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Acute discrimination between superficial-partial and deep-partial thickness burns in a preclinical model with laser speckle imaging

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ABSTRACT

A critical need exists for a robust method that enables early discrimination between superficial-partial and deep-partial thickness burn wounds. In this study, we report on the use of laser speckle imaging (LSI), a simple, non-invasive, optical imaging modality, to measure acute blood flow dynamics in a preclinical burn model. We used a heated brass comb to induce burns of varying severity to nine rats and collected raw speckle reflectance images over the course of three hours after burn. We induced a total of 12 superficial-partial and 18 deep-partial thickness burn wounds. At 3 h after burn we observed a 28% and 44% decrease in measured blood flow for superficial-partial and deep-partial thickness burns, respectively, and that these reductions were significantly different ($p = 0.00007$). This preliminary data suggests the potential role of LSI in the clinical management of burn wounds.

1. Introduction

1.1. Clinical evaluation

According to the American Burn Association, 450,000 people receive medical attention for burn wounds, with 40,000 undergoing hospitalization and 3400 deaths attributed to burn wounds [1]. Typical causes of burn wounds include flame, hot water, electricity, and chemicals [2]. Clinical observation is the most common method of diagnosing the severity of a burn wound [3]. The clinician performs tests that provide visual and tactile information of the burn wound, such as wound appearance (edema, color, and blisters), capillary blanching and refill, and sensibility to pinprick and touch [4].

Based on this information, the clinician typically categorizes the burn wound into one of four categories: superficial, superficial-partial, deep-partial, or full thickness burns. Each category is associated with varying healing periods and characteristics. Superficial burn wounds only involve injury of the epidermis and are associated with hyperemia, a dry surface, a diffuse pink or red color, and an absence of blisters.

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These wounds heal within fourteen days. Full thickness burn wounds involve injury of the epidermis, dermis, and hypodermis; and are associated with thrombosis; a dry, leathery, and rigid appearance; a white, black, and cherry red color; and an absence of blanching with pin pricks. These wounds take longer than 21 days to heal [5].

While superficial and full thickness burns are straightforward to diagnose based on visual appearance, clinicians have difficulty with accurate differentiation between superficial-partial and deep-partial thickness burns. The experience of the clinician and the fact that these burns can dynamically increase in severity (i.e., burn wound conversion) during the initial 48 h period, both lead to higher clinician error [4]. Both wound types are associated with similar characteristics, and both involve the epidermis and dermis. Superficial-partial burns involve injury of the papillary dermis and are associated with intact blisters, moderate edema, a moist surface under the blisters, a bright pink or red color, and blanching with fast capillary refill after pressure is applied. Deep-partial thickness burns involve injury of both the papillary and reticular dermis and are associated with broken blisters, substantial edema, a wet surface, a mixed red or waxy white color, and blanching with slow capillary refill after pressure is applied [5].

With superficial or superficial-partial burn wounds, patients receive antimicrobial creams for treatment. With deep-partial or full thickness burn wounds, excision and grafting is beneficial [6]. The decision on treatment protocol is typically based on clinical observation and hence remains subjective. Unfortunately, the reported accuracy of this approach is ~67% [4]. Hence, a critical need exists to develop technologies and methodologies that improve the accuracy of burn-wound assessment. Recently, we reviewed various technologies that have been investigated within the context of burn wound severity assessment [7]. One set of techniques that appears to be promising in this regard is based on measurements of skin blood flow.

1.2. *Laser Doppler imaging*

Researchers have studied the use of laser Doppler imaging (LDI) to assess burn wounds. Stern first demonstrated that LDI provides measurements related to the Doppler shift resulting from interactions of incident coherent light with moving red blood cells [8]. With LDI, researchers have reported accuracies greater than 90% after 72 h after-burn [9–11], demonstrating the potential efficacy of LDI to characterize burn wounds.

Despite the high accuracy associated with LDI, it unfortunately remains largely a research tool, in part because of limitations including long scan times, cost, system size, and artifacts associated with patient movement [7]. Laser speckle imaging (LSI) is a relatively low-cost approach to gather similar blood-flow information that offers faster acquisition speeds, day-to-day reproducibility [12], and simple instrumentation [13]. We previously used LSI for several biomedical applications [14–17]. In the present work, we characterized the performance of LSI to evaluate perfusion dynamics associated with burn wounds of graded severity. Based on the results from several studies using LDI [9,11,18,19], our central hypothesis was that a decrease in blood-flow values measured with LSI correlate with increasing burn severity, and hence enable differentiation of superficial-partial and deep-partial thickness burns.

2. Materials and methods

2.1. *Animal model*

We performed experiments involving nine male Sprague Dawley rats (300 to 400 g in mass). The burn model and study protocol (no. 1999-2064) were approved in accordance with the University of California, Irvine Institutional Animal Care and Use Committee.

2.2. *Burn wounds*

We used an identical burn wound protocol to one we described previously [20]. Briefly, burn wounds were induced using a previously established brass comb [21] that consisted of four prongs each 10×20 mm with 5 mm gaps between each prong, as shown in Fig. 1.

One day prior to burn induction, rats were shaved on the lateral dorsal region and Nair (Church and Dwight, Princeton, NJ) was applied to remove excess hair. The rats were anesthetized with a combination of ketamine hydrochloride (0.87 mL/kg) and xylazine (0.65 mL/kg) via intraperitoneal (i.p.) injection, with booster injections used as necessary. The brass comb was immersed in 100 °C boiling water and applied to the hairless lateral dorsal region without any additional applied force other than gravity. Burn times ranged from two to ten seconds with the intent of creating burn severities of superficial-partial and deep-partial thickness burns.

During the three hours after burn wounds were created, images were acquired approximately every 20 to 25 min. The rats were then euthanized with euthasol via i.p. injection. A skin pelt of the burned region from the lateral dorsal side of each rat was removed and placed into 10% buffered formalin for 24 h, followed by dehydrating and embedding the tissue in paraffin. Cuts were then made with a microtome to separate and examine each burn site individually. A vertical section was then cut through the middle of each burned region that bisected each burn site along the long axis from top to bottom. Normal skin was also taken sufficiently far from the burned region, to not be affected by the burn induction, yet still be taken from the lateral dorsal region of the rat. Normal sections and burn severity were verified using hematoxylin and eosin (H&E) staining and optical microscopy (Olympus BH2, Tokyo, Japan).

2.3. *LSI instrumentation, data acquisition and analysis*

For an excitation source, we used an 808 nm laser (Ondax, Monrovia, CA) with the collimator removed. We used a ground glass diffuser (ThorLabs, Inc., Newton, NJ) to expand the beam and achieve near-uniform illumination of the tissue (Fig. 2). For each image collection timepoint, we used a multispectral CCD camera (Nuance, Cri, Inc., Woburn, MA) to collect 50 raw speckle images with an exposure time of 10 ms.

We used custom-written MATLAB code (MathWorks Inc., Natick, MA) to process the raw speckle images. We used a