Novel Burn Device for Rapid, Reproducible Burn Wound Generation

Kim, J.Y.^{a,b}, Dunham, D.M.^c, Supp, D.M.^{d,e}, Sen, C.K.^{b,f}, and Powell, H.M.^{a,b,c,d*}

^aDepartment of Biomedical Engineering, The Ohio State University, Columbus, OH

^bCenter for Regenerative Medicine and Cell-Based Therapies (CRMCBT), The Ohio State University, Columbus, OH

^cDepartment of Materials Science and Engineering, The Ohio State University, Columbus, OH

^dResearch Department, Shriners Hospitals for Children, Cincinnati, OH

^eDepartment of Surgery, University of Cincinnati, Cincinnati, OH

^fDepartment of Surgery, The Ohio State University, Columbus, OH, Comprehensive Wound Center and CRMCBT

*Corresponding Author:

Heather M. Powell, Ph.D.

Department of Materials Science and Engineering

116 W. 19th Ave

243 Fontana Labs

The Ohio State University

Columbus, OH 43210

(T)1-614-247-8673

powell.299@osu.edu

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Abstract

Introduction

Scarring following full thickness burn injuries leads to significant reductions in range of motion and quality of life for burn patients. To effectively study scar development and the efficacy of anti-scarring treatments in a large animal model (female Red Duroc Pigs), reproducible, uniform, full-thickness, burn wounds are needed to reduce variability in observed results that occur with burn depth. Prior studies have proposed that initial temperature of the burner, contact time with skin, thermal capacity of burner material, and the amount of pressure applied to the skin need to be strictly controlled to ensure reproducibility. The purpose of this study was to develop a new burner that enables temperature and pressure to be digitally controlled and monitored in real-time throughout burn wound creation and compare it to a standard burn device.

Methods

A custom burn device was manufactured with an electrically heated burn stylus and a temperature control feedback loop via an electronic microstat. Pressure monitoring was controlled by incorporation of a digital scale into the device, which measured downward force. The standard device was comprised of a heat resistant handle with a long rod connected to the burn stylus, which was heated using a hot plate. To quantify skin surface temperature and internal stylus temperature as a function of contact time, the burners were heated to the target temperature ($200 \pm 5^{\circ}\text{C}$) and pressed into the skin for 40 seconds to create the thermal injuries. Time to reach target temperature and elapsed time between burns were recorded. In addition, each unit was evaluated for reproducibility within and across three independent users by generating burn wounds at contact times spanning from 5-40 seconds at a constant pressure and at pressures of 1 or 3 lbs with a constant contact time of 40 seconds. Biopsies were collected for histological analysis and burn depth quantification using digital image analysis (ImageJ).

Results

The custom burn device maintained both its internal temperature and the skin surface temperature near target temperature throughout contact time. In contrast, the standard burner required more than 20 seconds of contact time to raise the skin surface temperature to target due to its quickly decreasing internal temperature. The custom burner was able to create four consecutive burns in less than half the time of the standard burner. Average burn depth scaled positively with time and pressure in both burn units. However, the distribution of burn depth within each time-pressure combination in the custom device was significantly smaller than with the standard device and independent of user.

Conclusions

The custom burn device's ability to continually heat the burn stylus and actively control pressure and temperature allowed for more rapid and reproducible burn wounds. Burn injuries of tailored and repeatable depths, independent of user, provide a platform for the study of anti-scar and other wound healing therapies without the added variable of non-uniform starting injury.

Introduction

Severe scarring is estimated to affect as many as 70% of burn patients [1] and results in both cosmetic and functional deformities that negatively impact on the quality of life for those affected [2-3]. As a result, significant research has been conducted to develop therapies to prevent and treat scarring [3-10]. Despite the volume of scar research, a highly effective treatment has not yet been developed. A complication to scar research is the availability of an *in vivo* testing environment. Longitudinal studies in the human patient population lack controlled burn depth, size and location along with proper negative controls [3,5]. Rodent models do not possess the complex mechanical and biological environment observed in human skin and do not naturally form hypertrophic scars [11-15]. More recently, female, red Duroc pigs (FRDPs) have been proposed as a model for scarring [3,5,16-17]. Studies have confirmed that FRDPs form robust scars following deep cutaneous wounds and that these scars are similar in appearance to human hypertrophic scars. FRDP scars are thick, raised above surrounding skin, lack hair and contain elevated populations of myofibroblasts and mast cell [3,16,18]. Additionally, the pattern of inflammatory cytokine expression is similar between humans and female red Duroc pigs [3,16,18,19]. These structural and biological similarities between human hypertrophic and the thick scars on FRDPs provide a new platform for the study of scar development and anti-scar therapies.

One of the main obstacles in studying burn wounds in animal models is the difficulty in producing burns with uniform depth [20]. For consistent wound generation, it has been proposed that the initial temperature of burner, contact time, thermal capacity of burner material, and the amount of pressure applied to the skin all need to be tightly controlled [20]. A number of different burning devices have been created to satisfy this set of needs. Heating a metal or glass stylus in a water bath has been used in prior studies to generate cutaneous burn wounds [20-22].

One study created contact burns placing water heated to 92°C in a plastic bottle on the skin surface for 15s [23]. Although the operator and procedure were kept consistent, variability in injury depth and subsequently differences in healing time of local areas within each wound were reported [23]. To improve reproducibility, the addition of a pressure monitor to the standard burn device has been employed [20] resulting in more uniform wounds.

The goal of this study was to develop a new burn device that could provide real time pressure monitoring, in addition to real time control and monitoring of device temperature, for rapid generation of reproducible burn wounds. This device was then compared to a standard burn stylus comprised of a heated block. Time required to bring the device to temperature, ability to raise and maintain skin surface temperature throughout the burn experiment, and elapsed time between generation of consecutive burn wounds was examined. In addition, burn depth was assessed as a function of burn device, time, pressure and user.

Materials and methods

Burn Devices

To create full-thickness wounds, 1 x 1 in stainless steel blocks were placed onto pig skin to create a thermal injury. Two different burn devices were utilized. The first was a standard burn stylus consisting of a stainless steel block (1in.³) connected to a metal rod and handle (Figure 1A), which can be heated using a hot water bath or using a hot plate. A hot plate (Corning PC-420D, Tewksbury, MA) was used in this study to heat the block to 200°C (Figure 1A). The second device was a custom burner fabricated in house that consisted of a stainless steel block (2.5 cm³) connected to a metal stylus, an electronic microstat (JUMO GmbH and Co. KG, Fulda, Germany) and electronic scale (Guangzhou WeiHeng Electronics Co. Ltd.,

Guangdong, China) (Figure 1B). The custom burn device was electronically heated internally, and the electronic scale allowed for precise measurements of pressure applied to the skin surface. The housing surrounding the heating element and pressure gauge was constructed from aluminum to keep the added weight to a minimum (custom unit was 2.5 lbs heavier than the commercial unit). The housing unit can be handled without the use of thermally insulated gloves during operation.

Temperature Logging System

Internal, mid-block temperature of the standard burner was logged in real time during initial heating, burning, and post-burn using a thermocouple (J-type, OMEGA, Stamford, CT) inserted in a centrally-located hole in the stainless steel block (Figure 1A). The thermocouple was connected to a Hi-Speed USB Carrier (National Instruments, Austin, TX) and a computer for data acquisition. LabVIEW™ SignalExpress 2010 (National Instruments, Austin, TX) recorded temperature of the thermocouple at 0.5 second intervals. To obtain mid-block temperature of the custom burner during heating, burning, and post-burn, a thermocouple connected to an electronic microstat controller was inserted in the stainless steel block (Figure 1B). Video footage was taken of the microstat controller during the burning procedure and was used to log temperature changes every 1 second. To quantify the surface temperature of the pig skin throughout the entire burn procedure, an additional thermocouple, connected to the same USB carrier, was placed directly in between the skin surface and burner and temperature recorded each second. Time-temperature plots were utilized to quantify time to reach initial temperature, maximum skin surface temperature and time required to bring the burn device back to temperature after each burn. Average elapsed time data was plotted $\pm$ standard error and statistical differences assessed via student's t-test.

Burn wound creation, anesthesia, animal care

All experiments and data collection were performed following The Ohio State University Institutional Laboratory Animal Care and Use Committee (ILACUC) approved protocols. Four female, Red Duroc pigs were utilized for the study: two pigs for analysis of heating time and temperature using a fixed pressure and contact time, and two pigs for analysis of time, pressure, and intra-user variability on burn depth. Pigs were anesthetized with Telazol followed by isoflurane and the dorsal trunk shaved and surgically prepared with alternating chlorohexidine 2% and alcohol 70% scrubs (Butler Schein, Columbus, OH). On the first two pigs, full-thickness wounds were generated using the standard burner heated via hot plate and the custom designed burner (n = 8 per group). Thermal injury was induced by heating each stylus to $200 \pm 5^{\circ}\text{C}$ and applying the stylus to the surface of the skin for a contact time of 40 seconds. During the burn wound generation, time–temperature data was logged within the block and on the pig skin surface continuously. Three pounds of pressure was applied with the custom-made burner. To deliver a roughly equivalent of pressure with the standard burner, users practiced by pressing the commercial device onto a scale to achieve the desired pressure, prior to wounding the pigs. Sixteen total wounds were generated on each pig, eight with the standard burner and eight with the custom-made burner. Photographs of the wounds were taken immediately after injury and day 0 biopsies taken from half of the wounds. Wounds were then covered with non-stick gauze pads (Curad) and Elastikon™ (3M). Taffeta dressings were placed over wounds and secured with Elastikon. A fentanyl patch (NOVAPLUS patch, Watson Laboratories Inc, 100 mcg) was placed in the pig ear pinna and removed three days post wounding. The pigs were maintained on standard chow *ad libitum*, fasted overnight before the procedures, housed individually and euthanized 7 days post-burn.

The second two pigs were used to assess the ability of the devices to control burn depth via contact time or applied pressure, and to assess intra-user variability. In the first experiment, contact time was examined (5, 10, 20 and 40 sec) while holding contact pressure at 3 lbs. Three independent users created three wounds per contact time. In the second experiment, pressure was examined (1 or 3 lbs) using a constant contact time of 40 seconds (n = 3 per user and a total of 9 per group). Following wound generation, pigs were euthanized and biopsies collected.

Histology

Pig skin was excised from each wound in 1cm x 2cm strips to collect the wound margin and the midpoint of each wound. These biopsies were fixed in formalin for 6 hours prior to processing and paraffin embedding. Sections were stained with hematoxylin and eosin (H&E) and imaged with light microscopy (Nikon Eclipse 90i with NIS-Elements AR3.1 software) at 4X. Individual images were digitally stitched together (Adobe Photoshop Elements 6.0) and representative composite images shown for each group. Wound depth (at the wound center and at the margins) was quantified using ImageJ. Box-and-Whisker plots were generated from each burn depth data set using SigmaPlot software, with the upper boundary of the box representing the upper quartile of burn depths for each group, the lower boundary showing the lower quartile, the end of the upper whisker identifying the maximum value and the lower end of the whisker identifying the minimum value. The solid line within the box represents the mean value of the data. Statistical analyses were performed using SigmaPlot software (One-way ANOVA with post-hoc test of Tukey).

Results

Initial Heating Time

Internal burn stylus temperature as a function of time post heating was collected to compare the time required to bring each burner device from room temperature ($20 \pm 5^\circ\text{C}$) to the desired stylus temperature for thermal injury ($200 \pm 5^\circ\text{C}$). The standard burn stylus heated on standard hotplate (Figure 1A) took an average of 13.4 ± 2.1 minutes to reach target temperature (Figure 2). The custom designed burn device with an electrically controlled internal heater and pressure gauge (Figure 1B) took an average of 16.9 ± 2.9 minutes to reach desired temperature ($p > 0.05$, Figure 2).

Stylus and Skin Surface Temperature During Use

In order to examine the ability of each burner device to raise and maintain skin surface temperature throughout the entire burn procedure, mid-block burn stylus temperature and skin surface temperature measurements were simultaneously collected on each burn. Temperature data acquisition was repeated for a total of 16 burns per pig (4 sequential burns per device in each of the two pigs, before switching to other device, 8 total wounds per pig). The internal temperature of the standard device decreased linearly at an average rate of $0.4^\circ\text{C}/\text{sec}$ while in contact with the porcine skin (Figure 3A). In contrast, the custom device maintained the internal burn stylus temperature within 7°C of target temperature throughout contact with the skin surface. It took the standard burner between 7 and 20 seconds to raise the external skin temperature to 97°C (Figure 3B). In addition, there was significantly greater variability in the skin surface temperature during device contact when using the standard device versus the custom device (Figure 3B). The custom burn device was able to quickly raise the skin surface temperature to 97°C (Figure 3B), and consistent skin surface temperature was maintained during the full contact time of 40 seconds (Figure 3B).

Elapsed Time

The elapsed time between each of the four consecutive burns showed how quickly each burner was able to return internal stylus temperature to $200 \pm 5^{\circ}\text{C}$ after each burn wound. The average elapsed time between burns for the custom design device was 2.8 minutes (Figure 4). The standard device took approximately 4.5 minutes longer ($p < 0.005$), an average of 7.3 minutes between each burn, than the custom design device to heat up to target temperature in between burns (Figure 4). This resulted in the standard burner taking more than 2.5X as long as the custom design burner to generate 4 subsequent burn wounds.

Burn Depth as a Function of Time, Pressure and Device

Histological analysis of wounds at day 0 showed necrotic tissue at increasing depths as a function of time in both groups (Figure 5). At 5 seconds, the standard device damaged only the upper epidermis whereas the custom device destroyed the full epidermis. At each contact time point, the thickness of tissue damage appeared to be greater in the custom device group compared to the standard group. In addition, wound depth appeared to be more sensitive to time in the custom group compared to the standard group where the difference in observed burn depth was less drastic between 5 and 20 seconds (Figure 5). A similar trend was observed with increasing pressure and constant contact time. Intact collagen fibrils and other indications of non-damaged tissue were observed higher in the dermis in the standard device group compared to the custom device (Figure 6). To quantitatively assess burn depth, images were processed using ImageJ with burn depth calculated from the top of the tissue section to the point at which clear collagen fibrils could be observed (Figure 7). No significant differences were observed

among different users when either time or pressure were varied. Therefore, data from all users within a group (time-pressure-device combination) was pooled and box-and-whisker plots of burn depth as a function of time or pressure and device were constructed. A trend of increasing mean burn depth with increasing time was observed in both the standard and custom device group (Figure 8A&B). With the standard device, a wider variance in intragroup burn depths was observed compared to the custom group and this intragroup variance increased with contact time (Figure 8A). No significant difference in burn depth was found in the standard group between 5 and 20 seconds of contact time ($p = 0.529$). Burn depth at 40 seconds was significantly greater than 5, 10 or 20 seconds of contact time ($p < 0.005$). Using the custom burn device, intragroup variance was low at all contact time points (Figure 8B). Burn depth also increased significantly with contact time ($p < 0.01$). Similar trends were observed when device pressure was examined. With both devices, burn depth increased with increasing pressure. Mean burn depth was similar with both devices (standard = $140.4 \pm 82.9 \mu\text{m}$, custom = $190.3 \pm 11.2 \mu\text{m}$) at 1 lb of force. Intragroup variance was substantially greater with the standard device compared to the custom device (Figure 8C&D).

Discussion

One of the most significant differences in the performance of the custom burn device compared to the standard device was its ability to generate reproducible, user-independent, controlled-thickness burn wounds at a rapid pace. The custom burner's ability to continuously be heated internally not only significantly shortened the overall amount of time required to create the wounds, but also made the custom burn device more convenient to use. Whereas the standard burn device needed to be transported to and from the hot plate to be heated between

burns, a delay that can allow temperature to drop before contact with the pig's skin, this was not required for the custom burner. When studying burn wound healing, it is important to maintain consistency within the burns that are generated. This not only includes burn depth but also the initial time point of injury because the healing cascade begins instantaneously following injury [24]. Therefore, it is ideal to use a burner that is capable of generating quick, highly reproducible burn wounds. Recently, this custom burner design was utilized successfully to create large (2" x 2") full-thickness burn wounds in a Yorkshire pig model to facilitate the study of biofilm infected wounds [25]. The ability to exchange the burn stylus with another stylus of any shape and size provide unlimited tunability of burn shape and size in addition to controlling burn depth using temperature and pressure.

Initial temperature of burner, contact time, thermal capacity of burner material, and applied pressure all play a role in burn depth [20]. Burn depth scaled positively with both contact time and contact pressure using either unit; however the custom burn device generated burns with much greater reproducibility. Both the standard and custom burners were heated to the same initial temperatures, and both could control burn depths by altering contact times. Thus, it is most likely the ability to control applied pressure and maintain burner temperature while in contact with the skin that improves the precision of the custom burn device. Both burners utilize a stainless steel stylus, therefore are presented with the same low thermal conductivity of 24 watts per meter Kelvin [26] and heat transfer capabilities. The difference lies within the custom burner's ability to be heated continuously and internally during burning. This constant heat source provided continuous heat transfer from the stylus to the skin, resulting in the maintenance of a more uniform skin surface temperature throughout burning. This contributed to greater damage and consistency in wound depth, both between and within wounds, compared to the

wounds created by the standard burner. The standard burner immediately started losing heat once detached from the hot plate and continued to cool down at a rapid rate while in contact with the skin. The amount of pressure applied to the skin during thermal injury has been suggested to be a key indicator of extent of damage with greater tissue damage resulting from increased pressure. Compression of the underlying tissue is believed to decrease the rate of heat dissipation [20]. Though each of the three independent users practiced controlling applied pressure with the standard device by pressing the device onto a scale, it is highly likely that the magnitude of applied pressure varied from burn to burn. The ability to see the digital pressure gauge in the custom device allowed the user to monitor the applied pressure initially and maintain this pressure throughout the duration of contact. This level of control is not achievable with the standard device and provides a distinct advantage in generating reproducible burn injuries of tailored depths.

Conclusions

When creating burn wounds to study wound healing and scarring in an animal model, it is ideal to have a burn device that can quickly create consistent, consecutive burn wounds of uniform depth and damage. In this study, a new custom burn device was developed that allowed control and monitoring of real time pressure, internal burner temperature, and elapsed time throughout burn wound generation. In comparison to the standard burner, the custom burner not only was quicker and more convenient to use, but also created burn wounds of tailorable depth and high reproducibility. The burner design used for these studies can be further customized to produce burns of different sizes or shapes for studies in other animal models.

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Figure Captions

Figure 1. Photograph of A) standard burn stylus heated to temperature using a laboratory hotplate (*inset*: close up of stylus and port for thermocouple) and B) a custom designed burn device with an electrically controlled internal heater and pressure gauge (*upper inset*: close up of burn stylus with thermocouple in port, *lower inset*: electronically controlled microstat).

Figure 2. Time required to bring each burn stylus to temperature (200°C).

Figure 3. Time-temperature traces for both the inside of the burn stylus (A) and the skin surface (B). Note that the internal temperature of the burner decreases linearly at a high rate in the standard device whereas the custom device held temperature to within 7 degrees throughout contact with the skin surface. The external skin temperature was rapidly raised to 97°C using the custom burn device and held constant at this temperature until the device was removed. In contrast, the standard device required between 7 and 20 seconds to reach peak temperature and had only a short period of steady surface temperature.

Figure 4. Elapsed time between subsequent burns.

Figure 5. Representative H&E stained sections of burns generated using the standard and custom device heated to 200°C and applied to the skin at 3 lbs of pressure for 5, 10, 20 or 40 seconds.

Figure 6. Representative H&E stained sections of burns generated using the standard and custom device heated to 200°C and applied to the skin for 40 seconds at either 1 or 3 lbs of pressure.

Figure 7. H&E stained histological section of a burn injury showing methodology for quantifying burn depth. Burn depth quantified by measuring distance from top of tissue section to the depth where distinct collagen fibers can be visualized.

Figure 8. Box and whisker plot of burn injury depth using the standard (A&C) and custom (B&D) device. Burn depth increased as a function of contact time while pressure is held at a 3lbs (A&B). Burn depth increased with increasing pressure during a 40 sec contact time (C&D).

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8