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The progression of burn depth in experimental burns: a histological and methodological study

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Abstract

This study was designed to create a reproducible model for experimental burn wound research in pigs. Previously, the thicker paraspinal skin has been used. We used the more human-like ventral skin to create burns of different depths.

Contact burns were created to 11 pigs using a brass plate heated to 100 °C in boiling water. Different contact times were used to create burns of different depths. In pigs 1–6, the follow-up time was 72 h and in pigs 7–11 24 h. Burn depth was determined by histology. Histologically, samples were classified into five anatomical layers: epidermis, upper one-third of the dermis, middle third of the dermis, deepest third of the dermis and subcutaneous fat. The location of both thromboses and burn marks were evaluated, respectively.

The 1 s contact time lead to a superficial thermal injury, 3 s to a partial thickness and 9 s to a full thickness injury. A progression of burn depth was found until 48 h post-injury. The intra-observer correlation after repeated histological analyses of burn depths by the same histopathologist and the repeatability of burn depth creation yielded kappa coefficients 0.83 and 0.92, respectively.

Conclusion: a reproducible burn model for further research purposes was obtained.

Keywords: Burn; Depth; Histology; Progression; Pig

1. Introduction

Treatment of a burn wound is dictated by its depth. In practice, the depth of the injury is evaluated by clinical judgement [1]. Superficial and very deep burns are not a challenge for burn depth evaluation. However, burns of indeterminate depth create challenges both in diagnostic matters and treatment modalities.

Histological wound biopsy would seem to be the most precise diagnostic tool for burn depth evaluation [1]. However, it has never become a useful tool for clinical practice, since it is expensive, time consuming and leaves a scar in the site of the biopsy [2]. Also, the dynamic changes in the burn site make both the timing of the biopsy and the evaluation of the microscopic changes troublesome [1].

The development of oedema and progressive vascular damage are characteristic for burn injuries [3]. Vascular patency has shown to be a reliable method for determining the depth of burn injury [4]. Accordingly, a progression of burn depth was found in deep dermal burns in humans while superficial burns remained stable during the 48 h follow-up.

As a part of a larger study to examine local events in experimental burns, we aimed to create a reproducible burn model of inducing burns of different depths. Previously, the thick dorsal skin of the pig has been used to create experimental burns [5,6]. The skin of pig and human is almost indistinguishable by physiological, anatomical and physical resemblance [7]. In the present study, the thinner, more human-like, skin of the ventral side of the pig was used.

2. Materials and methods

2.1. Anesthesia and monitoring

This study was approved by the Institutional Animal Care and Use Committee of the University of Kuopio. Three-month-old female Finnish landrace pigs ($N = 11$, 28–38 kg) were fasted for 48 h with free access to water

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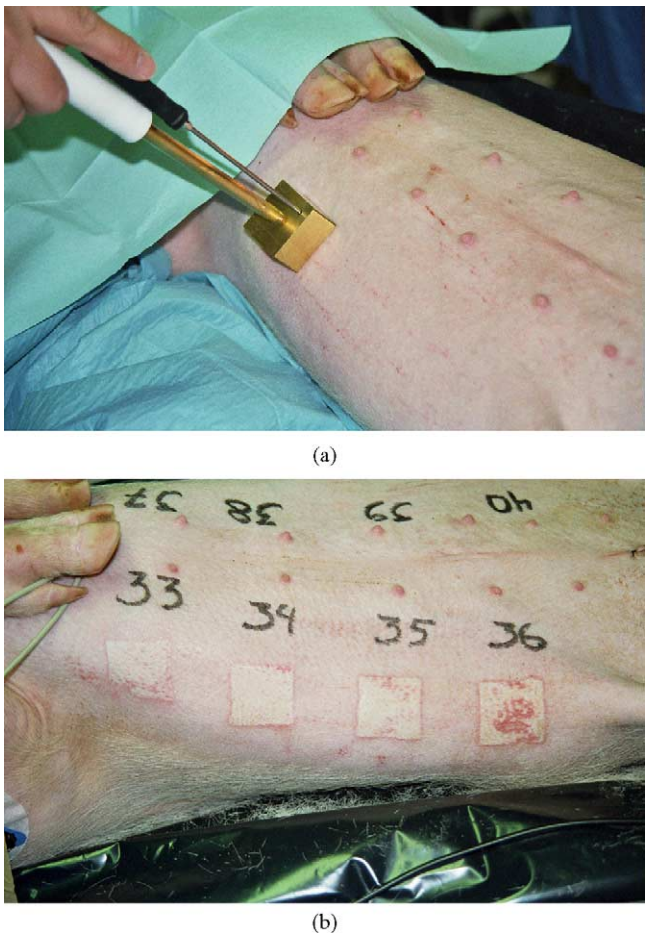
prior to the experiment. After premedication with atropine (0.05 mg/kg i.m.) and azaperone (8 mg/kg i.m) an ear vein was cannulated for administration of thiopenthal sodium (5–15 mg/kg i.v.) as induction of general anesthesia. Animals were intubated or tracheostomized and ventilated with a volume-controlled ventilator (Servo 900E, Siemens, Elema, Sweden) at a tidal volume of 10 ml/kg. Minute volume was adjusted to achieve normocapnia ($paCO_2$ 4.4–5.5 kPa, 34–41 mmHg). Fraction of oxygen in the inspiratory gas (FiO_2) was adjusted to keep arterial partial pressure of O_2 >13.3 kPa (100 mmHg). Positive end expiratory pressure of 5 cm H_2O was maintained throughout the study. Anesthesia was maintained with infusion of thiopentone (5 mg/kg/h). Pancurone (2–4 mg boluses i.v.) was administered for shivering when needed and fentanyl 30 μg/kg/h during the creation of burn wounds and 5 μg/kg/h thereafter for pain relief.

Right carotid artery and internal jugular vein were cannulated for blood pressure and central venous pressure (CVP) monitoring and blood sampling. Systemic and central venous pressures were recorded with quartz pressure transducers and displayed on a multimodular monitor and recorder (AS3, Datex-Ohmeda, Helsinki, Finland). Continuous information was collected automatically in two minute intervals (Clinisoft, Datex-Ohmeda, Espoo, Finland). All pressure transducers were zeroed to the level of the heart. Heart rate was continuously monitored with electrocardiogram. Haemodynamics were recorded at 15 min intervals. A urinary catheter was placed through a small incision in the lower part of the abdomen for urinary output measurements.

Animals received 50% glucose infusion which was adjusted to maintain normoglycaemia (5–7 mmol/l). Normovolaemia was maintained with Ringer's acetate (Ringersteril, Baxter, Vantaa, Finland) according to CVP 4–7 mmHg.



(a)

(b)

Fig. 1. (a) Creating a burn with a heated brass block. A digital thermometer is placed inside the block to measure the temperature of the block. (b) The 3 (marked 33), 6 (34), 9 (35) and 12 (36) second contact burn sites on right flank of the animal.

2.2. Experimental protocol

The ventral side of the body was shaved and washed with chlorhexidine solution (5 mg/ml). The burns were created by using a 4 cm × 4 cm brass block heated to 100 °C in boiling water (Fig. 1a). The temperature of the block was measured by having the tip of a digital thermometer (Lutron Thermometer, Taiwan TM-903) inserted in a hole of the block. By varying the contact time between the block and the animal skin, burns of different depths were created (Fig. 1b). Only the weight of the block was used to create the burns. No additional pressure was put on it. In order to avoid variations in creating the burns, one person (AP) created all burns. Two studies were performed.

Study 1: In pigs 1–5, four burns were created on each half of the ventral body 5 cm apart from each other using contact times 3, 6, 9 and 12 s. Contact times were modified from the previous investigation by Schomacker et al. [8] where thicker paraspinal pig skin was used. Thus, a total of eight burns were created on each animal, two of each contact time. Additional 10 burns (5 on each half of the ventral body) using a 1 s contact time were created on pig 6 to create more superficial burns and to test the repeatability of both the creation of superficial burns and the applied measurements. All burns were covered with moist saline dressings which were changed daily. There were two non-burned control sites in each animal, one in each side of the ventral torso. The follow-up time was 72 h. A 6 mm punch biopsy was taken at 2, 24, 48 and 72 h post-burn for histology, one from each quadrant of each burned site extending down to subcutaneous fat to evaluate the progression of burn depth. Thus, a total of four biopsies were taken from each burn site, one at each time point. The animals were kept sedated in the respirator throughout the study and were sacrificed with an i.v. overdose of magnesium sulphate.

Study 2: In pigs 7–11, three burns were similarly created on each side of the torso using 1, 3 and 9 s contact times. After creating the burns, the sites were covered with moist saline dressings. There was one control site in the middle of the ventral torso in each animal. The follow-up time was 24 h. The animals were kept sedated in the respirator throughout the study and sacrificed with an intracardial

injection of magnesium sulphate. A punch biopsy was taken after sacrifice from the center of the burned site for histological evaluation.

An experienced histopathologist (KK) analyzed the first 10 samples from Study 1 together with another pathologist, after which they agreed on the criteria of burn-related changes. Following this, all samples were analyzed by using standard hematoxylin and eosin-stained histological sections of 5 μm-thickness cuts from paraffin embedded samples by the same histopathologist. Evaluation of the samples was done blinded to the details of the sample. After the primary evaluation, a repeated analysis of 35 randomly selected samples was performed 18 months later by the same pathologist to test intra-observer reliability by calculating the kappa coefficient as described by Cohen [9].

Histologically, samples were classified into five anatomical layers: epidermis (level 1), upper one-third of the dermis (level 2), middle third of the dermis (level 3), deepest third of the dermis (level 4) and subcutaneous fat (level 5). The location of both thromboses and burn marks were evaluated respectively. Vascular patency was described as intact vessel walls with normal endothelial cells and no signs of cellular debris or tightly packed erythrocytes [4]. Epidermis was evaluated for burn artifacts (distortion of cell contour) and separation of epidermis from dermis (subepidermal blistering). Dermis was evaluated for histological separation or destruction of different cell layers of hair folliculi and vessel walls, microthrombi and neutrophils. Subcutaneous fat was evaluated for identical adnexal findings as in the dermis, thrombosis and fat necrosis. Burn depth at each evaluation was graded as 1–5 according to the depth of the deepest burn-related histological finding of each sample. In addition, skin thickness was measured from three different points of every sample from the dermo-adipous junction to the surface of the epidermis and presented as mean of the three measured values.

2.3. Statistics

Values are presented as mean (range). We used the Helmert contrasts, which compare the average of the response variable in a time point with the average of the following time point means in sequence. Also, the non-parametric Wilcoxon test was used for statistical analysis. The kappa coefficient [4] was calculated to test the intra-observer reliability in Study 1 and the repeatability of burn creation between Studies 1 and 2. All analyses were performed using SPSS for Windows (SPSS Inc., Chicago, IL, USA) program. A P-value <0.05 was considered statistically significant.

3. Results

The weights and burned areas of the animals are presented in Table 1. The pigs in Study 2 were heavier than in Study 1. All animals survived the experiment until sacrifice. No wound infections occurred at burned sites. Pig 2 created a clinically obvious pneumonia at post-burn day 2, after which prophylactic antibiotic treatment (cefuroxime 375 mg × 3 i.v.) was started to pigs 3–6.

Table 1
Demografics of animals in Studies 1 and 2

Pig	Weight (kg)	TBSA (cm^2)	TBSA (%)
Study 1			
1	30	128	1.9
2	26	128	2.1
3	30	128	1.9
4	31	128	1.8
5	33	128	1.8
6	33	160	2.2
Mean	30.5		1.9
Study 2			
7	38	96	1.2
8	40	96	1.2
9	36	96	1.2
10	34	96	1.3
11	36	96	1.2
Mean	36.8		1.2

TBSA: total burned surface area.

3.1. Haemodynamics

The data of haemodynamic measurements, temperature and blood gas analysis are presented in Tables 2 and 3. There were no time-related changes in the measured parameters.

3.2. Clinical inspection

By inspection, the 1 s burns appeared redder than the burns with longer contact times. In the 1 s burns, a very narrow, sharp-edged oedema area was clinically apparent at and around the burned area at 2 h whereas in 3–12 s burns oedema was apparent at 4 h being most obvious in the 9 s burns. At 12 h oedema was clinically diminishing in all burn sites and only minimal oedema was left at 24 h.

3.3. Histology

3.3.1. Study 1

The main histological changes are presented in Fig. 2a–c and the progression of burn depth in Fig. 3. There was no progression of burn depth evident in the 1 s burns. They were all graded as superficial dermal burns at all time points. According to the Helmert contrasts, the 3 s burns were significantly more superficial than the burns created with longer contact times ($P = 0.001$). Similarly the 6 s burns were significantly more superficial than the 9 and 12 s burns ($P = 0.006$). However, no difference was seen between the 9 and 12 s burns ($P = 0.104$). Also, there was a time-related

Table 2
Haemodynamic data and core temperature at different timepoints post-burn

Time (h)	CVPm		$ETCO_2$		HR		SAPm		Tc	
	mmHg	Range	kPa	Range	b/min	Range	mmHg	Range	°C	Range
2	6.0	4.4–7.0	4.1	3.8–4.1	76	57–129	76	55–86	36.8	36.6–37.1
4	5.9	4.0–7.5	4.2	3.8–4.4	75	52–100	77	72–86	37.3	36.1–38.2
8	6.8	5.6–8.0	4.1	4.0–4.5	56	48–69	76	71–79	38.2	37.4–38.6
12	6.3	5.2–8.0	4.1	4.0–4.5	57	54–66	76	67–80	38.0	37.9–38.8
24	6.0	4.6–7.0	4.1	4.0–4.3	61	64–100	79	64–100	38.3	38.2–38.6
36	5.7	4.2–6.7	4.2	4.0–4.5	62	58–72	87	80–90	38.4	38.0–38.7
48	5.3	3.1–7.5	4.1	3.8–4.3	66	59–78	86	67–100	38.4	38.1–38.7
72	6.2	3.0–7.5	3.9	3.3–4.1	76	75–100	73	71–93	38.3	38.2–38.5

Values are presented as mean and range. CVPm: mean central venous pressure; $ETCO_2$: end-tidal carbon dioxide; HR: heart rate; b/min: beats per minute; SAPm: mean systemic arterial pressure; Tc: core temperature.

Table 3
Data of the blood gas analysis at different timepoints post-burn

Time (h)	aB-BE		$aB\text{-}pCO_2$		aB-pH		$aB\text{-}pO_2$		$aB\text{-}sHCO_3^-$	
	mmol/l	Range	kPa	Range	kPa	Range	kPa	Range	mmol/l	Range
2	2.1	−5.3 to 4.6	4.8	4.7–5.1	7.5	7.3–7.5	23.7	22.1–26.3	25.2	19.4–27.9
4	4.4	0.9–7.9	4.4	4.2–5.1	7.5	7.5–7.6	22.6	21.0–24.5	26.9	23.7–31.7
8	3.3	1.6–5.8	4.3	4.1–5.0	7.5	7.5–7.5	22.7	21.0–24.4	25.6	24.1–28.9
12	2.4	0.1–4.4	4.6	4.3–4.8	7.5	7.5–7.5	22.7	20.3–23.9	25.0	23.4–27.2
24	1.6	−0.1 to 2.6	4.3	4.3–4.6	7.5	7.5–7.5	21.1	18.5–23.6	24.2	22.9–25.2
36	1.7	−0.8 to 2.4	4.7	4.4–5.0	7.4	7.4–7.5	22.5	16.9–24.4	24.6	23.8–25.1
48	2.2	−0.1 to 3.7	5.0	4.4–5.7	7.5	7.4–7.5	16.4	10.1–22.8	25.0	24.1–27.1
72	3.1	2.3–4.5	4.8	4.5–5.4	7.5	7.5–7.5	14.4	11.3–16.0	25.6	25.0–27.8

Values are presented as mean and range. aB-BE: arterial base excess; aB-Hb: haemoglobin; $aB\text{-}pCO_2$: arterial carbon dioxide tension; aB-pH: arterial pH; $aB\text{-}pO_2$: arterial oxygen tension; $aB\text{-}sHCO_3^-$: arterial bicarbonate concentration.

progression of burn depth during the study (Fig. 3). The depth of the injury was more superficial in all burn sites at 2 h post-burn compared to any later time point ($P < 0.0005$), and also at 24 h compared to later time points ($P = 0.015$), but there was no difference between 48 and 72 h post-burn ($P = 0.397$). Accordingly, progression of burn depth was histologically evident until 48 h post-burn.

Histologically, at 24 h post-injury, the 1 s contacts lead to a superficial dermal injury (level 2), the 3 s burn to a partial thickness injury (levels 3–4) and the 9 s burn to a full thickness (level 5) injury. Accordingly, these contact times and the follow-up time of 24 h were chosen for study 2.

3.3.2. Study 2

There were significant differences in burn depths between different contact times at 24 h post-injury. The 3 s burn was significantly deeper than the 1 s burn ($P = 0.004$) and the 9 s burn was deeper than both the 1 s burn ($P = 0.006$) and the 3 s burn ($P = 0.005$). The mean burn depths of different contact times are presented in Fig. 4.

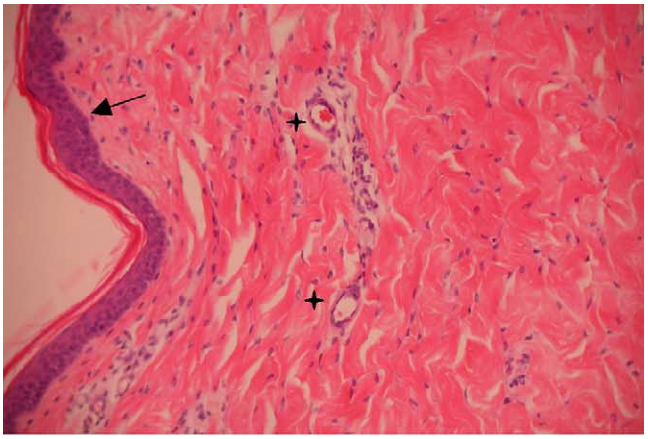
The kappa coefficient for intra-observer variation between two repeated analyses of 35 histological samples by the same pathologist in Study 1 was 0.83. The repeatability of burn depth creation between Studies 1 and 2 in the 1, 3 and 9 s burns yielded a kappa coefficient 0.92.

3.4. Skin thickness

The changes in absolute skin thickness in Study 1 are presented in Fig. 5. The skin thickness of the non-burned control site remained unchanged (1.6 mm, range 1.5–1.73 mm) throughout the study. The skin thickness in the 1 s burns was greater compared to both the control site ($P = 0.005$ at all time points) and to the 3, 6, 9 and 12 s burn sites ($P = 0.005$–0.012) throughout the study period. The skin thickness in the 3, 6, 9 and 12 s burns was greater than the skin in the control site at all times ($P = 0.005$-0.037) expect the 3 s burn at 2 ($P = 0.285$) and 72 h ($P = 0.92$) and the 12 s at 72 h ($P = 0.767$). There were no differences between the 3, 6, 9 and 12 s sites.

4. Discussion

Histological evaluation has been considered the golden standard in burn depth determination despite the fact that it has many problems [1]. Burn wound is a dynamic entity consisting of progressive time-related changes. Therefore, timing of the evaluation is troublesome. The very early evaluation of the depth of irreversible injury may be difficult due to the ensuing progressive tissue damage [10]. Histology is



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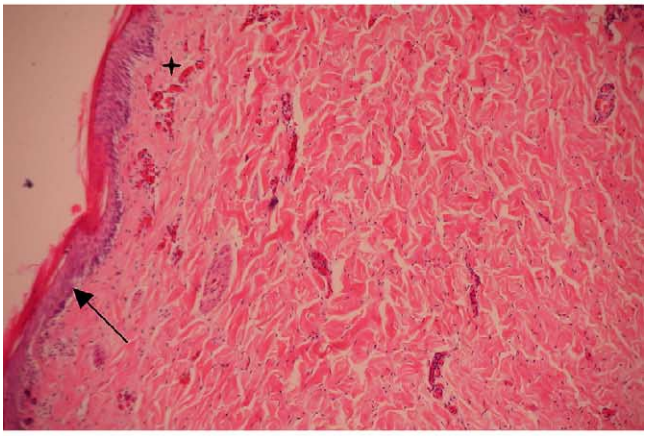

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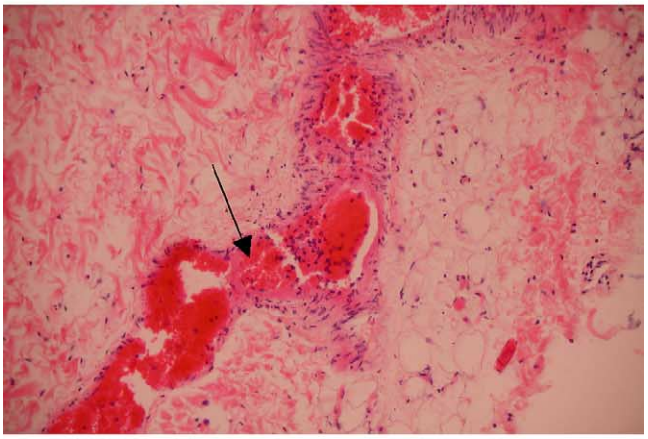

(c)

Fig. 2. (a) Normal pig skin from non-burned control area. Note the intact epidermal layer (arrow) and the patent vessels (asterix) (10×). (b) Subepidermal blistering (arrow) and hyperemia (asterix) in a 12 s burn 2 h post-burn (10×). (c) Thrombosis in the dermo-adipous junction. Note the mass (arrow) within the lumen of the vessel indicating old thrombosis. Also neutrophil infiltration present (20×).

also prone to errors due to the subjective nature of the assessment of different variables.

The main results in our study were that a reproducible model was obtained for creating experimental burns of different depths by using 1, 3 and 9 s contact times in pigs. It

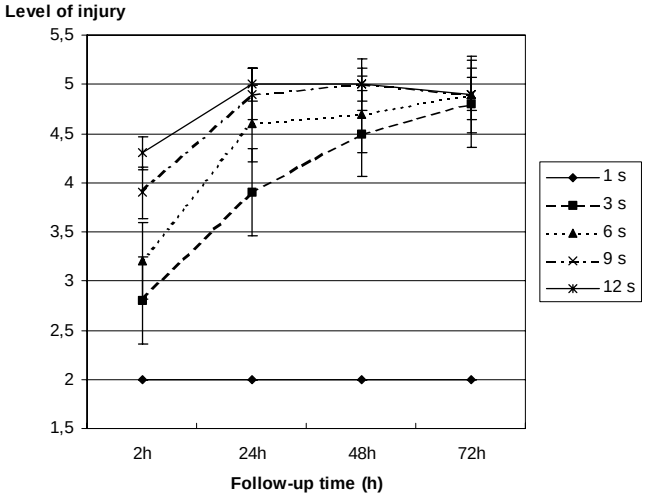

Fig. 3. Progression of histologic depth (±1 S.E.) of all burn sites during follow-up. Anatomic level of thermal injury is graded from 1 to 5.

was also found that there was no progressive tissue damage in the most superficial burns (1 s burns). In contrast, there was an increase in burn depth in the 9 and 12 s burns lasting up to 24 h post-burn and in 3 and 6 s burns lasting up to 48 h post-burn.

Several different animals have been used for burn research [5,6,10–17]. Pigs were used in the present study since pig skin has many similarities to human skin [5,7,17,18]. The absence of the intradermal muscular layer, panniculus carnosus, in pigs leads, as in humans, to closure of the wound by epithelial growth instead of contraction. Secondly, pig and human skin has the same relative thickness of epidermis and dermis, presence of epidermal ridges, distinct papillary dermis, similarities in both the vascularisation of the hair follicle and the structure of the collagenous tissue framework and a deep layer of subcutaneous fat [18]. In experimental burns, it is also important to choose an animal big enough to have flat surfaces to create uniform burns [17]. Previously, the thick dorsal paraspinal skin has been used [5,6]. We used the thinner ventral body skin as has been

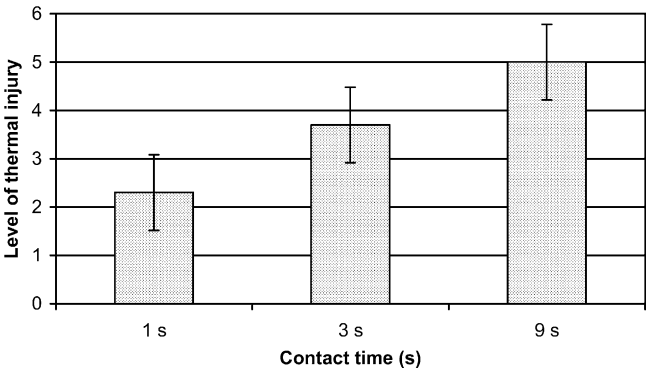

Fig. 4. Mean histological depth (±1 S.E.) of thermal injury in Study 2.

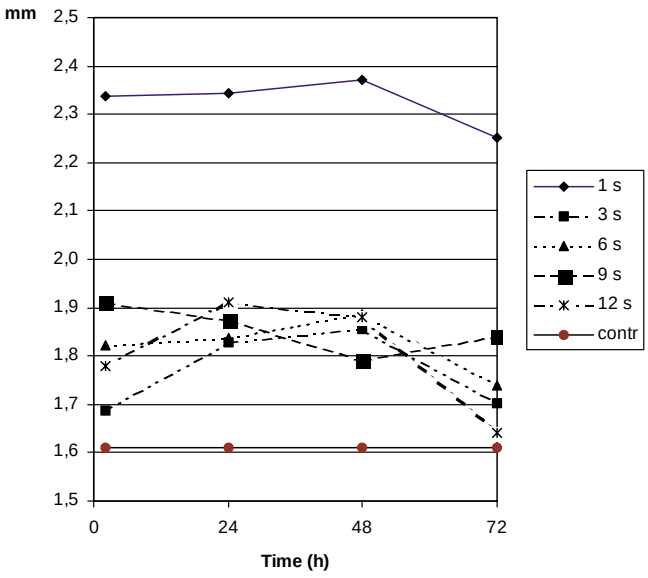

Fig. 5. Absolute skin thickness (mm) of different burn sites during follow-up.

used previously in small piglets [13]. The mean thickness of the ventral non-burned body skin was 1.6 mm, which is in accordance to previous studies, where skin thickness in 3–4-month-old pigs was 1.4 mm [19]. This is close to the thickness of human skin (1.44 ± 0.25 mm), measured from the thigh and forearm of 25–66-year-old people [20].

The mean surface area burned was 1.9% in Study 1 and 1.2% in Study 2. There was no burn-related need for fluid resuscitation and the animals were haemodynamically stable throughout the study (Table 2). Also, there were no changes in the thickness of the skin in the non-burned control sites indicating generalized oedema. Hence, it can be postulated that there were no significant systemic effects on the measured parameters caused by the thermal injury.

The importance of vascular patency in burn depth evaluation has been shown by Watts et al. [4], who found a good correlation between the histological evidence of vascular patency to clinical healing. Kahn et al. [21] suggested that it is important to evaluate blood vessels and their contents in determining the viability of the tissue. In Study 1, there were no thromboses in the 1 s burns and only in 15% of the samples in Study 2 within 24 h post-injury (10% in the superficial dermis and 5% in the middle dermis). When both vascular patency and other histological burn marks were evaluated, no progression of burn depth in these superficial burns was found. This is in accordance with the study of Watts et al. [4] where the depth of the superficial burns remained stable with time. However, in the 3–12 s burns we found a clear increase in depth with time, which has also been described before [4,12]. The final burn depth in the 9 and 12 s burns was obtained in 24 h post-injury when there was an increase in burn depth in the 3 and 6 s burns until 48 h. This might reflect the fact that in the 9 and 12 s burns the initial damage due to longer contact time already extends deep into tissue. In the 3 and 6 s burns, however, there must be a more clear ‘zone of stasis’ in the deeper part of dermis, where the ensuing vascular flow impairment leads to tissue ischemia, which might be devastating for already compromised cells.

The pattern of vascularisation of pig skin differs somewhat from human skin. It displays a lower, mid-dermal and subepidermal network, where the latter is less dense than in human [18]. Therefore, the direct interpretation of the results in human is limited. Due to the lack of pathologists experienced in evaluation thermal injuries histologically, an inter-observer reliability analysis could not be performed. However, the repeated analyses after 18 months by the same pathologist yielded an intra-observer correlation of 83%, which according to the definition of Landis and Koch [22] is considered almost perfect. Although all 1 s burns were created to the same animal and no comparisons could be made between different animals, the histological depth of all the 1 s burns were the same indicating a good repeatability of creating such burns. A good repeatability was also found in creating the 1, 3 and 9 s burns in Studies 1 and 2 yielding a kappa correlation of 92%, which is also considered almost perfect [22].

In conclusion, a reproducible model for creating superficial, partial thickness and full thickness thermal injuries in the skin of ventral body of a pig was obtained for further research purposes. This model is useful to examine more closely the local events and differences between burns of different depths in such an animal model where the skin closely resembles to human skin.

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