

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

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Summary

What is already known

Burn injuries are very common worldwide. First aid treatment consists of cooling with water, but little is known about the effects of cooling burns in humans, and there is a need for controlled clinical and mechanistic studies.

What is new

An accurate, reproducible, ethically acceptable model of burn injury in humans was developed. As proof of principle, it was shown for the first time that cooling has a measurable effect on depth and progression of burn injury, salvaging 25% of dermal thickness.

Potential impact on future practice

This work confirms and quantifies the importance of cooling as a first aid measure, and should be used to reinforce public health messaging.

Furthermore, the model provides a platform for future research into new agents aimed at reducing the impact of burn injury.

Abstract

Background

Burn injuries are a major cause of morbidity and mortality worldwide. Cooling of burns is widely practiced 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 female patients who were anaesthetised for breast reconstruction surgery using a deep inferior epigastric artery perforator flap. Burns were excised between one and three hours after creation and analysed using histopathological assessment. Differences between groups were tested for statistical significance using ANOVA or paired t-tests as appropriate.

Results

All 25 patients approached agreed to take part in the study. There were no adverse events. Increased contact duration lead to increased burn depth, with a contact time of 7.5 seconds 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 minutes. On average, cooling salvaged 25% of dermal thickness.

Conclusions

This is the first study to demonstrate favourable effects of cooling on human burns. Public health messaging should further emphasise cooling in the first

aid management of burns. This model system will allow dissection of the molecular effects of cooling burns, and provide a platform for testing novel therapies aimed at reducing the impact of burn injury.

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 illness¹. 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-grafting². 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 scarring⁷. However, there are significant micro-anatomical and physiological differences between animal and human skin that mean the translation of mechanistic findings to humans is unreliable⁸.

There has been no quantification of the effect of cooling in humans. Experimental human *in vivo* studies were last attempted in the 1960's, 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 for 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 utilise a reproducible model in human skin that was acceptable to subjects, associated with no morbidity, 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. Also, the model should have the ability to assess therapeutic interventions, such as cooling, using simultaneous within subject controls¹¹. 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, and this model will form the basis of future research efforts aimed at developing and testing novel interventions for burn injured patients.

Patients and methods

Ethical approval

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

Patients

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

Apparatus

Two pieces of apparatus were designed, one to create consistent burns, 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 176mm², and the force applied was 1.96N, meaning that the pressure exerted at the skin during burning was 11kPa, or 84mmHg. 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 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 1,345g. Due to the natural curvature of the abdomen, the cooling device was applied at $\sim 45^\circ$ to the skin. This means that the pressure exerted on the skin during cooling was 932 Pa, or 7mmHg.

Burn injury

The design of a DIEP flap is shown in Fig 1e and f. The central part of the abdominal ellipse is used to reconstruct the breast, whilst the lateral parts are discarded during surgery to achieve an aesthetic abdominal closure. These lateral zones of skin are symmetrical, consistent, and predictable. We selected these areas for 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 10mm between a burn and a control area. Burns were created using the template and the heated hand-piece at 70°C with consistent pressure and contact times of between 5 and 60 seconds. 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 minutes, commencing two minutes after burn creation, as this has been shown to be the most effective temperature in an animal model¹². Finally, each control and burned area was harvested at time-points from one to three hours after burn creation using a 12mm biopsy punch.

Tissue sectioning, staining, and analysis

Samples were formalin fixed and processed in an automated tissue-processor. Fixed skin sections were paraffin-embedded and cut in transverse sections at 4µm thickness, with the aid of CellSoft™ (Cellpath) tissue softening solution. Sections were dried onto charged slides and stained with Masson's trichrome stain (Sigma Aldrich) as previously described¹³. The standardised burn depth, that is the distance to the deepest occluded dermal blood vessel as a proportion of the dermal depth, was calculated as described previously¹⁴.

Statistical analysis

Comparisons were made between the standardised burn depth created with differing contact times using the one-way ANOVA test. Paired comparisons between cooled and uncooled burns from the same individuals were made using the paired t-test. All analyses were performed using GraphPad Prism 8 (GraphPad Software Inc, California, USA).

Results

Participant Recruitment

Twenty-five of 25 subjects identified agreed to participate and were successfully recruited to the study. There were no adverse events or complications related to participation in the study.

Increasing duration of burn contact increases burn depth

Representative clinical appearances of burns created with different contact-times are shown in Figure 2a. Contact durations of 5, 7.5, and 10 seconds produced clinically superficial partial-thickness burns, with blister formation, and a vascularised base after blister removal. Central fixed staining clinically consistent with a deep-dermal burn was visible after 15 seconds' contact duration, whilst white, leathery, clinically full thickness burns were produced after 30 and 60 seconds' contact durations.

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

Next, data was combined from all uncooled burns created with 7.5 seconds' contact duration harvested at three hours after creation of the burn. There were a total of 26 burns from 16 individuals. The mean standardised depth of these burns was 43% (95% confidence interval 37-49%) validating that this methodology is able to produce a highly consistent, reproducible, mid-dermal burn depth in human abdominal skin.

Cooling reduces the clinical and histological depth of burn injury

The clinical and histological appearance of paired uncooled and cooled burns in five independent patients, created at 70°C with a contact time of 7.5 seconds, were compared at three hours after injury. Cooling occurred two minutes after the burn creation for 20 minutes at 16°C on one side of the patient. We noted that this cooling temperature and duration consistently reduced blistering, erythema, and oedema of the burned skin (Fig 3a,b).

The standardised burn depth in the same cooled and uncooled burn samples was analysed. Within each individual, the mean standardised burn depth was less in cooled burns than non-cooled burns (Fig 3c). When considered as a group, the mean standardised burn depth of all non-cooled burns was 44.9%, while that of cooled burns was 19.7% ($P < 0.0001$); equating to a mean reduction of burn depth of 25.2% of the thickness of the dermis.

Histological depth of burn increases after burning but its rate is slowed by cooling

Paired cooled and uncooled burn and control specimens from five patients were harvested at 1, 2, and 3 hours after burning. There was a significant increase in burn depth between one, two and three hours 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 human skin in a normally perfused environment. It makes use of skin normally discarded during surgery, but that remains perfused *in vivo* for up to three hours after induction of anaesthesia. This approach does not interfere with the nature or timing of the breast reconstruction operation, and was highly acceptable to the patients with 100% recruitment amongst those eligible. There was no morbidity from the procedure across the 25 subjects recruited. Importantly, the depth of first microvascular patency proved to be a reliable marker for depth of injury, and demonstrated progression over three hours following injury. This correlates with clinical observations by Jackson that burns progress in the period after burning¹⁵ and observational clinical and histological studies¹⁶, 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 burnt skin in the model responds to standardised cooling in a predictable manner. Previous studies in human burns have used a variety of outcomes that would not be applicable for the three hour time frame within which the burnt tissue is left *in vivo*, for example healing time¹⁷ or assessment of late granulation tissue formation¹⁷. Our choice of deepest occluded microvascular vessel to judge burn depth and response to treatment reflect the original observations of Jackson's classical three zone model of a burn with stasis of microvascular flow¹⁵. Furthermore, vascular patency measurements were quantifiable. All patients had received pre-operative enoxaparin according to the clinical venous thromboembolic

prevention protocol. There is a theoretical possibility that this might have reduced the depth of deepest microvascular occluded vessel, but previous animal work has shown Heparin does not have an effect on dermal perfusion after burning.

An alternative approach is to study human burn patients admitted to hospital^{14,16}. This has notable disadvantages compared to 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, depth of skin burnt, 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 subjects⁸. 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 subsequent translation to clinical practice.

The burn creation apparatus and mode of application worked reliably: it was established that 7.5 seconds of contact produced a stereotypical mid-dermal injury. Previous work has indicated that 7.5 seconds of contact at 70°C would produce a partial-thickness burn¹⁸. 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 due to cellular necrosis and protein coagulation. Studies using skin samples from excised

burns are limited in the insights that they can provide for this reason^{19,20}. It is the mid-dermal, partial thickness depth of injury that has greatest potential for therapeutic modulation. Clinically and histologically the burns with a contact duration of 7.5 seconds bordered that boundary between those burns which 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 life-long scar².

The cooling apparatus also worked reliably. It was possible to apply a temperature of 16°C for 20 minutes 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 three hours 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 three hours after burn injury, as after this time, the skin is excised. It is therefore not possible to firmly comment on the effect of interventions on late outcomes, such as healing time and scarring. 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. Also, due to the nature of breast reconstruction, the experiment can only be conducted on adult females. There is evidence that male skin is thicker than female skin, and that skin thickness decreases with age²¹. This suggests that a

given thermal injury may produce a more superficial burn in males, and a deeper burn in the elderly. This could affect the generalisability of the model, but is unlikely to affect the conclusions of the study. The reproducible depth of injury and clear effect of cooling in an *in vivo* human setting are powerful arguments in favour of its utilisation for elaborating pathogenesis of burn progression and response to treatment. The cellular and molecular response to cooling in this model is the subject of on-going studies.

In conclusion, the safety and feasibility of utilising redundant DIEP free flap abdominal skin as the basis for research into the effects of burning and cooling in human subjects have been demonstrated. The consistent, symmetrical areas of redundant skin available, combined with the purpose-built apparatus, have allowed a high level of standardisation and safety. As a marker of depth of injury, microvascular occlusion has a dose-response relationship to contact time with the burning device, and correlates to the clinical appearance of the burns. The depth of microvascular injury was consistent within and between individuals. The model is biologically active, as evidenced by the progression of the histological features between one and three hours after injury. Cooling protects the microvasculature and reduces the depth and progression of microvascular occlusion.

In human subjects, cooling a mid-dermal burn to 16°C for 20 minutes – current evidence-based first aid guidance- reduces the depth of microvascular occlusion in the dermis and attenuates subsequent progression, saving 25% of the thickness of the dermis when measured at 3 hours. This is the first time that objective histological evidence for the beneficial effects of this form of first aid has been shown in human skin.

Declarations of interests

The authors declare no conflicts of interest regarding this study.

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Figure Legends

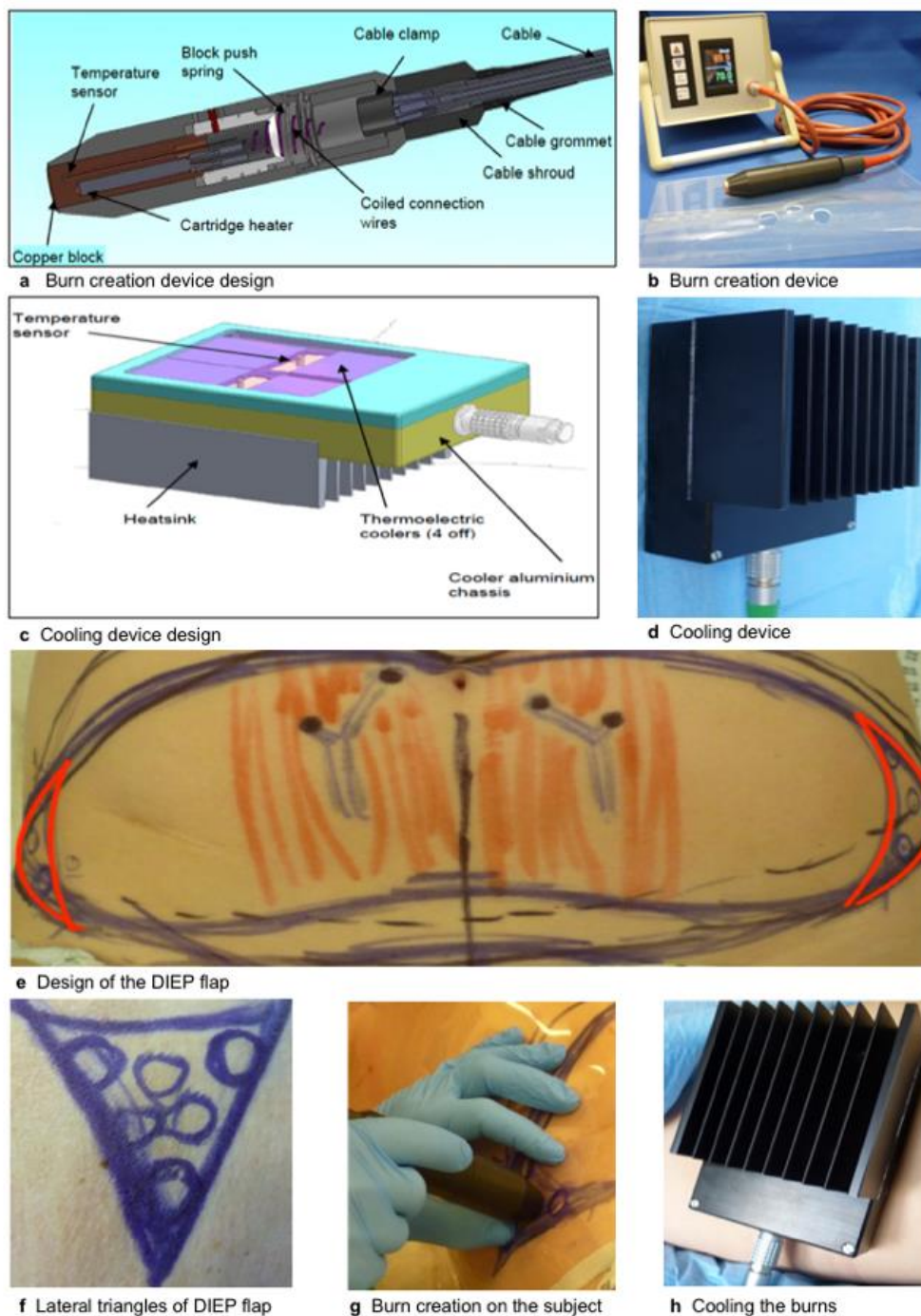
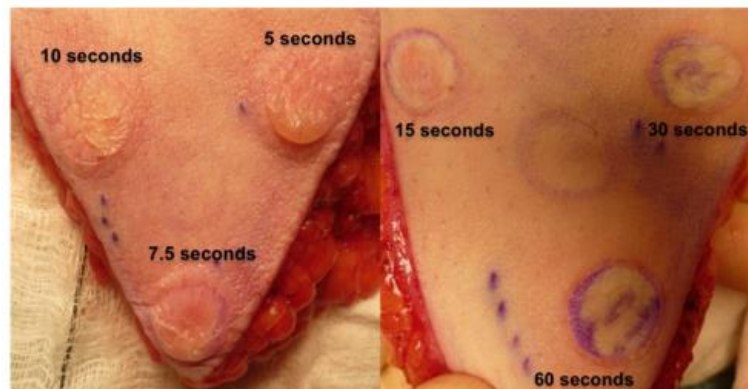
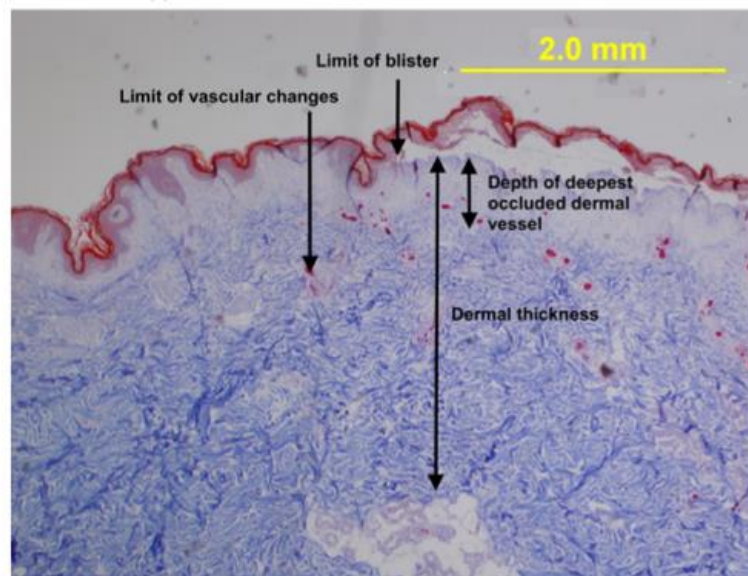


Figure 1. Experimental system. **a** Diagram of the burn creation device. **b** The burn creation device with silicone template used to create stereotyped burns whilst protecting the other skin. **c** Diagram of the cooling device. **d** The cooling device. **e** Pre-operative markings of the DIEP flap on the lower abdomen. The

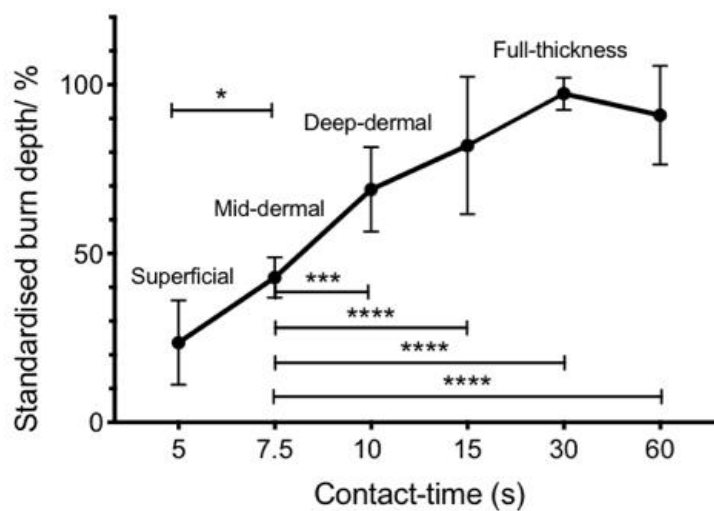
areas outlined in red are the lateral triangles of the flap that are usually discarded, and were used for burn creation in this study. **f** Close up of one lateral triangle of the DIEP flap demonstrating the site of planned burns and control areas (circles). **g** Burn creation through a hole in the protective silicone sheet allowing protection of adjacent skin. **h** The cooling device in place over the burned lateral triangle of a DIEP flap.



a Clinical appearance of burns



b Histopathological appearance of burns



c Quantification of burn depth

Figure 2. Clinical and histopathological appearance of standardised human burns. **a** Clinical appearance of burns on the lateral triangle of a DIEP flap after contact durations between five and 60 seconds at 70°C. **b** Histopathological appearance of harvested skin showing a typical mid-dermal burn. 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 seconds; n=6 for 7.5, 30 and 60 seconds). Differences between groups were compared with a one-way ANOVA test; *p<0.05, **p<0.01, ***p<0.001, ****P<0.0001.

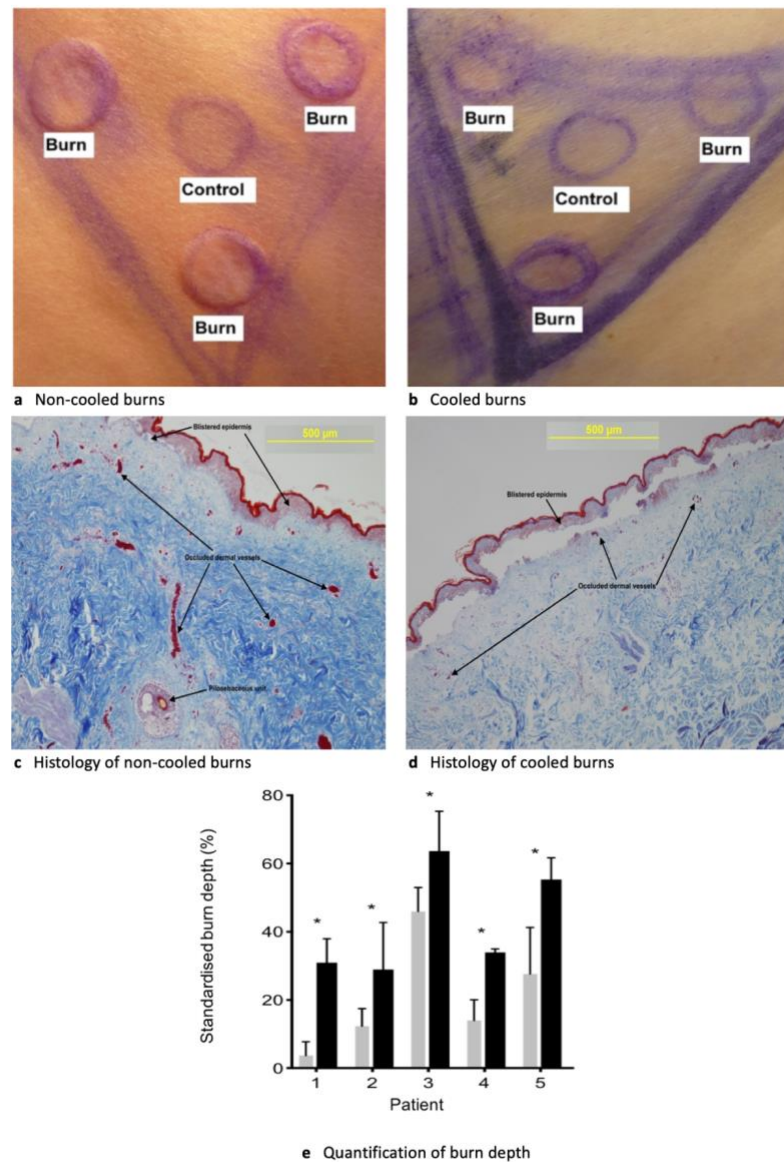


Figure 3. The effects of cooling on human burns. Clinical appearance of **a** non-cooled, and **b** cooled burns and control (non-burnt areas) three hours after injury with a contact time of 7.5 seconds at 70°C; cooling at 16°C for 20 minutes. Histological appearance of the **c** non-cooled and **d** cooled burns shown in a and b. The non-cooled burns demonstrate greater depth of microvascular occlusion than the cooled burns. **e** Quantification of burn depth with (grey) and without (black) cooling in five independent patients three hours after injury with

a contact time of 7.5 seconds at 70°C. Bars represent mean depth +/- SD of three independent burns in each group. * $p < 0.0001$ (paired t-test).

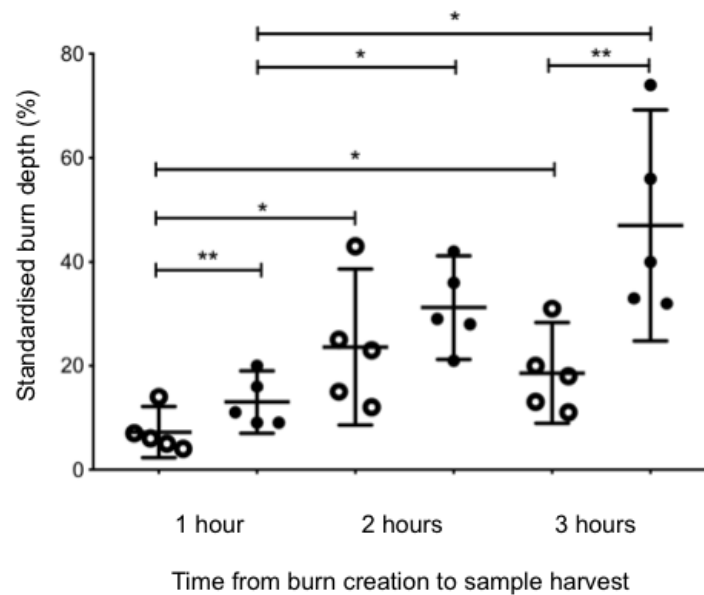


Figure 4. Cooling attenuates the progression of burn depth over time. Five patients were burned in triplicate bilaterally with a contact time of 7.5 seconds at 70°C, with one side being subjected to cooling at 16°C for 20 minutes. Burns were then harvested at one, two, and three hours after injury, and burn depth assessed histologically. Note the progression in burn depth over time in both cooled (open circles) and non-cooled (closed circles) burns, but with a significant reduction in cooled compared to non-cooled at three hours. Each circle represents an individual burn from an individual patient. Differences between groups were compared with a paired t-test; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $P < 0.0001$.

