Original article

Human model of burn injury that quantifies the benefit of cooling as a first aid measure

E. H. Wright[1,2], M. Tyler[1], B. Vojnovic[4], J. Pleat[5], A. Harris[2] and D. Furniss[3]

[1]Department of Plastic Surgery, Stoke Mandeville Hospital, Aylesbury, [2]Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, [3]Nuffield Department of Orthopaedics, Rheumatology, and Musculoskeletal Science (NDORMS), Botnar Research Centre, and [4]Gray Institute for Radiation Oncology and Biology, University of Oxford, Oxford, and [5]Department of Plastic Surgery, Southmead Hospital, Westbury-on-Trym, UK
Correspondence to: Professor D. Furniss, Botnar Research Centre, Windmill Road, Headington, Oxford OX3 7HE, UK
(e-mail: dominic.furniss@ndorms.ox.ac.uk)

Background: **Burn injuries are a major cause of morbidity and mortality worldwide. Cooling is widely practised as a first aid measure, but the efficacy of cooling burns in human skin has not been demonstrated. A safe, consistent, ethically acceptable model of burning and cooling in live human skin *in vivo* was developed, and used to quantify the effects of cooling.**

Methods: **Novel apparatus was manufactured to create and cool burns in women who were anaesthetized for breast reconstruction surgery using a deep inferior epigastric artery perforator flap. Burns were excised between 1 and 3 h after creation, and analysed using histopathological assessment.**

Results: **All 25 women who were approached agreed to take part in the study. There were no adverse events. Increased duration of contact led to increased burn depth, with a contact time of 7·5 s at 70°C leading to a mid-dermal burn. Burn depth progressed over time following injury, but importantly this was modified by cooling the burn at 16°C for 20 min. On average, cooling salvaged 25·2 per cent of the dermal thickness.**

Conclusion: **This study demonstrated the favourable effects of cooling on human burns. Public heath messaging should emphasize cooling as first aid for burns. This model will allow analysis of the molecular effects of cooling burns, and provide a platform for testing novel therapies aimed at reducing the impact of burn injury.**

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Introduction

Worldwide, burn injuries are a major cause of morbidity and mortality, leading to an estimated 180 000 deaths annually. Non-fatal burns can have profound consequences, from functional debility to disfigurement, social isolation and psychological illness[1]. The prevention of scarring after burn injury remains a major unmet clinical need.

Cooling with cold water is the most widely employed first aid measure, and has been shown to reduce the severity of acute symptoms and the need for skin grafting[2]. There has been extensive study of the duration and temperature of cooling in porcine models[3–6]. In animal models, cooled burns heal with reduced scarring[7]. However, there are significant microanatomical and physiological differences between animal and human skin that mean the translation of mechanistic findings to humans is unreliable[8].

There has been no quantification of the effect of cooling in humans. Experimental human *in vivo* studies were last attempted in the 1960s, and were unable to demonstrate any therapeutic benefit, most likely because of the use of full-thickness burns as the basis for the model, and the clinically ineffective interventions of ice–water or topical antihistamines[9,10]. Therefore, the clinically important variables of temperature of water and duration of cooling required to achieve adequate first aid in humans remain unknown. Furthermore, establishing the molecular mechanism of the effects of cooling may reveal new therapeutic

pathways, allowing treatment in environments where cold water treatment is not readily available.

Ideally, the molecular investigation of burn injury and cooling would use a reproducible model in human skin that was acceptable to subjects, was associated with no morbidity, was easy to complete and produced a depth of injury that could be assessed with simple, objective laboratory techniques. The depth of injury should be to the mid-dermal level, as epidermal burns heal simply without intervention or scarring, and full-thickness necrosis cannot be modified by intervention. Furthermore, the model should have the ability to assess therapeutic interventions, such as cooling, using simultaneous within-subject controls[11]. Finally, there are ethical and logistical barriers to creating such stereotyped burn injuries in healthy human subjects. These issues have been addressed in this new model of human burn injury; the model will form the basis of future research efforts aimed at developing and testing interventions for burned patients.

Methods

Ethical approval for the study was obtained from the South Central Research Ethics Committee (13/SC/0518).

Patients undergoing deep inferior epigastric artery perforator (DIEP) free-flap breast reconstruction were recruited to the study. All subjects were women, aged under 65 years, without significant medical co-morbidities, and gave written informed consent. All patients received preoperative enoxaparin at a prophylactic dose the night before surgery, as dictated by clinical protocol.

Apparatus

Two pieces of apparatus were designed, one to create consistent burns and the second to deliver uniform consistent cooling. The device for creating a burn consisted of an integrally heated, thermostatically controlled, spring-mounted copper rod, housed within an insulated handle (*Fig. 1a,b*). The design allowed the application of a consistent temperature and constant pressure at the interface with skin. The area of the handpiece was 176 mm^2, and the force applied was 1·96 N, meaning that the pressure exerted at the skin during burning was 11 kPa, or 84 mmHg. A transparent silicone template (*Fig. 1b*) was manufactured with defined apertures for the creation of each burn, ensuring that the burn and control areas were created in a fixed spatial relationship to one another, and preventing accidental damage to the surrounding skin.

The cooling device consisted of a Peltier thermoelectric effect, thermostatically controlled metal block (*Fig. 1c,d*). This was placed over the burns selected for cooling, under its own mass of 1345 g. Owing to the natural curvature of the abdomen, the cooling device was applied at approximately 45° to the skin. This means that the pressure exerted on the skin during cooling was 932 Pa, or 7 mmHg.

Burn injury

The design of a DIEP flap is shown in *Fig. 1e,f*. The central part of the abdominal ellipse is used to reconstruct the breast, whereas the lateral parts are discarded during surgery to achieve an aesthetic abdominal closure. These lateral zones of skin are symmetrical, consistent and predictable. These areas were selected for the creation of consistent burns after the induction of general anaesthesia in the anaesthetic room before commencement of surgery. The transparent silicone template with holes was placed over the lateral abdominal triangle of surplus skin, allowing burns and control areas to be created in a consistent and symmetrical pattern (*Fig. 1g*). There was a minimum of 10 mm between the burn and the control area. Burns were created using the template and the heated handpiece at 70°C, with consistent pressure and contact times of between 5 and 60 s. The unburned control skin had pressure only for the same contact time from the unheated probe. The burns and controls from each side of the body were then either cooled or not cooled depending on the experiment (*Fig. 1h*). Cooling was administered at 16°C for 20 min, commencing 2 min after burn creation, as this has been shown to be the most effective temperature in an animal model[12]. Finally, each control and burned area was harvested at time points from 1 to 3 h after burn creation using a 12-mm biopsy punch.

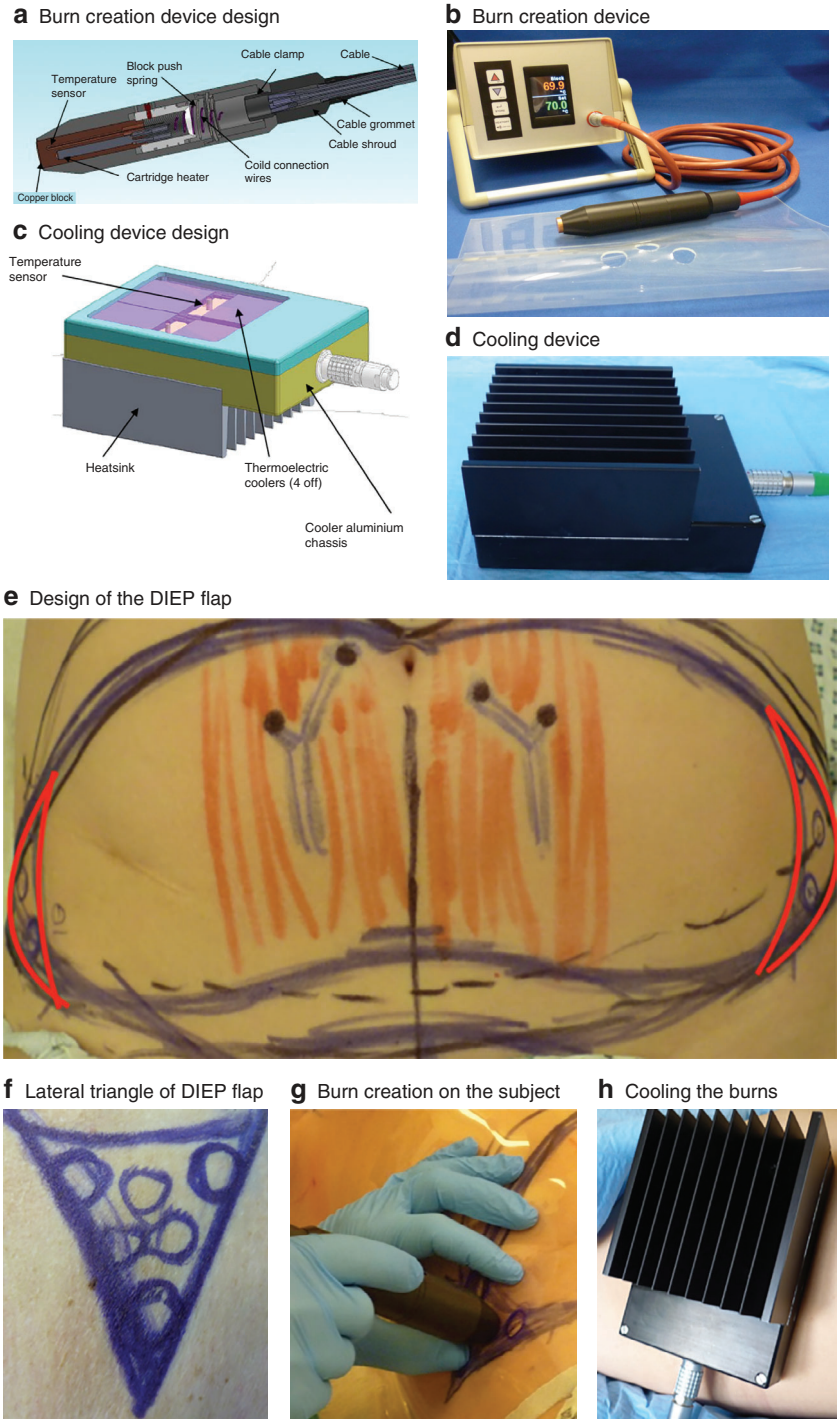
Tissue sectioning, staining and analysis

The samples were formalin-fixed and processed in an automated tissue processor. The fixed skin sections were paraffin-embedded and cut in transverse sections at 4 µm thickness, with the aid of CellSoft™ (Cellpath Newtown, UK) tissue softening solution. Sections were dried on to charged slides and stained with Masson's trichrome stain (Sigma Aldrich Gillingham, UK) as described previously[13]. The standard burn depth, that is the distance to the deepest occluded dermal blood vessel as a proportion of the dermal depth, was calculated as described previously[14].

Statistical analysis

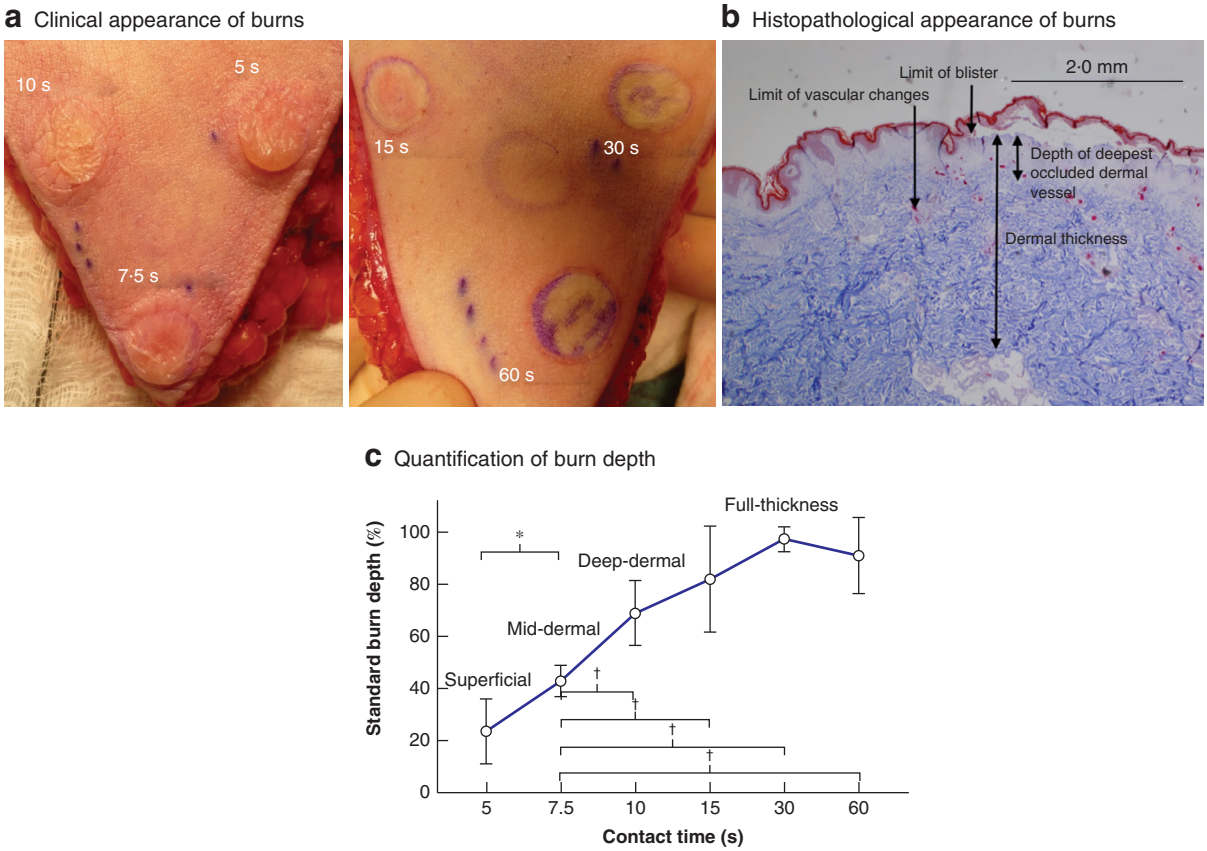
Comparisons were made between the standard burn depth created with differing contact times using one-way ANOVA. Paired comparisons between cooled and uncooled burns from the same individuals were made

Fig. 1 Experimental system



a Diagram of burn creation device. **b** Burn creation device with silicone template used to create stereotyped burns while protecting other skin. **c** Diagram of cooling device. **d** Cooling device. **e** Preoperative markings of a deep inferior epigastric artery perforator (DIEP) flap on the lower abdomen. Areas outlined in red are lateral triangles of the flap that are usually discarded, and were used for burn creation in this study. **f** Close up of one lateral triangle of the DIEP flap demonstrating the site of planned burns and control areas (circles). **g** Burn creation through a hole in the protective silicone sheet allowing protection of adjacent skin. **h** Cooling device in place over the burned lateral triangle of a DIEP flap.

Fig. 2 Clinical and histopathological appearance of standard human burns



a Clinical appearance of burns on the lateral triangle of a deep inferior epigastric artery perforator (DIEP) flap after contact for between 5 and 60 s at 70°C. **b** Histopathological appearance of harvested skin showing a typical mid-dermal burn (Masson's trichrome stain). The depth of the deepest occluded dermal vessel, skin thickness and limit of the vascular changes induced by burning are demonstrated. **c** Quantification of depth of burn injury by contact time ($n = 8$ for 5, 10 and 15 s; $n = 6$ for 7·5, 30 and 60 s). *$P < 0{\cdot}050$, †$P < 0{\cdot}001$ (1-way ANOVA).

using the paired *t* test. All analyses were performed using GraphPad Prism® 8 (GraphPad Software, San Diego, California, USA).

Results

All 25 women agreed to participate and were recruited. There were no adverse events or complications related to participation in the study.

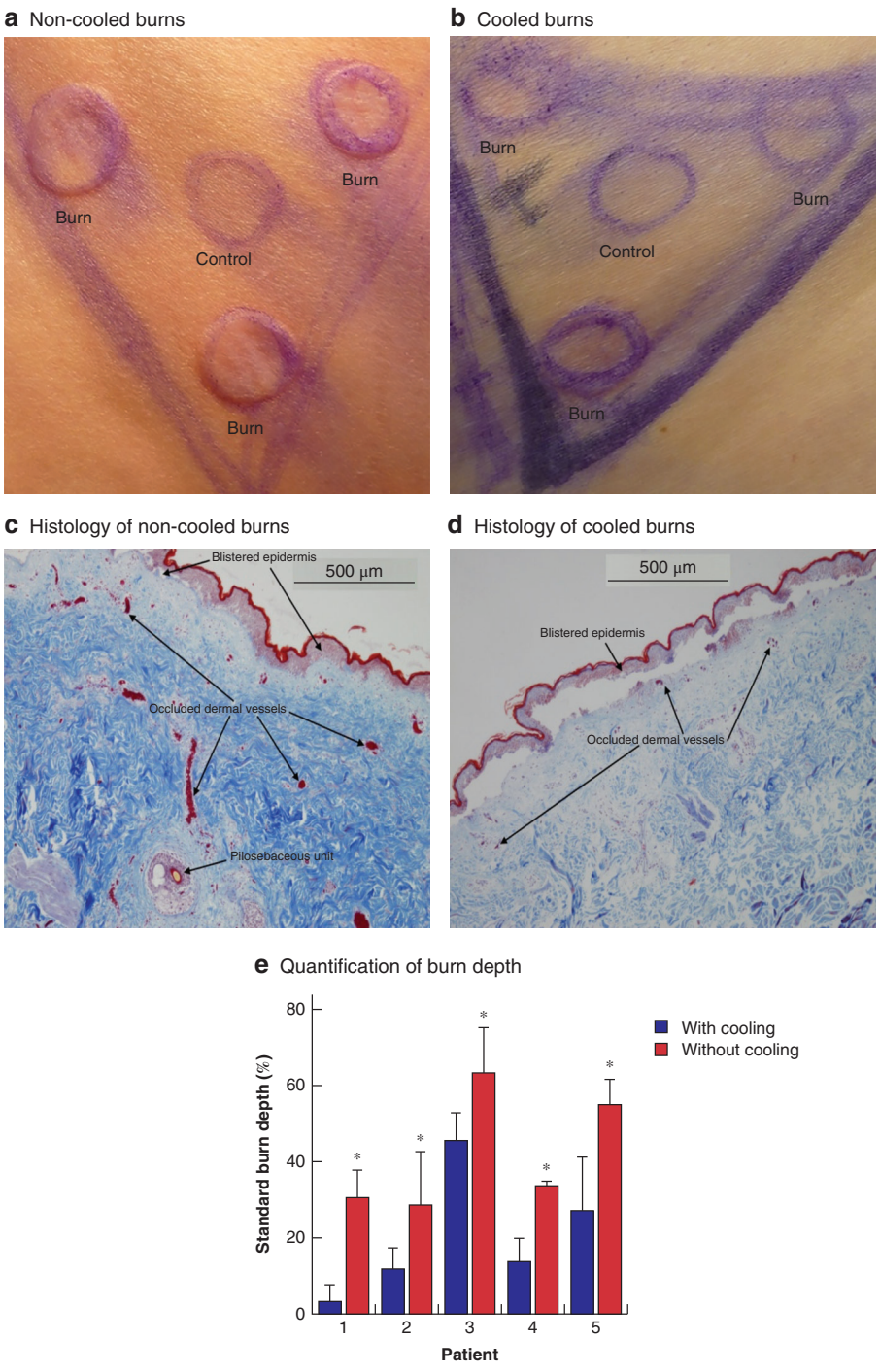
Increasing duration of burn contact increased burn depth

Representative clinical appearances of burns created with different contact times are shown in *Fig. 2a*. Contact durations of 5, 7·5 and 10 s produced clinically superficial partial-thickness burns, with blister formation and a vascularized base after blister removal. Central fixed staining clinically consistent with a deep dermal burn was visible after contact for 15 s, whereas white, leathery, clinically full-thickness burns were produced after 30 and 60 s.

Histological assessment of the burn depth was consistent with these clinical observations (*Fig. 2b,c*). There was a linear increase in the standard burn depth with contact for up to 10 s, after which the burn caused a full-thickness injury. Each increase in the duration of contact produced a statistically significant increase in the standard burn depth up to 15 s.

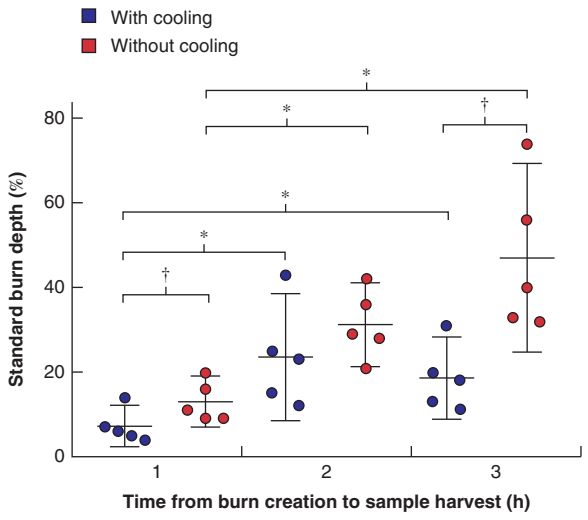
Next, the data were combined from all uncooled burns created with contact for 7·5 s and harvested at 3 h after creation of the burn. There were a total of 26 burns from 16 women. The mean standard depth of these burns was

Fig. 3 Effects of cooling on human burns



Clinical appearance of **a** non-cooled and **b** cooled burns and control (non-burned areas) 3 h after injury with a contact time of 7·5 s at 70°C, and cooling at 16°C for 20 min. Histological appearance of **c** non-cooled and **d** cooled burns shown in **a** and **b** respectively (Masson's trichrome stain). The non-cooled burns demonstrate a greater depth of microvascular occlusion than the cooled burns. **e** Quantification of burn depth with and without cooling in five independent patients 3 h after injury with a contact time of 7·5 s at 70°C. Values are mean(s.d.) of three independent burns in each group. *$P < 0{\cdot}001$ *versus* with cooling (paired *t* test).

Fig. 4 Cooling attenuated progression of burn depth over time



Five patients were burned in triplicate bilaterally with a contact time of 7·5 s at 70°C, with one side subjected to cooling at 16°C for 20 min. Burns were then harvested at 1, 2 and 3 h after injury, and burn depth assessed histologically. Note the progression in burn depth over time in both cooled and non-cooled burns, but with a significant reduction in cooled compared with non-cooled at 3 h. Each circle represents an individual burn from an individual patient. Mean(s.d.) values are also shown. *$P < 0{\cdot}050$, †$P < 0{\cdot}010$ (paired t test).

43 (95 per cent c.i. 37 to 49) per cent, validating that this methodology is able to produce a consistent, reproducible, mid-dermal burn in human abdominal skin.

Cooling reduced the clinical and histological depth of burn injury

The clinical and histological appearances of paired uncooled and cooled burns in five independent women, created at 70°C with a contact duration of 7·5 s, were compared at 3 h after injury. Cooling occurred 2 min after burn creation for 20 min at 16°C on one side of the patient. This cooling temperature and duration consistently reduced blistering, erythema and oedema of the burned skin (*Fig. 3a*,*b*).

The standard burn depth in the same cooled and uncooled burn samples was analysed. Within each individual, the mean standard burn depth was less in the cooled burns than in the non-cooled burns (*Fig. 3c*). When considered as a group, the mean standard burn depth of all non-cooled burns was 44·9 per cent, whereas that of cooled burns was 19·7 per cent ($P < 0{\cdot}001$), equating to a mean reduction of burn depth of 25·2 per cent of the thickness of the dermis.

Histological depth of burn increased after burning but its rate was slowed by cooling

Paired cooled and uncooled burn and control specimens from five women were harvested at 1, 2 and 3 h after burning. There was a significant increase in burn depth between 1, 2 and 3 h after burn injury, but this response was attenuated by cooling (*Fig. 4*).

Discussion

A novel *in vivo* human model of burn injury is described in this study. The model provides a way of creating a burn on normally perfused human skin. It makes use of skin normally discarded during surgery, but remains perfused *in vivo* for up to 3 h after the induction of anaesthesia. This approach does not interfere with the nature or timing of the breast reconstruction operation, and was acceptable to the patients with 100 per cent recruitment amongst those eligible. There was no morbidity from the procedure. Importantly, the depth of first microvascular patency proved to be a reliable marker for the depth of injury, and demonstrated progression over 3 h following injury. This correlates with clinical observations by Jackson[15], that burns progress in the period after burning, and observational clinical and histological studies[16], and represents an important therapeutic opportunity. This also explains why delayed first aid is less effective than immediate intervention.

Furthermore, it was demonstrated that the depth of burned skin in the model responds to standard cooling in a predictable manner. Previous studies in human burns used a variety of outcomes that would not be applicable for the 3-h time frame within which the burned tissue is left *in vivo*, for example healing time[17] or assessment of late granulation tissue formation[17]. The choice of deepest occluded microvascular vessel to judge burn depth and the response to treatment reflected the original observations of Jackson's classical three-zone model of a burn with stasis of microvascular flow[15]. Furthermore, vascular patency measurements were quantifiable. All patients had received preoperative enoxaparin according to the clinical venous thromboembolic prevention protocol. There is a theoretical possibility that this might have reduced the depth of the deepest microvascular occluded vessel, but previous animal work suggested that heparin does not have an effect on dermal perfusion after burning.

An alternative approach is to study burned patients admitted to hospital[14,16]. This has disadvantages compared with the approach described here; in particular, there are a range of variables that are difficult to normalize. For example, there are variations in the source and type of energy imparted, the depth of skin burned, anatomical

location, initial treatment and time to presentation at hospital.

Animal models have also been used extensively; however, findings are inconsistent between species, and often do not translate to human subjects[8]. Human skin is unique with respect to the relative lack of hair, the presence of sweat glands, the absence of a panniculus carnosus layer, the presence of a well developed superficial dermal vascular plexus, and the potential to form hypertrophic and keloid scars. It provides the most direct setting for the investigation of new therapeutics in burn injury, and the subsequent translation to clinical practice.

The burn creation apparatus and the mode of application worked reliably: it was established that 7·5 s of contact produced a stereotypical mid-dermal injury. Previous work[18] has indicated that 7·5 s of contact at 70°C would produce a partial-thickness burn. Clinically, most superficial injuries to the epidermis and upper dermis heal rapidly with minimal need for intervention, whereas full-thickness injuries are not amenable to intervention owing to cellular necrosis and protein coagulation. For this reason, studies using skin samples from excised burns are limited in the insights that they can provide[19,20]. It is the mid-dermal, partial-thickness depth of injury that has the greatest potential for therapeutic modulation. Clinically and histologically the burns with a contact duration of 7·5 s bordered the level between those that might require a skin graft and those that would heal without surgery. At this key depth, good first aid can make the difference between surgery and no surgery, and thus healing with or without a lifelong scar[2].

The cooling apparatus also worked reliably. It was possible to apply a temperature of 16°C for 20 min using a compact unit that did not interfere with surgery. The effect of cooling was apparent within the first hour after injury, with the difference between a cooled and uncooled burn becoming greatest at 3 h as the depths of the cooled and uncooled burns diverged. This phenomenon suggests that the model is dynamic, and that the microvasculature initially remains patent and perfused. It is possible that cooling helps to maintain this perfusion, allowing the survival of more dermis.

The main limitation of the model is that it can only be used to observe changes in the skin until 3 h after burn injury, as after this time the skin is excised. It is therefore not possible to comment on the effect of interventions on late outcomes. The burn is sustained while the subject is under general anaesthesia, which may affect the response to burn injury, although the effects of general anaesthesia are a common, constant factor throughout each sample, and between each individual. In addition, because of the nature of breast reconstruction, the experiment can be conducted only on adult women. There is evidence that male skin is thicker than female skin, and that skin thickness decreases with age[21]. This suggests that a given thermal injury may produce a more superficial burn in men, and a deeper burn in the elderly. This could affect the generalizability of the model, but is unlikely to affect the conclusions of the study. The reproducible depth of injury and the clear effect of cooling in an *in vivo* human setting are arguments in favour of its use for elaborating the pathogenesis of burn progression and response to treatment. The cellular and molecular response to cooling in this model is the subject of ongoing studies.

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The authors confirm that they are willing to make their data, analytical methods and study materials available to other researchers in order to reproduce the results reported in this manuscript. Some clinical samples may not be available, and release would be dependent on suitable material transfer agreements between institutions, and Human Tissue Authority governance arrangements being in place at the receiving institution. This work was not preregistered in an independent institutional registry. The data reported in this paper are available from the corresponding author on request.

Disclosure: The authors declare no conflict of interest.

References

1 Mock C, Peck M, Peden M, Krug E, Ahuja R, Albertyn H *et al. A WHO Plan for Burn Prevention and Care*. WHO: Geneva, 2008.

2 Nguyen NL, Gun RT, Sparnon AL, Ryan P. The importance of immediate cooling – a case series of childhood burns in Vietnam. *Burns* 2002; **28**: 173–176.

3 Jandera V, Hudson DA, de Wet PM, Innes PM, Rode H. Cooling the burn wound: evaluation of different modalites. *Burns* 2000; **26**: 265–270.

4 Cuttle L, Kempf M, Kravchuk O, Phillips GE, Mill J, Wang XQ *et al.* The optimal temperature of first aid treatment for

partial thickness burn injuries. *Wound Repair Regen* 2008; **16**: 626–634.
5 Rajan V, Bartlett N, Harvey JG, Martin HC, La Hei ER, Arbuckle S *et al.* Delayed cooling of an acute scald contact burn injury in a porcine model: is it worthwhile? *J Burn Care Res* 2009; **30**: 729–734.
6 Bartlett N, Yuan J, Holland AJ, Harvey JG, Martin HC, La Hei ER *et al.* Optimal duration of cooling for an acute scald contact burn injury in a porcine model. *J Burn Care Res* 2008; **29**: 828–834.
7 Boykin JV Jr, Eriksson E, Sholley MM, Pittman RN. Cold-water treatment of scald injury and inhibition of histamine-mediated burn edema. *J Surg Res* 1981; **31**: 111–123.
8 Domergue S, Jorgensen C, Noël D. Advances in research in animal models of burn-related hypertrophic scarring. *J Burn Care Res* 2015; **36**: e259–e266.
9 Sevitt S, Bull JP, Cruickshank CN, Jackson DM, Lowbury EJ. Failure of an antihistamine drug to influence the course of experimental human burns. *Br Med J* 1952; **12**: 57–62.
10 Raghupati N. First-aid treatment of burns: efficacy of water cooling. *Br J Plast Surg* 1968; **21**: 68–72.
11 Finnerty CC, Jeschke MG, Branski LK, Barret JP, Dziewulski P, Herndon DN. Hypertrophic scarring: the greatest unmet challenge after burn injury. *Lancet* 2016; **388**: 1427–1436.
12 Venter TH, Karpelowsky JS, Rode H. Cooling of the burn wound: the ideal temperature of the coolant. *Burns* 2007; **33**: 917–922.
13 Chvapil M, Speer DP, Owen JA, Chvapil TA. Identification of the depth of burn injury by collagen stainability. *Plast Reconstr Surg* 1984; **73**: 438–441.
14 Watts AM, Tyler MP, Perry ME, Roberts AH, McGrouther DA. Burn depth and its histological measurement. *Burns* 2001; **27**: 154–160.
15 Jackson DM. The diagnosis of the depth of burning. *Br J Surg* 1953; **40**: 588–596.
16 Tyler MP, Watts AM, Perry ME, Roberts AH, McGrouther DA. Dermal cellular inflammation in burns. An insight into the function of dermal microvascular anatomy. *Burns* 2001; **27**: 433–438.
17 Cuttle L, Kempf M, Liu PY, Kravchuk O, Kimble RM. The optimal duration and delay of first aid treatment for deep partial thickness burn injuries. *Burns* 2010; **36**: 673–679.
18 Bull JP. Burns. *Postgrad Med J* 1963; **39**: 717–723.
19 Greco JA III, Pollins AC, Boone BE, Levy SE, Nanney LB. A microarray analysis of temporal gene expression profiles in thermally injured human skin. *Burns* 2010; **36**: 192–204.
20 Pollins AC, Friedman DB, Nanney LB. Proteomic investigation of human burn wounds by 2D-difference gel electrophoresis and mass spectrometry. *J Surg Res* 2007; **142**: 143–152.
21 Seidenari S, Pagnoni A, Di Nardo A, Giannetti A. Echographic evaluation with image analysis of normal skin: variations according to age and sex. *Skin Pharmacol* 1994; **7**: 201–209.