialization arising from adjacent surrounding uninjured skin rather than epithelium deep to the burn. Full-thickness 6 mm punch biopsies were taken from the center of each wound on day 28 prior to euthanasia with a pentobarbital-based solution (Fatal Plus, Vortech Pharmaceuticals, Michigan). The biopsies were marked for alignment, bisected, then placed in labeled cassettes and fixed in formalin for 24 hours. Processed by alcohol-dehydration, xylene-clearing and paraffin-embedding, tissue samples were sectioned at 5 mm and stained according to previously published protocols with hematoxylin and eosin (H&E), hematoxylin and an antibody to high mobility group box 1 (HMG1B1), a marker for cell necrosis (1 and 24 hour biopsies only) and Masson’s trichrome (day 28 biopsies only). Histomorphometric analyses were done by a board certified dermatopathologist, who was blinded to burn and excision protocol.

Outcomes

Depth of injury (1 and 24 hours post-burn): The overall depth of injury (abnormal collagen, blood vessel plugging, and cell necrosis) was determined on all specimens (24 replicates) using H&E-stained sections. In addition, the depth of injury as judged by abnormal collagen, blood vessel plugging, and epithelial cell necrosis was determined separately using a subset of the H&E-stained sections (8 replicates) according to previously published methods. HMGB1-stained sections (8 replicates) were used to confirm the depth of injury. Briefly, an ocular lens with a grid was used to make straight-line vertical measurements in millimeters from the dermal surface of the wound to the deepest point of injury. Depth of injury to collagen was determined by measuring to the deepest level of blue or magenta-tinged discoloration (normal collagen stains red). The depth of injury to follicular and/or apocrine epithelial cells was judged to the deepest level of nuclear pyknosis and similarly recorded for blood vessel plugging and endothelial cell pyknosis. On analysis, a correction factor of 0.73 mm was added to all wounds that were tangentially excised to account for the thickness of the excised skin.

Reepithelialization (7 and 10 days post-burn): Reepithelialization was determined on H&E-stained sections using a calibrated ocular micrometer to measure the total length in cross-section and the length of the neopidermis. Percentage of reepithelialization was calculated and reported.

Data analysis

Data were reported as the mean ± standard deviation (SD). Data analysis was done using Prism 4 software (GraphPad Software, Inc.). For parametric data sets, one-way analysis of variance (ANOVA) followed by Tukey post-hoc test was used to detect statistical significance with p<0.05. For nonparametric data sets, the Kruskal-Wallis test followed by the Dunn’s post-hoc test was used to detect statistical significance with p<0.05. A sample size of 24 wounds in each group is required to have an 80% chance of detecting an 18% change among five groups (PTW, 70/20, 70/30, Ex70/20, Ex70/30) when the standard deviation of the population is 19% with 95% confidence.

Results

Two types of wounds were created on each pig: 16 burns and 4 excisional wounds. Thus there were twenty wounds created on each of the six Yorkshire pigs, resulting in a total of 120 wounds. Burn wounds were created using an aluminum bar pre-heated to 70°C and applied to the skin for either 20 or 30 seconds (abbreviated as 70/20 or 70/30, respectively) (representative photographs are shown in Fig. 1). The 70/20 burns had a red-colored appearance, whereas the 70/30 burns were pale, white-colored, which is similar to Wang et al., who showed that pale-colored burns were deeper than pink-colored burns. Next, half of the 16 burns were tangentially excised (0.73 mm in depth) 30 minutes after injury using a dermatome (abbreviated Ex70/20 or Ex70/30). This resulted in four wounds per injury condition per pig. Similarly, a total of 4 control partial-thickness tangential excisional wounds (abbreviated PTW) were studied per pig. This experimental design (Table I) resulted in 24 replicates corresponding to each injury condition.

The biopsies taken at 1 and 24 hours were used to microscopically assess the depth of injury using both H&E and Scar area (28 days post-burn): The perimeter of the discolored (red or purple) scar with no hair present was traced in photographs using ImageJ software (National Institute of Health, Bethesda, MD). The calculated surface area was reported in square centimeters.

Scar depth (28 days post-burn): The depth of scar was determined using sections stained with Masson’s trichrome. An ocular lens with a grid was used to make straight-line measurements from the epidermal-dermal junction to the bottom of the scar. Three measurements were made per bisected half of the sample; one measurement was made at the center of the section, and two more were made 1 mm left and right of the center. A total of six measurements were taken per sample. The mean depth of scar was reported in millimeters.

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**Table I** - Types of wounds that were created on each of six Yorkshire pigs. Twenty wounds were created per pig, resulting in a total of 120 wounds

<table>
<thead>
<tr>
<th>Sample Description</th>
<th>Temperature / Time of exposure to heated bar</th>
<th>Abbreviation</th>
<th># of wounds per pig</th>
<th>Total # of wounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partial thickness wound</td>
<td>0 °C / 0 sec</td>
<td>PTW</td>
<td>4</td>
<td>24</td>
</tr>
<tr>
<td>Mid-dermal burn</td>
<td>70 °C / 20 sec</td>
<td>70/20</td>
<td>4</td>
<td>24</td>
</tr>
<tr>
<td>Tangentially excised mid-dermal burn</td>
<td>70 °C / 20 sec</td>
<td>Ex70/20</td>
<td>4</td>
<td>24</td>
</tr>
<tr>
<td>Mid-dermal burn</td>
<td>70 °C / 30 sec</td>
<td>70/30</td>
<td>4</td>
<td>24</td>
</tr>
<tr>
<td>Tangentially excised mid-dermal burn</td>
<td>70 °C / 30 sec</td>
<td>Ex70/30</td>
<td>4</td>
<td>24</td>
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</tbody>
</table>
HMGB1 staining. First, H&E-stained sections were used to microscopically assess the overall depth of injury to the tissue. Fig. 2 shows that the overall depth of injury was measured into the mid-dermal to deep dermal range for all burns regardless of excision. At 24 hours, 70/30 burns were significantly deeper than 70/20. Likewise, Ex70/30 burns were significantly deeper than Ex70/20 burns at 1 and 24 hours. The depth of injury did not significantly change as a function of time (1 vs. 24 h), except for 70/30 burns which progressed from 1.5 ± 0.3 mm (1 h) to 1.8 ± 0.3 mm (24 h). In both cases, immediate excision of the burns did not reduce the depth of injury; rather Ex70/30 burns were significantly deeper than 70/30 burns (2.1 ± 0.4 vs. 1.8 ± 0.3 mm at 24 h, respectively). Furthermore, all burns were significantly deeper than PTW controls at both 1 and 24 hours. No significant change in the depth of injury was measured for the PTW controls as a function of time, which confirms that damage to the vasculature during excision was not responsible for burn progression. Note that normal dermis in unburned control samples measured 1.90 mm, of which 0.10 mm or about 5% was papillary dermis.

Second, H&E-stained sections were used to assess the effects of burn injury and excision on the depth of vessel plugging, epithelial necrosis and injury to collagen (Table II). At 1 hour post-injury, epithelial cell necrosis and blood vessel plugging were measured into the mid-dermal to deep dermal range for all burns regardless of excision. On the other hand, collagen injury was limited to the upper dermis for both 70/20 and 70/30 burns, where it is likely that tangential excision (0.73 mm) debrided ~0.6 mm of viable collagen.

HMGB1 staining was then used to confirm the depth of injury to both endothelial and epithelial cell-containing dermal structures.33 HMGB1 is a small (~30 kDa) non-histone nuclear protein normally present in viable cells, but is passively released from necrotic cells into the cytoplasm and extracellular space. In control specimens (normal skin and PTW), HMGB1 staining of most intact nuclei was observed throughout the skin, but especially visible in epidermis and epithelial structures, i.e., hair follicles (Figs. 3a and 3b) and apocrine glands. In both 70/20 and 70/30 burns (regardless of excision), there was a portion of the specimens where obliteration of HMGB1-staining and/or leakiness of the HMGB1 from the cell nucleus into the cell cytoplasm or extracellular space was observed, confirming cell necrosis and loss of nuclear integrity (Figs. 3c-f). Injury depth by HMGB1 was comparable to H&E-stained sections (Table II), where non-excised 70/20 and 70/30 burns underwent significant burn injury progression. On the other hand, no significant difference in depth of injury as noted by damage to endothelial and/or epithelial cells as a function of time was measured in Ex70/20 and Ex70/30 burns. Overall, immediate tangential excision of 70/20 and 70/30 burns 30 minutes after injury did not significantly reduce the depth of injury at 24 hours.

The biopsies taken at 7 and 10 days were used to micro-
Table II - Histological evaluation of the depth of injury at 1 and 24 hours post-injury [mean (SD)]. Statistical differences between injury conditions at a specific time point are indicated by lower case letter superscripts. Statistical differences for each wound condition between 1 and 24 hour time points are indicated by an asterisk (p<0.05)

<table>
<thead>
<tr>
<th>Injury Condition</th>
<th>Depth of Injury at 1 hour (mm)</th>
<th>Depth of Injury at 24 hours (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Endothelial and/or epithelial cell necrosis</td>
<td>Blood vessel plugging</td>
</tr>
<tr>
<td>70/20</td>
<td>1.1 (0.2) ±s</td>
<td>1.1 (0.2) ±s</td>
</tr>
<tr>
<td>Ex70/20</td>
<td>1.5 (0.3) ±s</td>
<td>1.3 (0.2) ±s</td>
</tr>
<tr>
<td>70/30</td>
<td>1.2 (0.3) ±s</td>
<td>1.3 (0.2) ±s</td>
</tr>
<tr>
<td>Ex70/30</td>
<td>1.6 (0.2) ±s</td>
<td>1.7 (0.3) ±s</td>
</tr>
<tr>
<td>PTW</td>
<td>0.8 (0) ±s</td>
<td>0.8 (0) ±s</td>
</tr>
</tbody>
</table>

 superscript

> Depth of injury indicated by endothelial and/or epithelial cell necrosis was determined using HMGB1-stained sections.

† Depth of vessel plugging, epithelial necrosis, and injury to collagen was determined using H&E-stained sections.

Table III - Total number of wounds that were completely closed (100% reepithelialized) at 7 and 10 days post-injury determined using H&E-stained sections. There were 24 replicates per injury condition per time point

<table>
<thead>
<tr>
<th>Number of wounds with 100% reepithelialization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-excised</td>
</tr>
<tr>
<td>70/20</td>
</tr>
<tr>
<td>Day 7</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>Day 10</td>
</tr>
<tr>
<td>6</td>
</tr>
</tbody>
</table>

Fig. 4 - Microscopic evaluation of reepithelialization 7 and 10 days post-injury. Burns were created by an aluminum bar preheated at 70°C for 20 or 30 seconds (70/20 or 70/30, respectively), with and without partial-thickness excision. Reepithelialization of control partial-thickness wounds (PTW) is indicated by a dotted line. Mean ± SD is shown with *p<0.05. Statistical analysis was performed using the Kruskal-Wallis test followed by Dunn’s multiple comparisons post-hoc test with *p<0.05.

Fig. 5 - Photographs of wounds healed 28 days post-injury: (A) 70/20, (B) Ex70/20, (C) 70/30, (D) Ex70/30, and (E) PTW. Wound contracture is noted with 70/30 mid-dermal burns and tangentially excised 70/30 mid-dermal burns. Rulers shown in photographs are in centimeters.
PTW control wounds, however, no statistically significant difference was detected between 70/20 and 70/30 burns regardless of tangential excision. The depth of scarring slightly increased with greater exposure times (20 vs. 30 seconds), however, no significant difference was detected between 70/20, Ex70/20, 70/30, and Ex70/30. However, all burns, regardless of excision, were significantly deeper than PTW controls (Fig. 6).

**Discussion**

Tangential excision followed by grafting is the standard of care for deep partial and full thickness burns. However, it is unclear whether tangential excision without grafting of superficial and mid-dermal burns provides a benefit to the patient. In addition, a major barrier to topical administration and absorption of burn therapies is the presence of the thick burn eschar. Thus, we proposed that early removal of the burn eschar with tangential excision would allow greater dermal penetration of topical therapies. Therefore, the objectives of the study were to develop a mid-dermal burn model that undergoes tangential excision without grafting, and to determine the effects of immediate excision (30 minutes post-burn) on burn wound healing in this model. This model could then be used in future studies to evaluate topical burn therapies that improve healing and reduce scarring, but cannot penetrate the burn eschar.

This research is an extension of our recently reported work on the development of a porcine vertical burn injury progression model where we correlated the temperature of an aluminum bar and exposure time with depth of injury. The fundamental difference between the previous and current work is the inclusion of tangential excision post-injury, with the hope to inhibit burn progression, accelerate healing and reduce scarring. Furthermore, a narrowly focused window of burn conditions (70/20 and 70/30) was selected to study the effects of immediate excision on mid-dermal burn healing.

Here, we have demonstrated the ability to create a reproducible tangentially excised, mid-dermal burn in swine. It was determined that exposure of porcine skin to a preheated aluminum bar at 70°C for 30 seconds (tangentially excised and non-excised) resulted in a mid-dermal, partial-thickness burn that healed with a contracted, discolored scar. The finding that the depth of injury to collagen was limited to the upper to mid-dermis at 1 and 24 hours, but resulted in nearly full-thickness scars at day 28, suggests that burn injury progression did occur in this model. Moreover, depth of injury to endothelial and epithelial cells significantly increased for 70/20 and 70/30 burns from 1 to 24 hours. This is supported by a recent publication by our group where both H&E and immunohistochemical staining for apoptosis and necrosis was used to demonstrate that 70/30 burns had a partly viable dermis at 1 hour and significant contraction on day 28. Unfortunately, tangential excision 30 minutes after injury did not significantly reduce burn depth at 24 hours. Rather, excised burns have a deeper injury than non-excised burns. A possible explanation is that there may be human error in the utilization of the dermatom, which contributed to variability in the depth of excision and resulted in overcorrection of burn wound depth. However, all burns in the present study had significantly deeper vessel plugging, endothelial and epithelial cell necrosis compared to the injury to collagen at 1 and 24 hours. This discrepancy between levels is consistent with previous reports and is probably due to more heat conduction through blood vessels and down hair follicles as compared to collagen, which acts as an insulator. In addition, it is likely that surgical intervention via tangential excision of 0.73 mm in depth overestimated the depth of injury to collagen but underestimated the depth of injury to epithelial and endothelial cell-containing dermal structures.

In this work, it was also shown that immediate tangential excision of 70/20 and 70/30 burns 30 minutes after injury had a substantial effect on the total number of wounds that were 100% reepithelialized by day 10, especially for 70/20 burns. Even in the excised 70/30 burns, 7 of 24 burns completely healed by day 10 compared to 1 of 24 non-excised 70/20 burns. It is likely that immediate excision eliminated the physical impedance caused by eschar, which promoted faster cell migration. However, statistical analysis did not demonstrate significant differences on reepithelialization rates at days 7 and 10, probably due to the large variability in the data and relatively small sample size. Davis et al. showed that partial tangential excision (0.4 mm deep) 24 hours after injury was effective in speeding epithelialization of mid-dermal porcine burns (initially 0.8 mm deep). This difference may be attributed to the difference in timing of excision (1 vs. 24 hours) or the initial depth of injury, where the burns we created were much deeper than those of Davis et al. We also observed that reepithelialization of all burns, regardless of excision, was significantly slower than PTW controls at both 7 and 10 days, except Ex70/20 burns at day 10. This finding agrees with Shaffer et al., who showed that non-excised porcine burns (1.1 - 1.3 mm deep) heal significantly more slowly than excisional wounds, i.e., delayed reepithelialization, decreased fibroblastic proliferation, inhibition of early angiogenesis, and depressed matrix metalloproteinase expression.

Despite major enhancements in the total number of wounds completely reepithelialized at day 10, immediate tangential excision did not significantly reduce burn depth at 24 hours, scar contraction and scar depth at 28 days for both 70/20 and 70/30 burns. This is consistent with our previous report in the porcine hot comb burn model, where immediate excision of full-thickness burns 30 minutes post-injury neither inhibited horizontal burn injury progression nor significantly reduced scar formation. This is also supported by human clinical studies that showed no significant difference in scar formation and the function of joints in burns treated with early excision and
grafting versus conservative burn management protocols. In addition, a meta-analysis done using human clinical data showed that the reduction in mortality with early excision (<144 hours within injury) was not statistically significant compared to traditional treatment of burns, except in patients without inhalation injury. However, a previous report by Wang et al. demonstrated that immediate tangential excision of deep partial-thickness porcine burns (initial depth of injury was not specified) reduced the thickness of scar tissue and the elevation of the scar, but had no effect on scar color, wound size and contraction. In that study, the burns were larger in size (40–50 cm²) and treated with a hydrated gel (Solosite Gel™, Smith & Nephew, Queensland Australia), whereas our burns (6.25 cm²) were covered with dry non-adherent wound pads (Telfa, Kendall Healthcare Products Company, Mansfield, MA) and polyurethane film (Tegaderm, 3M Health Care, St. Paul, MN). These differences may be responsible for the difference in scar formation.

Further studies are required to determine suitable hydrating agents that can reduce scar formation in our model.

We demonstrated that immediate excision resulted in faster complete wound closure, but did not reduce burn progression and scarring, possibly due to several reasons. First, a conservative approach (mid-dermal) to surgical debridement was used. While avoiding over-excision, mid-dermal excision may not be deep enough to be effective since the depth of injury to both epithelial and endothelial cell-containing dermal structures noted at 1 hour was deeper than the excision. Recently our group reported that endothelial cell necrosis, not injury to collagen, predicts the depth of zone of apoptosis, and ultimately the depth of viable dermis. Second, it is possible that the presence of the eschar and the passive leakage of cellular content from necrotic cells is not the sole cause of progression. Recently Purschke et al. demonstrated that bystander cells (those that are close to the injury but not heated) experience significant injury and DNA damage. They propose that this injury is a result of an active cellular process where viable, heat-injured cells (potentially in the zone of stasis) undergo molecular changes and/or release mediators that injure or kill nearby bystander cells. Third, high concentrations of HMGB1 from the cell nuclei passively leaking into the extracellular space are known to act as proinflammatory cytokines and potentially mediate burn injury progression. This hypothesis is supported by Hohne et al. and Chen et al. who reported that HMGB1 plays a role in the progression of pneumococcal meningitis and rheumatoid arthritis, respectively. Fourth, it is possible that immediate excision eliminated the physical impediment of the eschar, thereby promoting faster wound closure. However, it is likely that immediate excision alone was not sufficient to intervene with burn progression. Since initial burn depth directly correlates with scar formation and immediate excision did not inhibit burn progression, it is not surprising that immediate excision also did not reduce scar depth.

**Limitations**

There are several limitations of the study design that should be noted. First, although Sullivan et al. showed that pig skin mimics human skin, burns on Yorkshire pigs rarely blister. Therefore, the necrotic epidermis was intentionally removed post-burn in order to stimulate blisters that burst, common in human burns but uncommon in swine burns. Despite this effort, porcine burn models are not exact mimics of human burn injury and the results presented in this paper may not be extrapolated to humans. Second, some investigators prefer using the red Duroc pig when studying scarring since the histomorphological characteristics of scars in this model more closely resemble those of human hypertrophic scars. However, we have previously shown that many deep dermal burns heal with significant contraction and discoloration in the domestic pig as evidenced in this study too. Third, standard dermatomes are not designed to excise thick pieces of skin. Although burn surgeons use sequential tangential excisions to remove burn eschar, this technique results in inconsistent depths of excision, which could result in an irreproducible study design. As a result, tangential excision was restricted to a single pass of 0.73 mm (maximum depth allowed by the equipment) in this study. Fourth, immediate excision 30 minutes post injury is not common in clinical situations. Since it is extremely difficult to determine initial burn depth especially while burn progression is underway, the accuracy of distinguishing between superficial and deep burns ranges from 50-70%. As new technologies are developed to determine initial burn depth, such as infrared thermal imaging, immediate burn excision may become a realistic part of the future standard of care. Finally, standard burn care in the clinic of deep-dermal and full-thickness burns includes excision followed by immediate wound closure with grafting. In this study, all burns healed spontaneously within 3-4 weeks, without autografting.

**Conclusion**

In conclusion, our results demonstrate that immediate tangential excision 30 minutes after injury enhanced complete wound closure, but failed to minimize the initial depth of injury and reduce scar formation in mid-dermal burns. In addition, this work suggests that immediate surgical intervention alone neither mitigates burn injury progression nor reduces scar formation. Therefore, it is critical that the field continues to develop novel therapies that can be used to inhibit burn progression, accelerate wound closure and decrease scarring. We believe that this work provides a suitable animal model to evaluate such new topical therapies; particularly those that can reverse dermal ischemia and prevent apoptosis, but are not able to penetrate burn eschar.

**BIBLIOGRAPHY**


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