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Partial thickness wound: Does mechanism of injury influence healing?

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ABSTRACT

Wound healing is a complex multistep process which is temporally and spatially controlled. In partial thickness wounds, regeneration is possible from the stem cells in the edges of the wound and from the remnants of the epidermal appendages (such as hair follicles and sebaceous glands). This study examines whether the mechanism of injury influences healing of wounds of similar depth. Burn and excisional wounds were created on the back of Hampshire pigs and harvested at 7, 14, 28, 44, 57 and 70 days after injury and processed for histology and immunohistochemistry. Quantitative analysis of re-epithelialisation, inflammatory response and thickness of the scar and semi-quantitative analyses of the architecture of the resultant scar were performed and subjected to statistical analysis. Results demonstrated a higher number of neutrophils, macrophages and lymphocytes present in the burn on day 7 compared to the excisional wounds. The inflammatory profile of burn wounds was higher than that of excisional wounds for the first month after injury albeit less marked than on day 7 after injury. Re-epithelialisation was markedly advanced in excisional wounds compared to burn wounds at day 7 after injury, corresponding to the higher number of hair follicles in the underlying dermis of excisional wounds at this time point. The thickness of the neo-epidermis increased with time and at day 70 after wounding, the neo-epidermis of the burn was significantly thicker than the neo-epidermis of the excisional scar. Interestingly, following partial thickness excision of skin, there was neo-dermal reformation albeit with an altered architecture, lacking the normal basket-weave pattern of collagen. The thickness of the dermis of partial thickness excisional scar was greater than that of the adjacent unwounded skin. The neo-dermis of the burn scar was even thicker, with the collagen arranged more compactly and disorganised compared to excisional scar and normal skin. This study provides evidence that the mechanism of injury does influence wound healing and the resultant scarring.

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1. Introduction

Wound healing is an intricate, well organised and well-co-ordinated process with temporal and spatial regulation. All wounds undergo common reparative processes including inflammation, granulation tissue formation, re-epithelialisation and scar remodelling [1]. Burn wounds are a common cause of injury with more than a quarter of a million people affected in England and Wales each year [2,3]. Although the majority of burn wounds are partial thickness wounds, often burn patients present with mixed depth burns, some of which undergo excision and grafting. This results in some patients having non-grafted partial thickness wounds and partial thickness excisional wounds (donor sites of skin grafts) concurrently. Clinicians have observed that these same depth wounds heal differently in the same patient.

Wound healing research to date has largely focussed on incisional and excisional wounds and there have been limited studies comparing the healing between different wound types, particularly burn and excisional wounds. As burn patients often have both types of wounds, it is important to understand similarities and differences in the healing and resultant scarring of both the burn and the donor site. Schaffer et al. [4] demonstrated delayed re-epithelialisation, fibroblastic response and subsequent granulation tissue formation in burn wounds caused by contact or lasers, compared to excisional wounds. In addition, new blood vessel formation (angiogenesis) and the expression of the proteolytic matrix metalloproteinases (MMP)-1, -2 and -9, necessary for wound remodelling, were also delayed. Interestingly, the two burn injuries (laser and contact burn) also differed in their healing responses suggesting the causation of the burn affected the healing response. However, these studies did not investigate the inflammatory profile or the long-term impact of the mode of injury.

Ross et al. [5] studied the differences within the neo-dermal architecture of excisional and burn wounds. They reported fine, fibrillar collagen fibres within the neo-dermis with fewer elastic fibres in both types of superficial wounds, with a more vertical orientation compared to the normal adjacent skin. In contrast, the neo-dermis of deep thermal wounds showed denser, more compact elastic and collagen fibres with a horizontal orientation. This suggested a firmer scar tissue following thermal injury. It is important to note, however, that this study only compared laser burn injuries with excisional injuries and this may not be representative of all modes of burn injury as observed by Schaffer et al. [4]

This longitudinal observational study sought to investigate the similarities and differences of wound healing and resultant scarring between partial thickness burn and partial thickness excisional wounds of similar depth by examining re-epithelialisation, neo-dermal architecture and the inflammatory profile of both types of wound. Ultimately, this information can provide opportunities for the development of targeted clinical interventions to modulate healing and reduce scarring.

2. Material and methods

2.1. Porcine model

Hampshire pigs were selected for their characteristic black colour with distinctive white stripe across the shoulders and front legs making them ideal for investigating wound healing in both pigmented and non-pigmented skin. All housing and wounding procedures were carried out at the University of Manchester in accordance with Home Office regulations (U.K. Animals (Scientific Procedures) Act, 1986) and appropriate licenses. Animals were acclimatised for a minimum of 2 weeks prior to wounding and were individually housed for the duration of the study. They were fed on a standard, antibiotic free diet (Oakes, Congleton, UK) twice a day and had access to water ad libitum.

Animals were fasted overnight prior to general anaesthesia and aseptic technique was used throughout all procedures. Fourteen female Hampshire pigs were anaesthetised using 2–4% isoflurane, nitrous oxide and supplemental oxygen via face mask initially and then intubated. The back and flanks were shaved and the skin cleaned with betadine and aqueous chlorhexidine before the wounding sites were marked on the skin using a 2.5 cm^2 template and permanent marker. All wounds (burn and excisional) were the same size. Superficial partial thickness burn wounds were created using a custom-designed thermo-regulated block heated to 80 °C and applied to the skin, under its own weight, for 10 s. Wounds were cooled immediately after wounding with sterile gauze soaked in cold saline. A similar depth of excisional wound was created using a hand held motorised dermatome with a 2.5 cm blade (Nouvag, Switzerland), at a setting of 0.5 mm. Intra- and inter-animal replication was achieved through varying the position of each type of wound to control for cranio-caudal and medio-lateral variances. Two superficial partial thickness burn and two equal depth excisional wounds (i.e. 4 wounds) were created per animal. At each time point there were a minimum of 2 animals and maximum of 3 animals. Haemostasis was achieved by light manual pressure with sterile gauze. The wounds were covered with Telfa™ non-adherent dressing (Covidien, Watford, UK) and surgical gauze which was secured in place with Bioclusive™ adherent dressing (Johnson & Johnson Ltd., Berkshire, UK) and Surgifix Colorline stocking (Bunzl Healthcare, Enfield, UK). Dressings were changed weekly until all wounds had reepithelialised. All wounds healed by secondary intention without infection. The progress of wound healing and scar formation was recorded and photographed at weekly intervals until the wounds were harvested.

Wounds and scars were harvested under terminal anaesthesia at 7, 14, 28, 44, 57 and 70 days after wounding. Wounds were excised with a scalpel down to adipose tissue with a 5 mm rim of surrounding unwounded skin. Each wound was divided as illustrated in Fig. 1. The medial tissue strip was placed in an embedding cassette to prevent curling, fixed in 10% buffered formal saline (FS) for a minimum of 48 h, including 1 change to fresh solution after 24 h and then transferred to 50% ethanol, for a minimum of 48 h, before being embedded in paraffin wax (Leica ASP300 processor, Leica Biosystems Ltd., Newcastle, UK); blocks were subsequently stored at room temperature. The central strip was cut in half (H and T) and each piece cryo

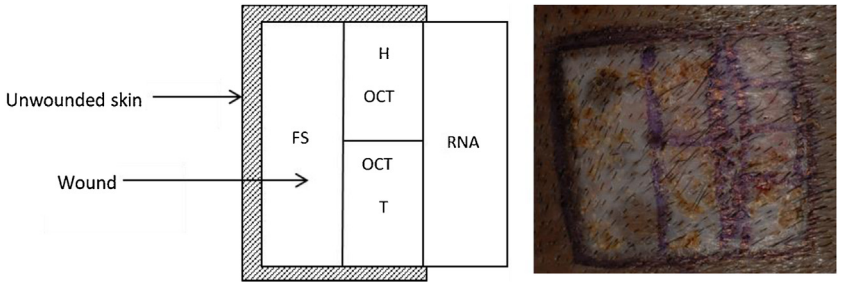


Fig. 1 – Harvested wounds were divided into strips and embedded in paraffin wax (FS), cryo preserved (OCT, Head and Tail) or placed in RNAlater®.

preserved by snap freezing in *iso*-pentane cooled with liquid nitrogen and embedded in optimal cutting temperature (OCT) embedding matrix (Cat No: KMA-0100-00A, Cell Path Ltd., Newtown, Powys, UK) and stored at −80°C. The lateral strip, without surrounding unwounded skin, was preserved in RNA*later*® (Cat No: R0901, Sigma-Aldrich Company Ltd., Gillingham, UK). Normal skin samples from the same animal were harvested at each time point for comparison. Normal unwounded skin samples were harvested cranial and caudal to the wounded areas. The skin removed with the dermatome when creating excisional wounds at day 0 was retained and preserved as described above.

2.2. Histology

Wax embedded samples were sectioned at 5 μm (Leica RM2155 microtome, Leica Biosytems Ltd.) and collected serially onto 3-aminopropyl triethoxysilane (APES) coated glass slides and dried overnight at 37°C. Sections were dewaxed in 2 changes of xylene, rehydrated and stained as required. Re-epithelialisation of wounds was analysed using Haematoxylin and Eosin staining. Tissue sections on slides were treated with Haematoxylin (Harris formulation) for 4 min, rinsed in running tap water for 5 min, 1% Eosin Y (v/v) for 1 min before dehydrating and mounting with Pertex mounting medium (Cell Path Ltd.). Deposition and orientation of new collagen within the scars was analysed using Picrosirius Red staining. Dewaxed and rehydrated slides were placed in 0.2% phosphomolybdic acid for 10 min, rinsed in water and stained with 0.1% Picrosirius red for 1 h before differentiating the stain in 0.01% hydrochloric acid for 10 min. Slides were dehydrated and mounted as above. Masson's Trichrome (Lillie's Modification) was used to analyse the depth of both the burn wound at 60 min after injury and the skin excised when creating the excisional wound. Dewaxed and rehydrated slides were stained with Harris Haematoxylin and rinsed in running tap water as above then treated with 1% picric acid for 30 s, rinsed, placed in 0.25% Biebrich Scarlet for 1 min, rinsed and treated with Phosphomolybdic acid (PMA)/phosphotungstic acid (PTA) solution (50/50) for 10 min. Slides were then placed directly into 1% Fast Green for 6 min rinsed in running tap water and dehydrated and mounted as above.

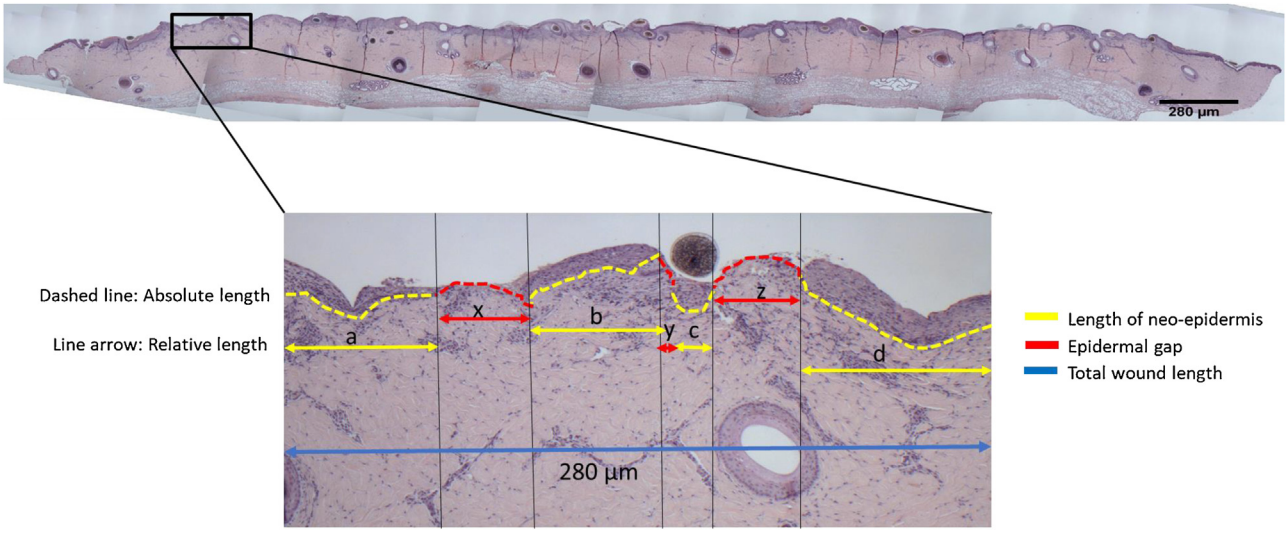
2.3. Immunohistochemistry

The inflammatory profile of the wounds was investigated using immunohistochemistry with markers against porcine neutrophils (myeloperoxidase activity), alternatively activated macrophages (cd163) and lymphocytes (cd8a). Myeloperoxidase activity was assessed in 5 μm wax embedded sections. Antigen retrieval was performed by microwaving in 0.01M citrate buffer, pH6.0, for 6 min. Cooled sections were washed twice in Tris buffered saline (TBS), endogenous peroxidase activity blocked in 0.3% hydrogen peroxide (v/v) in methanol, washed twice more in TBS before blocking with 1.5% normal donkey serum in TBS/0.1% bovine serum albumin (BSA) (Cat No: A7906, Sigma-Aldrich, Poole, UK) to reduce non-specific background. They were incubated overnight at 4°C with a 1/50 dilution of rabbit polyclonal antibody against myeloperoxidase (Cat No: ab9535, Abcam, Cambridge, UK). Sections were washed twice in TBS followed by 30 min incubation in biotinylated AffiniPure F(ab')$_2$ fragment donkey anti rabbit IgG (Jackson ImmunoResearch Cat No: 711-066-152, supplied by Stratech Scientific Ltd., Newmarket, UK) at a dilution of 1/200. After further washes in TBS sections were incubated with the peroxidase ABC reagent (Vectastain® Elite standard kit, Cat No: PK-6100, Vector Laboratories, Peterborough, UK) according to manufacturer's instructions for 30 min. Two further washes in TBS were carried out before applying the Vector® NovaRED™ peroxidase substrate (Cat No: SK-4800, Vector Labs); developed sections were placed in deionised water to stop the reaction, counterstained with Harris Haematoxylin, rinsed in running tap water for 5 min, dehydrated and mounted with Pertex mounting medium (Cell Path Ltd.).

Macrophages and lymphocytes were assessed using cryo preserved samples step-sectioned at 7 μm (Leica Cryocut 1800, Leica Biosystems Ltd.) and collected onto APES coated glass slides and stored at −20°C until required. Frozen sections were brought to room temperature, fixed for 10 min in acetone and air dried immediately prior to staining. Sections were then stained using peroxidase ABC as previously described [6] and mouse monoclonal antibodies against either cd163 for alternatively activated macrophages (Cat No: MCA2311GA, Bio-Rad AbD Serotec Ltd., Oxford, UK) or cd8a for lymphocytes (Cat No: MCA1223GA, Bio-Rad AbD Serotec Ltd.) at dilutions of 1/400 and 1/200 respectively.

2.4. Image capture and analysis

Re-epithelialisation and neo-epidermal thickness were measured on images captured using an Zeiss Axiostar plus microscope with digital camera and Image-Pro Insight® (Version 8.0 for Windows®) software. Re-epithelialisation



$$\text{Percentage (\%) re-epithelialisation} = \frac{\text{Total length of neo-epidermis (a+b+c+d...)}}{\text{Total wound length (a+b+c+d...)} + \text{(x+y+z+...)}} \text{ X } 100$$

Fig. 2 – Re-epithelialisation was calculated by measuring the length of each island of neo-epidermis (a+b+c+d) within each 280 µm field of view using ImageJ software. The cumulative length was then divided by the total length of the wound to give the percentage re-epithelialisation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

was measured on two burn wounds and two excisional wounds per animal at each time point: each image was divided into a grid of 280 µm fields of view using ImageJ software; the length of neo-epidermis and the length of the wound in each field of view were measured. The percentage re-epithelialisation across the whole wound was then calculated as shown in Fig. 2. The thickness of the neo-epidermis was measured in the middle of each field and an average epidermal thickness calculated for each wound and each normal skin section. The number of hair follicles present in each field of view was noted.

Similarly, the depth/thickness of the "neo-dermis" (i.e. scar) and total dermis down to but not including the fat was measured mid field, the same as the epidermal thickness. Total dermal thickness/depth of the dermis of the normal unwounded skin harvested along with the wounds/scars was also measured.

Slides stained for inflammatory markers were scanned with a digital slide scanner (Pannoramic 250 Flash II, 3DHISTECH Ltd.). Scanned images were viewed using the associated Pannoramic Viewer software and 1000 µm fields of view created. Positively stained cells were counted using HISTOQUANT analysis software (3DHISTECH Ltd.) and the number of positively stained cells/mm^2 of dermis in each section calculated.

A semi-quantitative analysis of the neo-dermal architecture of the wounds was performed on blinded Picrosirius red stained slides using a scoring system developed in our laboratory (Table 1). The length, thickness, orientation and spacing of collagen bundles were recorded both at the edges and the centre of the scars.

2.5. *Statistical analysis*

Data were analysed by GraphPad Prism 7.01 using non-parametric tests as the data were not normally distributed. The Wilcoxon Signed rank was used to compare wounds within the same animal (paired data). The Mann Whitney U test was used to compare the two wound types at each time point (unpaired data) while the Kruskal-Wallis test with post hoc Dunn's Multiple Comparisons test was used to compare both wound types with normal skin at the different time-points (unrelated data). A p value of $p<0.05$ was considered statistically significant.

Table 1 – Scoring criteria for semi-quantitative assessment of scar architecture.

Collagen bundle length	Collagen bundle thickness	Collagen bundle orientation	Collagen bundle spacing
1: ≤25% of normal dermis	1: ≤25% of normal dermis	1: ≤25% of normal dermis	−2: very tightly packed
2: >25%, ≤50% of normal dermis	2: >25%, ≤50% of normal dermis	2: >25%, ≤50% of normal dermis	−1: tightly packed
3: >50%, ≤75% of normal dermis	3: >50%, ≤75% of normal dermis	3: >50%, ≤75% of normal dermis	0: normal dermis
4: >75%, ≤100% of normal dermis	4: >75%,≤100% of normal dermis	4: >75%,≤100% of normal dermis	1: loosely packed
			2: very loosely packed

3. Results

3.1. *Validation of partial thickness wounding model*

To quality control the wound model, wax embedded paraffin sections of 13 burn wounds (60 min after injury) and the excised skin from 8 excisional wounds were stained with Masson's trichrome (Lillie's modification) stain. The depth of each wound was measured in the centre and at the mid-point between the centre and each edge, giving 3 measurements per wound. The mean depth per wound was then calculated. The depth of burn wound was measured down to the level of altered collagen and thrombi in the blood vessels. As shown in Fig. 3 there were no significant differences between the depth of the burn and the excisional wounds.

3.2. *Does the type of injury affect the inflammatory profile?*

There was a marked inflammatory response in the burn wound compared with the excisional wound. As Fig. 4 shows, there were significantly more neutrophils (A) per mm^2 of dermis in the burn than in the excisional wound at day 7 ($p=0.0002$). By day 14 after injury when the wounds were largely re-epithelialised, there were few myeloperoxidase positive neutrophils.

Interestingly, in the first 7 days after injury, there were more cd163 positive macrophages and cd8a positive lymphocytes in the burn wounds compared to the excisional wounds whilst this difference was much reduced thereafter (D and E).

3.3. *Does the mode of injury affect the rate of re-epithelialisation or the neo-epidermal thickness?*

Excisional wounds re-epithelialised faster than the burn wounds (Fig. 5). This difference was significant at day 7 after injury (excisional wound 77.97% ±6.7; burn wound 23.43% ±14.06) and was noted macroscopically but more marked microscopically. Interestingly by day 14 after injury almost all the wounds had re-epithelialised.

Wound edges and epidermal appendages both contributed to the process of re-epithelialisation. However, at earlier time points after injury, re-epithelialisation from epidermal appendages was more marked in excisional wounds compared to burn wounds. There were fewer hair follicles in the underlying dermis of burn wounds (mean=2.1/mm^2 of the dermis) 7 days after injury compared with the excisional wounds (mean=2.8/mm^2 of the dermis) (Fig. 6).

The neo-epidermis of both wound types was thicker than that of normal epidermis, once the wound had fully re-epithelialised. Fig. 7 shows that at day 70 after injury the

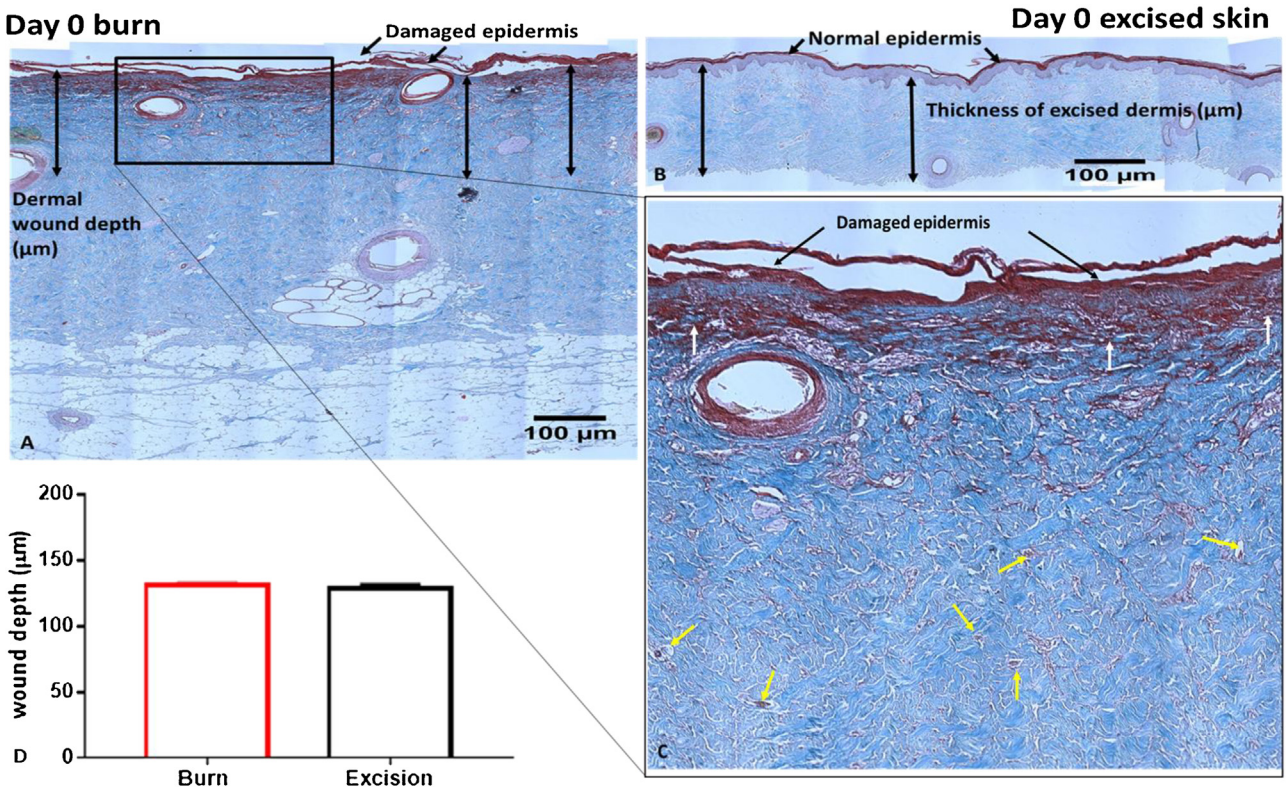


Fig. 3 – Masson's trichrome stained day 0 (60 min after wounding) burn (A) and excised skin (B) sections demonstrating the depth of burn wound (black double arrow) and the thickness of excised dermis (black double arrow). Heat damaged denatured collagen fibres (white arrows) and thrombosed blood vessels (yellow arrows) in burn damaged skin (C) There were no significant differences in wound depth observed between the burn wound and the excisional wound (D). Scale bar=100 μm (error bar=SEM). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

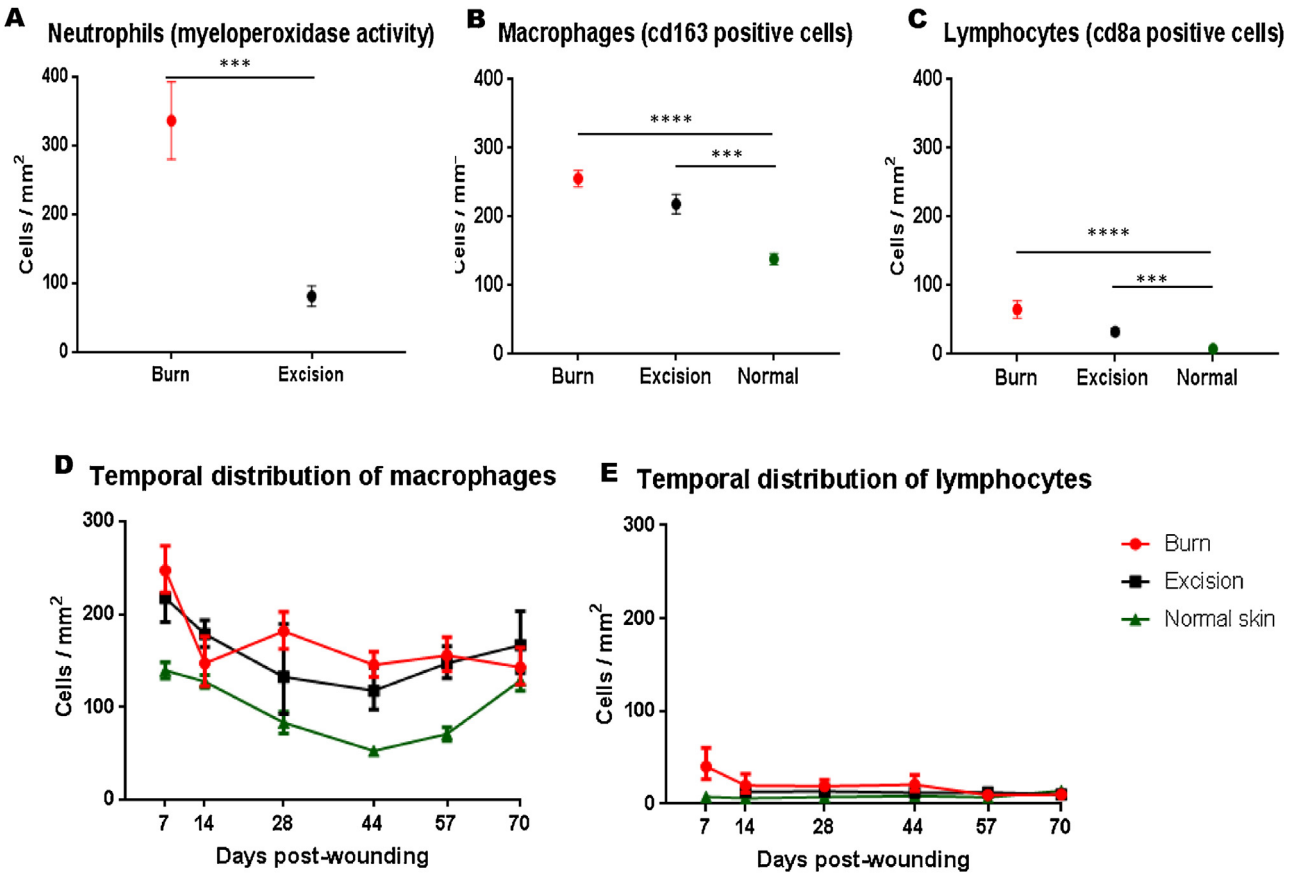


Fig. 4 – Significant differences in number of neutrophils (A) macrophages (B) and lymphocytes (C) between burn and excision wounds were observed at day 7 after injury. Temporal changes in number of macrophages (D) and lymphocytes (E). (Error bar=SEM, ***=p value <0.001, **=<0.0001 p value).**

neo-epidermis in burn wounds was thicker than in excisional wounds which was, in turn, thicker than normal epidermis.

3.4. *Does the mode of injury affect scarring and remodelling?*

Interestingly, following excision of partial thickness dermis, there was reformation of neo-dermis. These wounds did not leave a "dent" in the skin but appeared to fill up with neo-dermis. At day 7 after wounding, newly formed immature collagen fibres were present in the neo-dermis of excisional wounds but not in the burn, where the collagen fibres appeared to be damaged or lost.

At day 70 after wounding, the depth/thickness of the neo-dermis demonstrated significant differences between the two types of wounds. In the burn wound, the thickness of the neo-dermis comprised the upper third of the total dermis whilst it was less than a third of the dermal thickness in excisional wounds (Fig. 8). Interestingly, despite creating a partial thickness excisional wound, at day 70 after injury, the total dermal thickness of the resultant healed wound was actually thicker than the dermis of normal adjacent unwounded skin. As expected, the total dermal thickness of a healed burn was thicker than both the healed excisional wound dermis and normal unwounded dermis (Fig. 9).

The architecture of the neo-dermis of both wound types was distinctly different from the normal basket-weave pattern seen in un-injured dermis. However, the neo-dermis of the excisional wound was more akin to normal dermis. The collagen bundles in the excisional scars were better orientated and thicker than those in the burn scar where the fibres were crowded haphazardly and the bundles were of variable length and thickness (Fig. 8). Semi-quantitative analyses demonstrated that there were significant differences in the neo-dermal architecture of both types of scars compared to normal skin dermal architecture (Fig. 10). The collagen bundle spacing and orientation were significantly different between the excisional and burn scars at the later time points (p=0.0002 and p=0.0002 respectively).

4. Discussion

Partial thickness wounds generally have a spontaneous healing capacity due to the presence of epidermal stem cells in the basal layer of the surrounding interfollicular epidermis, bulge region of the hair follicle and base of the sebaceous glands: remnants of the epidermal appendages in the residual dermal wound bed [7]. It is therefore not uncommon to treat partial thickness excisional wounds (e.g. split thickness skin

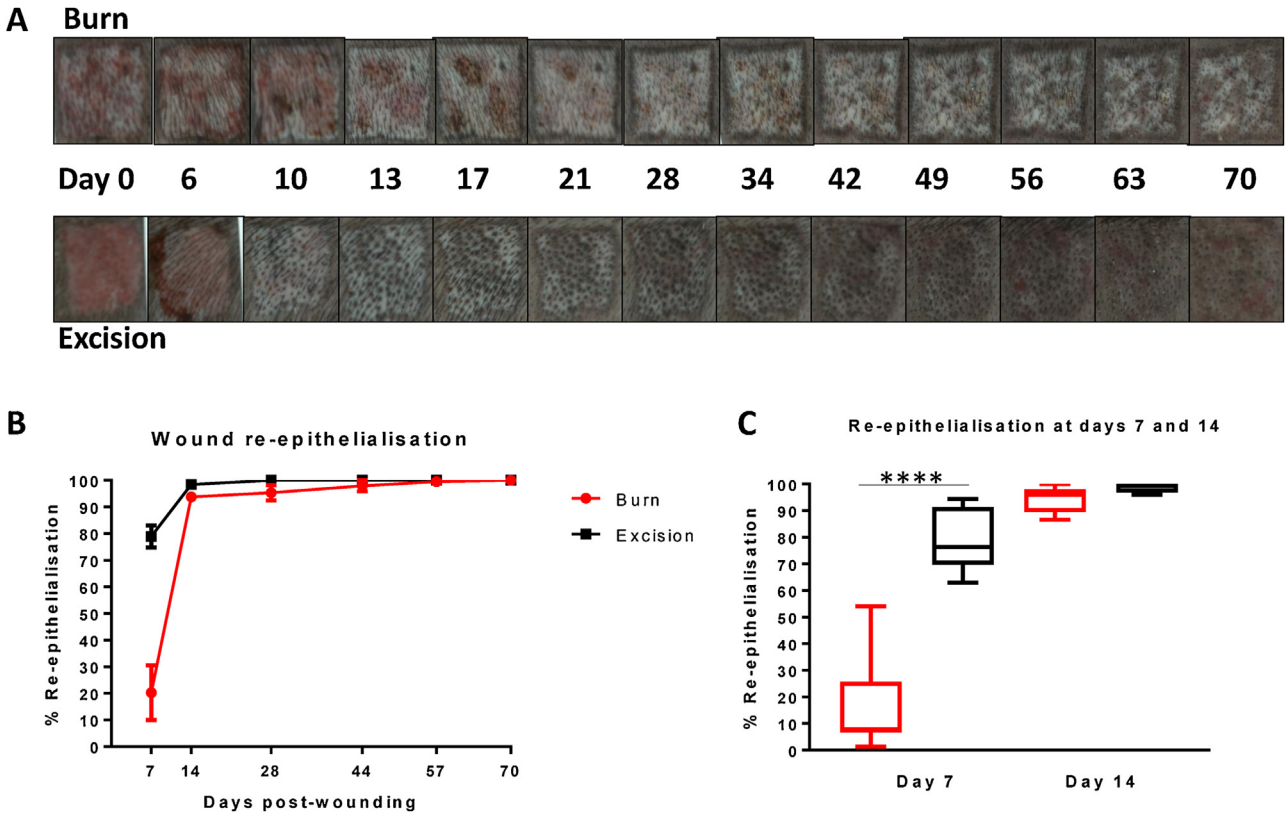


Fig. 5 – Macroscopic differences in re-epithelialisation and re-pigmentation of burn and excisional scars with time (A); microscopically re-epithelisation is significantly faster in excisional wounds compared to burn wounds at day 7 after injury ($p < 0.0001$) but is more than 90% complete in both excisional and burn wounds by day 14 (B, C). (Error bar = SEM (B) and min to max (C)).

graft donor site) and superficial partial thickness burn wounds in a similar manner. However, as clinicians, we have noticed differences in the outcome from these two types of wounds. Therefore, we set up a study to investigate whether the mechanism of injury in a partial thickness wound affects the wound healing and scarring. We used a porcine model for this study as porcine skin closely resembles human skin [8]. In order to validate our wound model, i.e. depth of partial thickness wounds created by burn and excision, we measured the thickness/depth of the excised skin (partial thickness excisional wounds) and compared that to the depth of the altered endothelium of the blood vessels and altered collagen in the burn wound at 60 min after injury. These depths were similar.

This study demonstrates that the mechanism of injury affects wound healing of partial thickness wounds and resultant scarring. In a partial thickness excisional wound, there is loss of part of the dermis along with the overlying epithelium causing the initial bleeding and subsequent blood clot over the surface of the wound. By contrast, there is no bleeding in the burn wound as thermal injury causes coagulative necrosis of the tissue; the epidermis separates from the dermis, the underlying dermal collagen is denatured and blood vessels thrombose [9]. The extent of injury in the burn wound is dependent on the temperature of the hot object, the duration and pressure of contact. Burns are dynamic wounds and the damage has been reported to progress vertically. However, Singer et al. [10] and Hirth et al. [11] have demonstrated that the depth of the endothelial damage in a burn wound at 60 min after injury predicts the depth of the burn at 7 days after injury [10,11].

The inflammatory response begins soon after wounding. Studies on incisional wounds have shown that different inflammatory cells follow a distinct temporal pattern of recruitment into the wound during normal healing [12]. An early dermal infiltration of neutrophils is vital for wound cleansing through several strategies including a burst of reactive oxygen species (ROS) to degrade the damaged tissue. Neutrophils are replaced by macrophages which phagocytose and clear the debris and promote collagen formation. Any delay or inhibition of macrophage dermal infiltration can disrupt the orderly healing process. The inflammatory profiles of partial thickness excisional and partial thickness burn wounds were examined by delineating myeloperoxidase activity in neutrophils, alternatively activated cd163 positive macrophages and cd8a positive lymphocytes. At day 7, the burn wounds had almost five times as many neutrophils as excisional wounds and this number was higher in the centre of the wounds compared to the wound edges. Although wound infection can be an important cause of raised neutrophils, none of our burn wounds showed clinical evidence of infection. The absence of neutrophils at later time-points

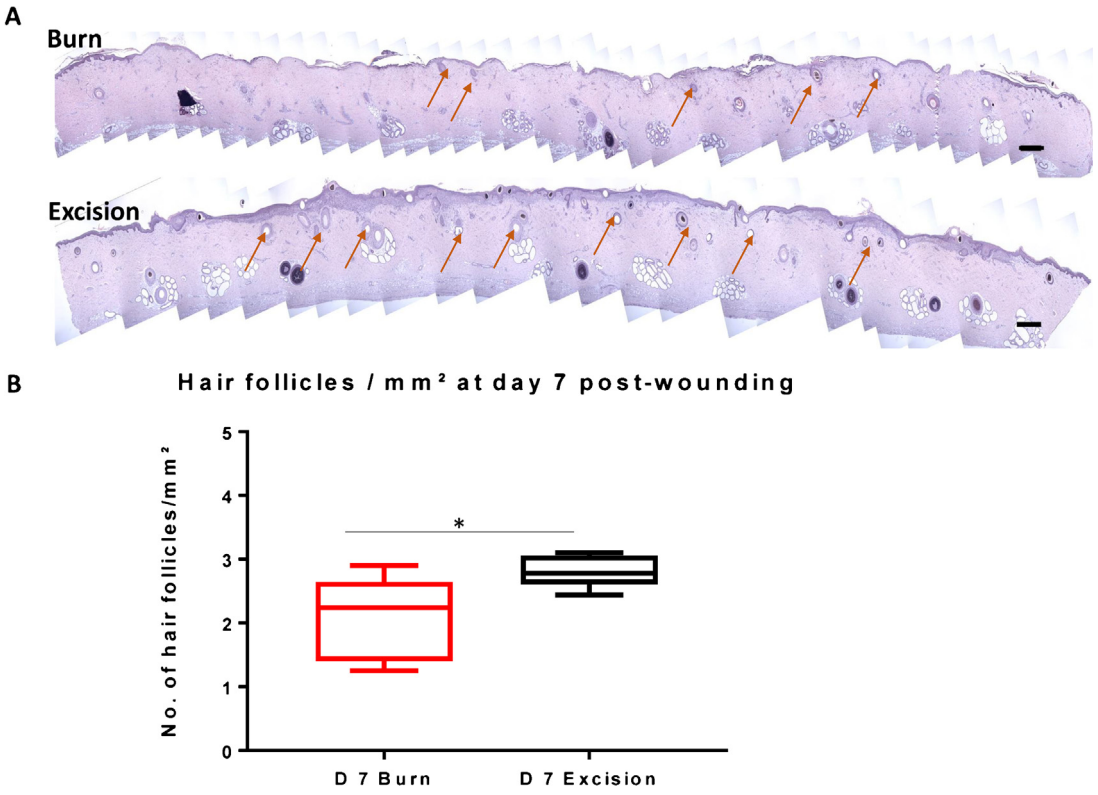


Fig. 6 – H & E stained day 7 burn and excisional wound sections (A) showing the hair follicles (brown arrows) in the upper dermis. There were fewer hair follicles in the underlying dermis of burn wounds at day 7 after injury compared with the excisional wounds (B). Scale bar=100 μm (error bar=SEM, **=p value 0.0059). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

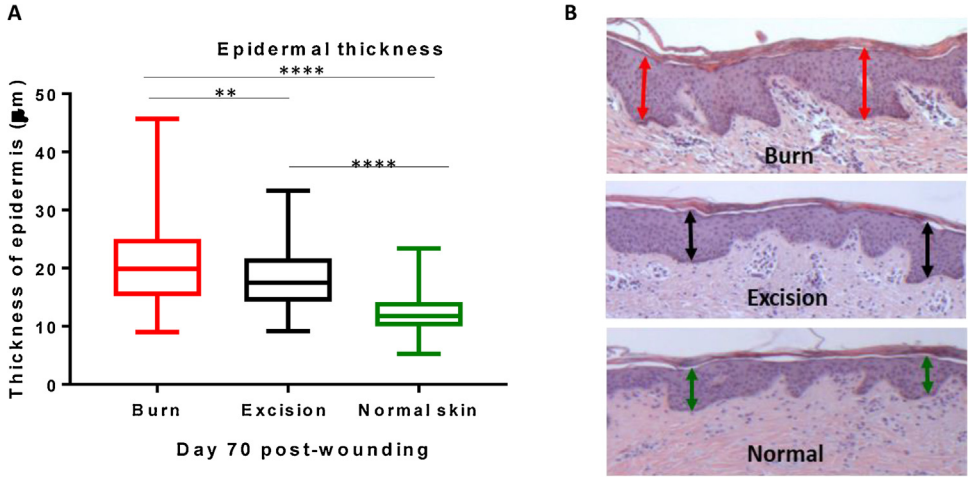


Fig. 7 – The thickness of the neo-epidermis in burn scar (red box) and excisional scar (black box) is significantly thicker than normal epidermis (green box) at day 70 after injury. (Error bar=min to max, **=p value 0.007, ****=p value <0.0001). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

coincided with re-epithelialisation of the wound. Faster re-epithelialisation in excisional wounds may have contributed to the lower neutrophil presence in these wounds compared to the burn wounds at day 7.

Macrophages also displayed a similar trend of dermal infiltration that was higher in the burn compared with the excisional wound at day 7 and mostly remained higher in the burn wounds until day 44 after injury. The temporal pattern of the cd163 positive macrophage infiltrate was different between the two wound types: in both wound types, the maximum infiltrate was noted at day 7 after wounding; however, in the burn, the macrophage numbers fluctuated up

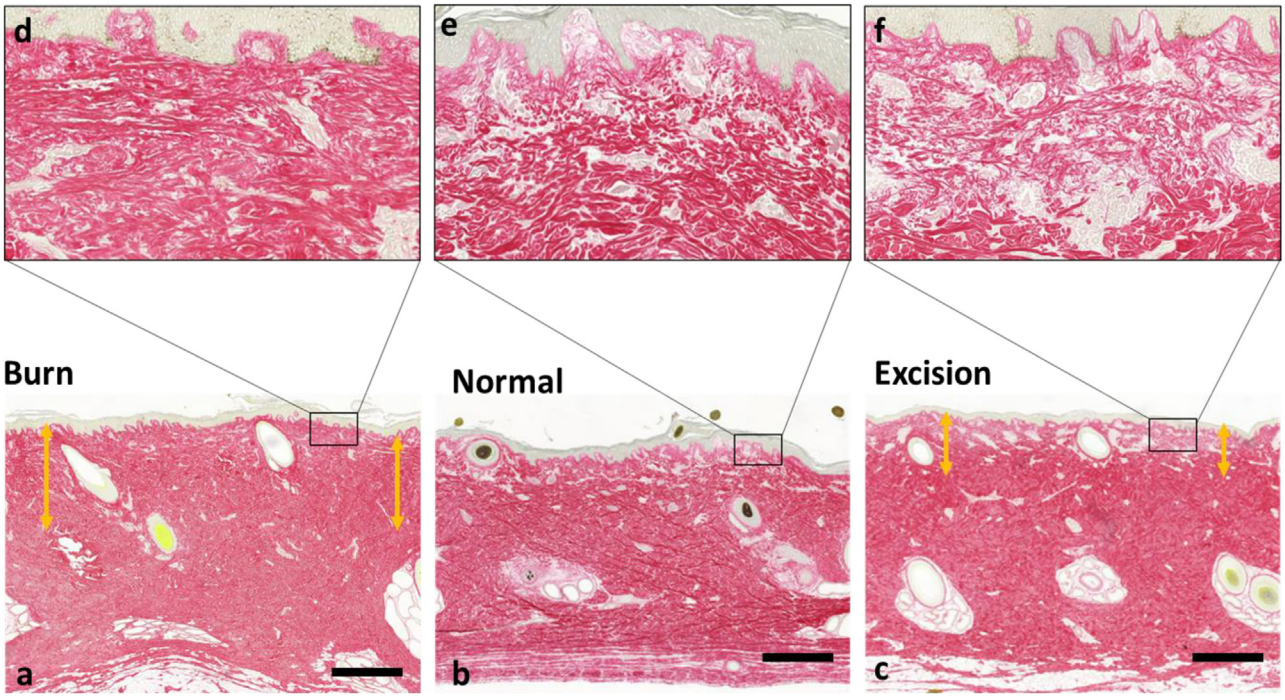


Fig. 8 – Dermal architecture of a burn scar (a), normal skin (b) and excisional scar (c) at day 70 after injury. Yellow arrows indicate the depth of the neo-dermis (scar). Magnified images of the burn scar (d), normal skin (e) and excisional scar (f) highlight how the architecture of the neo-dermis of excisional scars was more akin to normal dermis than the neo-dermis of a burn scar. Scale bar=500 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

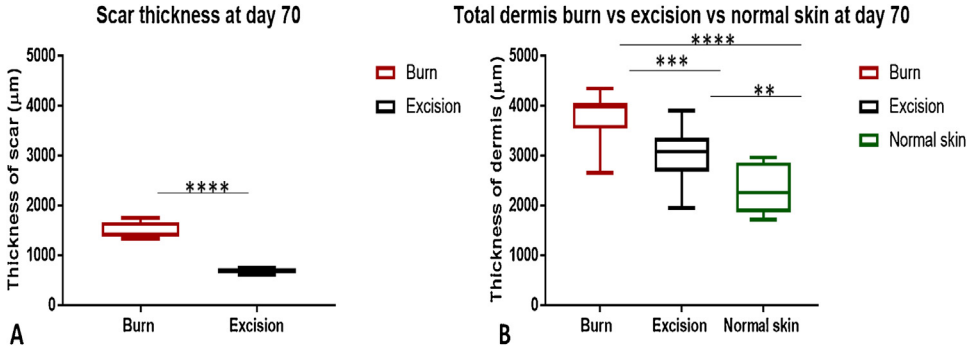


Fig. 9 – At day 70 after injury, the thickness of the scar (A) demonstrated significant differences between the two types of wounds. The total dermal thickness of a healed burn was more than both the healed excisional wound dermis and normal unwounded dermis (B). (Error bar=min to max, **=p value 0.001, ***=p value <0.0002 ****=p value <0.0001). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and down over time, increasing at day 28 and then reducing over time thereafter. By contrast, in the excisional wounds, the macrophage infiltrate reduced from day 7 after wounding to day 44 after wounding and thereafter increased up to day 70, the last time point studied. The sustained inflammatory response seen in burn wounds in this study may be due to the need to clear the increased necrotic tissue from the burn compared to the excisional wounds. However, it is worth noting that cd163 monoclonal antibody only identifies alternatively activated macrophages.

T-lymphocytes also regulate wound healing by producing several factors affecting the function of both macrophages and fibroblasts. The depletion of T-lymphocytes by means of specific cytotoxic monoclonal antibodies in mice causes impairment in wound healing [13]. $Cd8^+$ lymphocytes have been found to down-regulate the healing process thus, any reduction in lymphocyte numbers may prevent this down regulation and prolong the wound healing process. However, a non-specific trend in the lymphocytic infiltrate of human skin wounds has been previously reported by Tarran et al. [14]. In our study, burn wounds displayed twice as many lymphocytes as excisional wounds, these were found mostly in the wound bed rather than at wound edges which could explain the advanced healing observed at the burn wound edges.

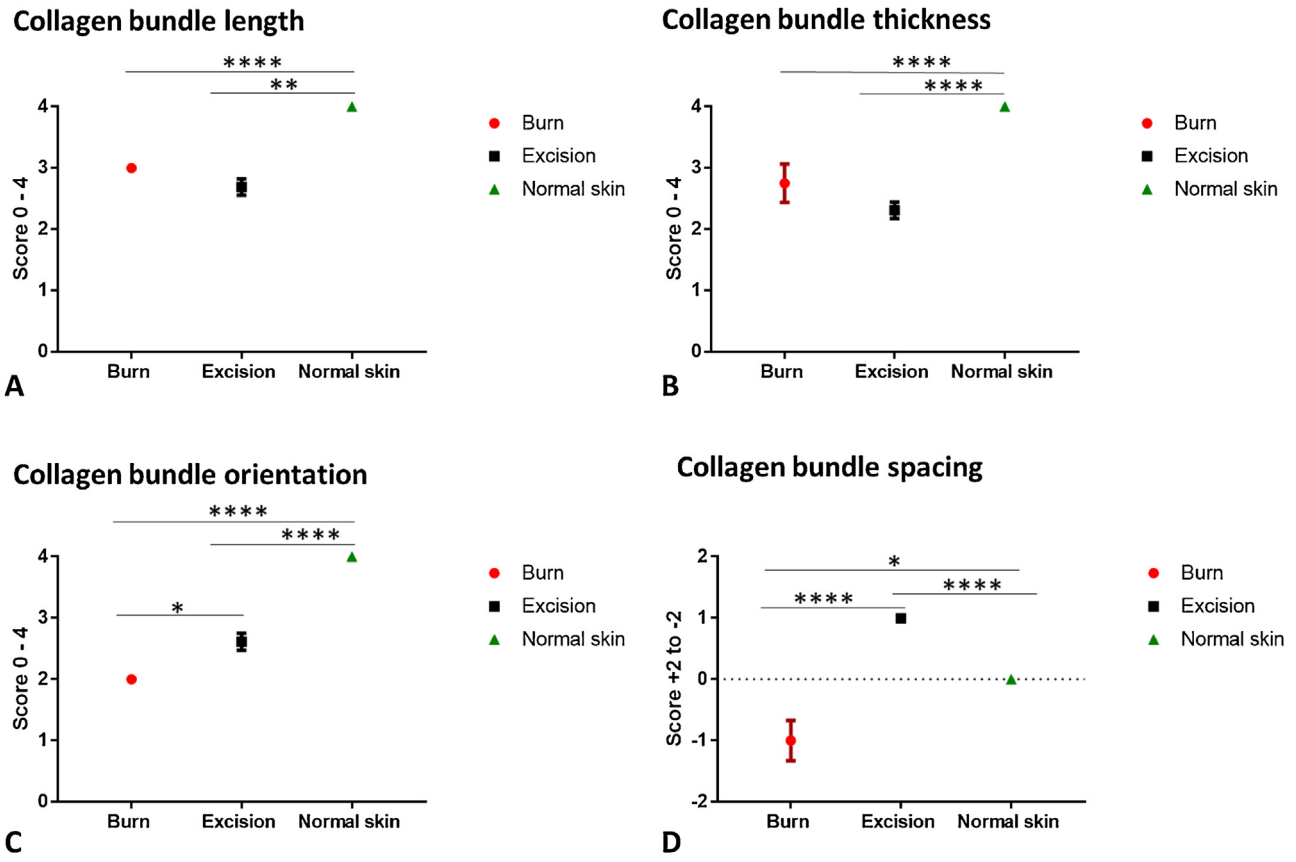


Fig. 10 – Semi-quantitative analyses of neo-dermal architecture at day 70 after injury demonstrating the differences of collagen bundle length, thickness, orientation and spacing in both types of scars (red: burn; black: excisional) compared to normal dermal architecture (green). The collagen bundle spacing and orientation were significantly different between the excisional and burn scars at the later time points (p=0.0002 and p=0.0002 respectively). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Partial thickness excisional wounds were found to be far more advanced in their re-epithelialisation at day 7 after injury compared to partial thickness burn wounds. However, by 14 days, the wounds had virtually re-epithelialised in both wound types. Hair follicle remnants within a partial thickness wound are considered to be an essential source of keratinocytes due to the presence of stem cells in the bulb region of the hair follicle [15]. Our study found a higher number of hair follicles under the wound surface in excisional wounds compared to burn wounds 7 days post-wounding. By day 14, the number of hair follicles under the wound surface in the burn was noted to be more than in the excisional wound. We speculate that the hair follicle cycle may be activated earlier following an excisional wound compared to the burn wound and therefore the number of hair follicles seen in the upper dermis after an excisional wound are more than burn. This in turn would contribute to a larger stem cell pool earlier in an excisional wound and therefore early re-epithelialisation. It has been previously suggested that the reduced rate of re-epithelisation in burn wounds could be due to the varying degrees of damage to the appendageal remnants caused by the thermal energy compared to the excisional wounds created with a dermatome; the thermal energy is thought to extend further down the hair follicle shaft causing damage or delay in the activation of the stem cell population. This may well be the reason for the delayed re-epithelialisation in the burn wound.

The epithelial tongues merged to form the neo-epidermis and keratinocytes differentiated into stratified layers over time. As expected, the thickness of the neo-epidermis of excisional wounds was greater than that of the burn wounds until day 14 after wounding, following which the neo-epidermis of the burn and excisional wounds became similar in thickness until day 44 after wounding. The thickness of the epidermis of both wound types was thicker than that of normal epidermis. The neo-epidermis of the burn scar was significantly thicker than that of the excisional scar. Epidermal thickness has been demonstrated to be correlated to the quality of the scarring by several investigators. Mustoe and Gurjala [16] hypothesised that the epidermis plays an important role in the quality of cutaneous scarring. They summarised lines of evidence from both human and animal studies demonstrating that the thickness of the epidermis and development of effective stratum corneum influenced the activity and quality of scarring. Machesney et al. [17] demonstrated that the epidermis of hypertrophic scars had activated keratinocytes, a phenomenon that is present during the re-epithelialisation process of normal wound healing. Limandjaja et al. [18] have recently reported the differences in

epidermal thickness between normal skin, normotrophic scars, hypertrophic scars and keloid scars with increasing thickness of the epidermis related to the severity of scarring. Whilst we have not looked for the activation markers of keratinocytes in this study at day 70 after wounding, the neo-epidermis of the burn is certainly much thicker than that of excisional wounds which is in turn thicker than normal epidermis and this is correlated with the thickness of the scar and total dermis as explained below.

The macroscopic appearance of the scars at day 70 show a thicker slightly raised burn scar and a flat excisional scar. Qualitative assessment of dermal architecture illustrated distinct differences in the appearance of scars from the two wound types. We found shorter and thinner collagen fibres in the excisional wound compared to the burn wound. The gross architecture of excisional scars at day 70 was closer to the architecture of normal skin when comparing the uniformity in size and spacing of the fibres. By contrast, the fibres within burn scars at day 70 were of variable size and were more crowded and disorganised across the whole width of the affected area of the dermis. In both wound scars, the normal basket weave orientation of fibres noted in normal skin was lost. Distinct differences between collagen fibre thickness and orientation between the edge and centre of the wound suggest the scar re-modelling process was more advanced in the wound edge as compared to the centre in both types of wounds. Ross et al. [5] also observed architectural differences within the neo-dermal regions of the two types of partial thickness wounds in their study. They observed fine, fibrillar collagen fibres within the papillary neo-dermis (as seen in normal papillary dermis) which were more vertically orientated but with fewer elastic fibres in excisional wounds and superficial burn wounds compared to normal adjacent skin. In contrast, the neo-papillary dermis of deep thermal wounds showed denser, more compact elastic and collagen fibres with a horizontal orientation. The findings in our study did not concur with those reported by Ross et al. as all burn injuries showed highly disorganised fibre patterns compared to excisional wounds. These differences may be due to the source of the burn injury, Ross et al. used laser burns while our study created a contact burn.

An intriguing finding was that despite excising part of the dermis in excisional wounds, the neo-dermal thickness at 70 days after injury was greater than that of normal, uninjured dermis, suggesting reformation of dermis albeit developing a scar. The neo-dermal thickness of the burn scar was significantly thicker than that of the excisional scar as well as the normal skin. It is possible that the rapid re-epithelialisation seen in excisional wounds at 7 days after injury, may have dampened down the inflammatory response and thereby improved the quality of the resultant scarring compared to the burn wound. From a clinical point of view, if there is reformation of neo-dermis as seen in this study, perhaps re-cropping of the skin graft from the same donor site could be delayed somewhat to ensure less morbidity of the donor site.

5. Conclusion

Despite sharing a common reparative process of inflammation, there are considerable spatial and temporal differences of re-epithelialisation and scar formation between similar thickness partial thickness burn and excisional wounds. This suggests that wound healing is influenced by the type of injury. It is therefore, inappropriate to use excisional wounds as surrogates for burn wounds to test the efficacy of therapeutic agents intended for optimising partial thickness burn wound healing.

Conflict of interest

None.

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REFERENCES

[1] Li J, Chen J, Kirsner R. Pathophysiology of acute wound healing. Clin Dermatol 2007;25:9–18.
[2] National Burn Care Review 2001, Standards and Strategy for Burn Care. http://www.specialisedservices.nhs.uk/library/23/National_Burn_Care_Review_2001.pdf.
[3] Benson A, Dickson WA, Boyce DE. ABC of wound healing burns. BMJ 2006;332(7542)649–52 Erratum in: BMJ 2006;332(7544):755.
[4] Schaffer CJ, Reinisch L, Polis SL, Stricklin GP. Comparisons of wound healing among excisional, laser-created, and standard thermal burns in porcine wounds of equal depth. Wound Repair Regen 1997;5:52–61.
[5] Ross EV, Naseef GS, McLinlay JR, Barnette DJ, Skrobal M, Grevelink J, et al. Comparison of carbon dioxide laser, erbium: YAG laser, dermabrasion, and dermatome: a study of thermal damage, wound contraction, and wound healing in a live pig model: implications for skin resurfacing. J Am Acad Dermatol 2000;42:92–105.
[6] Chadwick SL, Yip C, Ferguson MWJ, Shah M. Repigmentation of cutaneous scars depends on original wound type. J Anat 2013;223:74–82.
[7] Pastar I, Stojadinovic O, Yin NC, Ramirez H, Nusbaum AG, Sawaya A, et al. Epithelialization in wound healing: a comprehensive review. Adv Wound Care 2014;3:445–64.
[8] Sullivan TP, Eaglstein WH, Davis SC, Mertz P. The pig as a model for human wound healing. Wound Repair Regen 2001;9(2):66–76.
[9] Shakespeare P. Burn wound healing and skin substitutes. Burns 2001;27:517–22.
[10] Singer AJ, Hirth D, McClain SA, Clark RA. Validation of a vertical progression porcine burn model. J Burn Care Res 2011;32(6):638–46.
[11] Hirth D, McClain SA, Singer AJ, Clark RAF. Endothelial necrosis at 1h post-burn predicts progression of tissue injury. Wound Repair Regen 2013;21(4):563–70.

[12] Engelhardt E, Toksoy A, Goebeler M, Debus S, Brocker EB, Gillitzer R. Chemokines IL-8, GROalpha, MCP-1, IP-10, and Mig are sequentially and differentially expressed during phase-specific infiltration of leukocyte subsets in human wound healing. Am J Pathol 1998;153(6):1849–60.
[13] Davis PA, Corless DJ, Aspinall R, Wastell C. Effect of $CD4^+$ and $CD8^+$ cell depletion on wound healing. Br J Surg 2001;88:298–304.
[14] Tarran S, Langlois N, Dziewulski P, Sztynda T. Using the inflammatory cell infiltrate to estimate the age of human burn wounds: a review and immunohistochemical study. Med Sci Law 2006;46:115–26.
[15] Takeo M, Lee W, Ito M. Wound healing and skin regeneration. Cold Spring Harb Perspect Med 2015;5:a023267.
[16] Mustoe TA, Gurjala A. The role of the epidermis and the mechanism of action of occlusive dressings in scarring. Wound Repair Regen 2011;19(Suppl 1):s16–21.
[17] Machesney M, Tidman N, Waseem A, Kirby L, Leigh I. Activated keratinocytes in the epidermis of hypertrophic scars. Am J Pathol 1998;152(5):1133–41.
[18] Limandjaja GC, van den Broek LJ, Waaijman T, van Veen HA, Everts V, Monstrey S, et al. Increased epidermal thickness and abnormal epidermal differentiation in keloid scars. Br J Dermatol 2017;176:116–26.