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Quantitative Longitudinal Measurement in a Rat Model of Controlled Burn Severity Using Spatial Frequency Domain Imaging

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ABSTRACT

Background and Objective: Spatial Frequency Domain Imaging (SFDI) is a non-contact wide-field optical imaging technology currently being developed to investigate the feasibility of quantitative non-invasive evaluation of burn wound severity in a rat model. Our objective is to determine the potential of SFDI for mapping quantitative changes in spatially resolved tissue oxygen saturation and water concentration may be indicative of burn wound severity, healing, and further complications. In this portion of the investigation, we focus on the development of a rat burn model and the acute response of tissue to burn wounds.
Study Design/Materials and Methods: A controlled burn protocol involving a heated brass comb was applied to 6 rats. Imaging was acquired at 17 evenly spaced wavelengths in the near-infrared from 650 to 970 nm. Over the course of the 3 hour post-burn period, we were able to map quantitative changes in spatially resolved chromophores. Burn severities were verified post-experiment using standard H&E histology and optical microscopy.
Results/Conclusion: In total, we were able to induce 12 superficial-partial thickness burns, 8 deep-partial thickness burns, and 4 full thickness burns in our rat models. While several tissue chromophores were tracked, we found that changes in oxygen saturation and water concentration to be sensitive indicators of burn severity. Future work will include additional longitudinal studies over a period of days in order to investigate which parameters are correlated to tissue healing.

1. INTRODUCTION

1.1 Burn Wound Evaluation

Thermal injuries can be caused by exposure to a wide variety sources including heat, electricity, radiation, chemical, and friction. According to the American Burn Association, approximately 500,000 people seek treatment for burn injuries every year.(1) Of that population, about 45,000 have burn injuries requiring medical treatment with about 3,500 cases resulting in death.

Skin burns are normally characterized by depth of injury.(2) Starting with the least serious, "superficial burns" involve injury to the top epidermis layer. These often have a reddish non-blistering appearance (e.g.: sunburns) due to increased blood flow to the dermis. They are often hyper-sensitive to touch, and can naturally heal in less than 2 weeks via re-epithelialization. On the other end of the spectrum are full thickness burns which extend deep beyond the epidermal and dermal layers of the skin into the subcutaneous. These burns often appear leathery, firm, and depressed, and are insensitive to pinpricks due complete destruction the dermis including nerves and vasculature. Full thickness burns require full excision and grafting as treatment. Both superficial and full thickness burns are relatively easy to diagnose based on clinical observation.

In between these two extremes are "superficial-partial thickness" and "deep-partial thickness" burns in which damage extends to a fraction of the dermal layer.(2) Superficial-partial thickness burns extend to only the upper layers of the papillary dermis, and depending on the extent of damage and remaining vasculature, these injuries may naturally heal in 2 to 3 weeks with minimal to no scarring. Deep-partial thickness burns extend down to the reticular dermis and can often be found mixed with portions of non-charred full-thickness burns. These burns often require excision and grafting

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for optimal treatment. Both categories of partial-thickness often have a mottled pink and white appearance that can blanch with pressure, and are less sensitive to pinpricks than normal.

With visual clinical assessment as the current standard for diagnosis, it is not surprising that partial thickness burns are often a source of uncertainty when estimating depth of injury and its subsequent treatment.(3) Depending on the clinician's experience, visual assessment has been shown to have a field accuracy of about 50 to 70%. Overestimation results in unnecessarily invasive surgical treatment, while underestimation results in treatment delays, extended hospital stays, and increased chances of contracture and hypertrophic scar formation.

Further complicating the situation, burns also undergo dynamic burn wound conversions during the early 48 hour period in which superficial-partial thickness burns have been observed to progress to deep-partial thickness or full thickness burns.(4) The process is not fully understood, but it is generally agreed that continuous monitoring of early-stage burns is necessary for deciding optimal treatment and management.

Currently, our lab is investigating the use of Spatial Frequency Domain Imaging (SFDI) in order to quantitatively evaluate burn wound severities in a rat model. The objective of this study is to map quantitative changes in spatially resolved tissue oxygen saturation and water concentration that may be indicative of burn wound severity, healing, and further complications.

1.2 Spatial Frequency Domain Imaging (SFDI)

Spatial Frequency Domain Imaging (SFDI) is a non-contact wide-field optical imaging technology currently being developed at the Beckman Laser Institute and Medical Clinic in Irvine, CA. By combining 'structured illumination' projections with a camera based imaging system, SFDI is capable of quantifying subsurface optical properties, which can then be utilized to quantify chromophore concentrations in subsurface *in-vivo* tissue.(5) With the ability to accurately interrogate skin depths of about 1 to 5 mm, SFDI is able to measure, over a wide field-of-view, the concentrations of various clinically relevant chromophores including oxy-hemoglobin, deoxy-hemoglobin, lipid, water, and tissue oxygen saturation.

During imaging, a spatially-modulated pattern is illuminated onto the region of interest over a range of spatial frequencies. Diffuse light is recorded using a CCD camera and then demodulated in order to extract the diffuse reflectance at each wavelength and spatial frequency, which can then be further reduced into absorption (μ_a) and reduced-scattering (μ_s') coefficients by fitting to a known forward model.(5) These optical properties can then be fit to relevant biological chromophores using Beer's law.

2. MATERIALS AND METHOD

2.1 Animal Subjects

Six male Sprague-Dawley rats were used in this study. Housing and care for animals were in accordance with UC Irvine's University Laboratory Animal Resources (ULAR). Burn model and study protocol were approved according to the UC Irvine Institutional Animal Care and Use Committee (IACUC).

2.2 Burn Model and Study Protocol

Burn injuries were created using a previously established(6) heated "brass comb" shown in Figure 1. The custom-made comb weighs 313 grams and consists of 4 notches measuring 1 cm by 2 cm separated by 0.5 cm gaps.

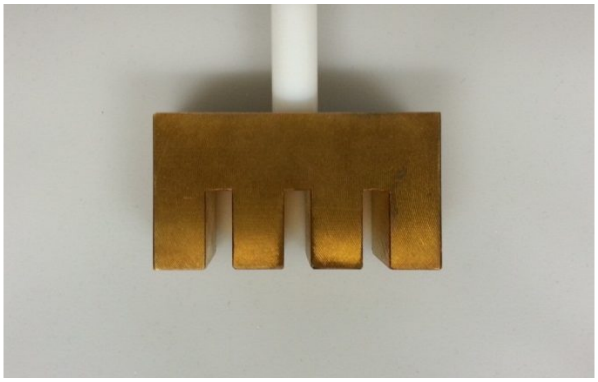 

Figure 1: Brass comb used to create burns (left). Example of burn wound immediately after injury (right).

On the day before experiments, each rat was shaved along the lateral dorsal region of the body using electric clippers and depilated with Nair (Church and Dwight, Princeton, NJ). During experiments, the rats were anesthetized using an intraperitoneal injected mixture of ketamine hydrochloride and xylazine with additional boosters administered as necessary. The brass comb was heated in boiling water (100°C) and applied onto the shaved lateral dorsal region without additional pressure (gravity only) for 2 to 15 seconds in order to create burns of graded severity, ranging from superficial-partial thickness to full thickness. However, due to the very thin thickness of the epidermal layer (13 to 22 µm), we were unable to reproduce any superficial burns.

After imaging the rats every 10 to 20 minutes for 3 hours post-burn, the rats were euthanized using pentobarbital and the burn region was excised into 10% buffered formalin where they fixed for 24 hours before being prepared for histology. In each case, burn severity was verified using standard H&E staining and optical microscopy (Olympus BH2, Tokyo, Japan).

2.3 Instrumentation and Data Acquisition

The SFDI instrument used in this study consists of a 250W tungsten lamp used to illuminate spatially modulated projections created by a digital micro-mirror device (DMD) (Texas Instruments, Dallas, TX). Diffusely reflected light was captured using a multispectral CCD camera (Cri, Inc., Woburn, MA) consisting of a liquid-crystal tunable filter for wavelength selection and a pair of linear cross polarizers to reject specular diffuse reflectance. Images were saved as binary files for post-acquisition processing via MATLAB (MathWorks, Inc., Natick, MA).

For the purpose of this study, a 65 mm by 86 mm field-of-view was measured over the course of 3 hours at 5 spatial frequencies equally spaced between 0 to 0.25 mm^{-1}. 17 spectral wavelengths between 650 and 970 nm were acquired in 20 nm intervals, and the diffuse reflectance images were calibrated for system response using tissue simulating phantoms with known optical properties ($\mu_a = 0.0176\ mm^{-1}$ and $\mu_s' = 1.024\ mm^{-1}$ at 650 nm). Effects related to body curvature were ameliorated using a 3D profile intensity correction as described by *Gioux et al.*[7] Pixel-by-pixel optical property values were calculated using a two-frequency look-up-table approach based on Monte-Carlo forward predictions [5] at 0 mm^{-1} and 0.1 mm^{-1}. Chromophore concentrations were calculated from the absorption spectrum according to Beer's law.

3. RESULTS

3.1 Histology

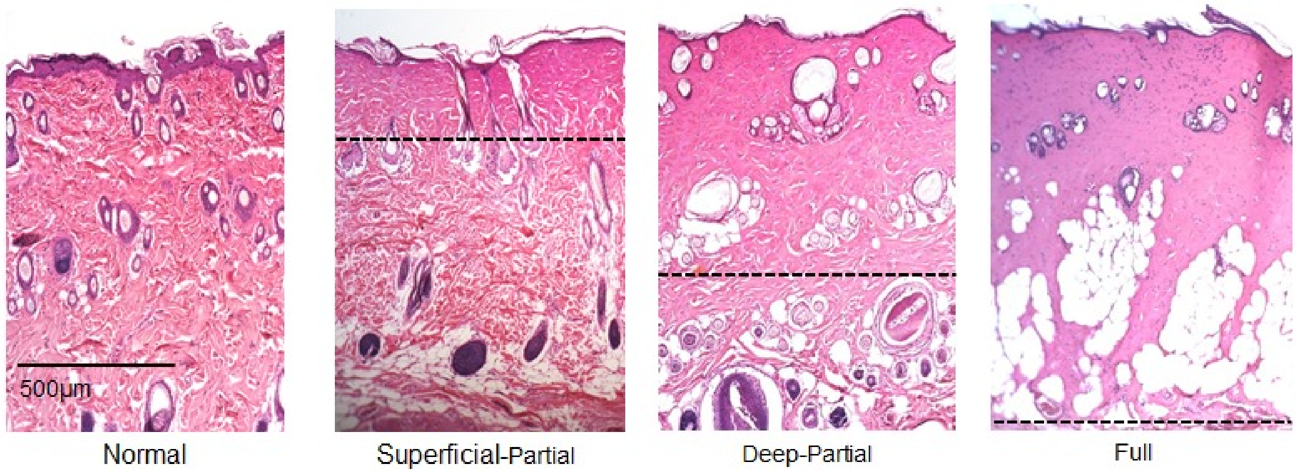

Figure 2: Examples of H&E stained cross-sections for each burn severity. Dotted lines represent estimated depth of burn

Burn severities for each sample were verified post-experiment via H&E staining and optical microscopy. Depth of burn was determined by examining for viable adnexal structures (such as hair follicles and sweat glands) and by examining for the appearance of glass-like collagen hyalinization.[8, 9] Examples of H&E stained cross-sections for each burn type can be seen in Figure 2. In total, we were able to induce 12 superficial-partial thickness burns, 8 deep-partial thickness burns, and 8 full thickness burns in our rat models**.**

3.2 SFDI Chromophore Time Plots

SFDI data analysis was concentrated on 2 parameters that may be indicative of burn depth: tissue oxygen saturation and water concentration. The following data are from 3 different rats with 3 different burn severities (4 samples per burn severity).

Oxygen Saturation (StO_2%):
As seen in Figure 3, an increase in oxygen saturation in the entire skin area was observed immediately after application of the burn in the data presented here. However, over the three hour post-burn period, the magnitude of change in tissue oxygen saturation varied dramatically depending on burn severity. The superficial-thickness burns generally stayed within ±10% of baseline, while the full-thickness burns gradually dropped to about 60% below baseline tissue oxygen saturation level. Oxygen saturation in deep-partial thickness burns stayed within ±10% of baseline saturation until about 2.5 hours into the post-burn period when it started to drop 20% below baseline.

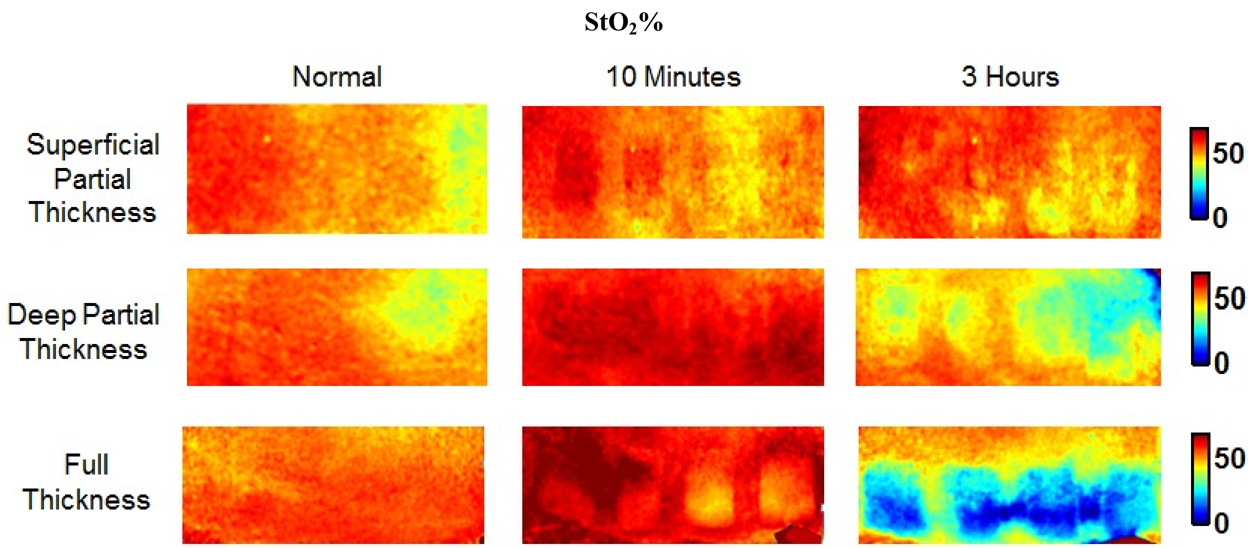

Figure 3: SFDI StO2% maps for different burn severities measured at normal baseline, 10 minutes after injury, and 3 hours after injury. Oxygen saturation scale (%) is at the far right.

Water Concentration (H_2O%):

As seen in Figure 4, there was an increase in water concentration for each type of burn presented here. The superficial-partial thickness burns remained about +10% elevated compared to baseline throughout the entire post-burn period. The full thickness burns exhibited a steady increase to about +33% of baseline, and the deep-partial thickness burns exhibited a steady increase to about +40% of baseline.

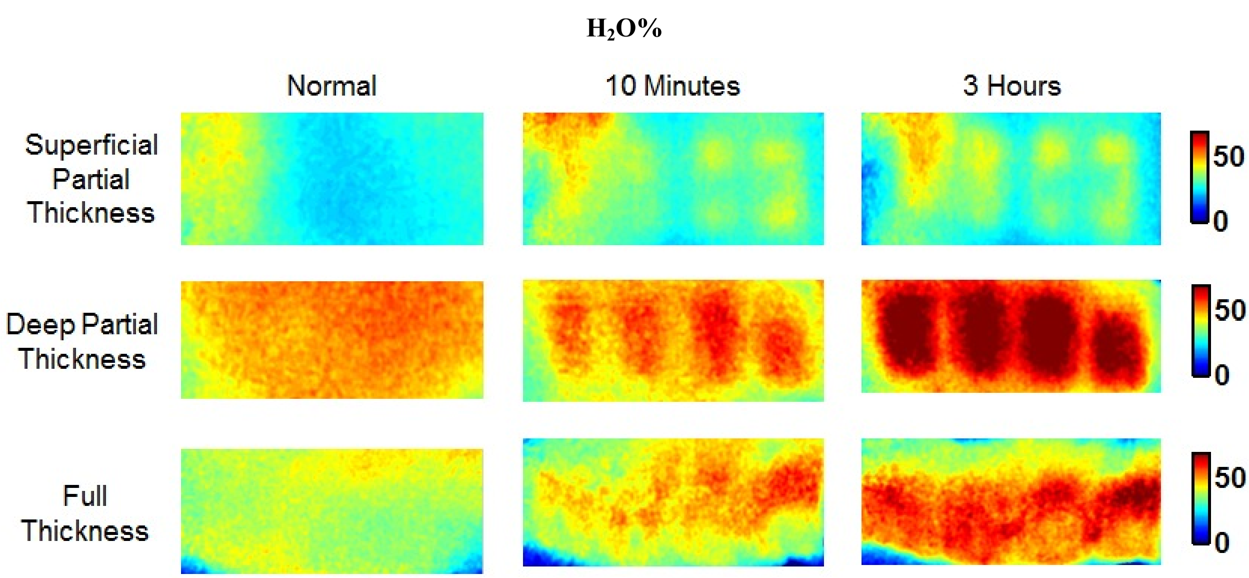

Figure 4: SFDI water concentration maps for different burn severities measured at normal baseline, 10 minutes after injury, and 3 hours after injury. Water fraction scale (%) is at the far right.

5. DISCUSSION

For the first time, we have applied SFDI to image a model of graded burn severity. As seen in the results, SFDI can be used to monitor chromophore changes over a wide field-of-view. During the 3 hour imaging period, we were able to observe changes in oxygen saturation and water concentration that may have potential of informing burn severities.

Oxygen Saturation (StO_2%):
SFDI oxygen saturation maps may be an indicator of vascular damage. As seen in Figure 3, we were able observe how tissue oxygen saturation varied depending on burn severity. Superficial-thickness burns and partial-thickness burns generally stayed close to baseline, while full-thickness burns dropped significantly within the first hour. This is expected, as full-thickness burns are characterized by complete damage of the epidermal and dermal layers including their vasculature.(2) It is interesting to note that the oxygen saturation in the deep-partial thickness burns stayed within 10% of baseline until about 2.5 hours into the post-burn before dropping about 20% below baseline. This may be indicative of negative burn wound conversion in which the partial-thickness burns' zone of stasis converted to necrosis due to possible factors including hypoperfusion, desiccation, edema, and infection.(10)

Water Concentration (H_2O%):
SFDI water concentration maps may be an indicator of edema formation. As seen in Figure 4, we were able to observe different increases in tissue water concentration for each type of burn presented. Superficial-partial thickness burns, though elevated, stayed close to baseline during the entire post-burn period, whereas deep-partial and full-thickness burns exhibited a large steady increase throughout the duration. Burn injuries often cause extravasation of plasma into burn wounds and surrounding tissues.(10) By nature of the collagen disruption, these injuries often exhibit abnormal osmotic and hydrostatic pressure gradients that worsen with burn depth and normal inflammatory response. It is interesting to note from the results in Figure 3 and Figure 4 that the oxygen saturation in the deep-partial thickness burns dropped at a time point when water concentration was at its highest. This may be indicative of edema related tissue ischemia.

6. CONCLUSION

The results of this study suggest that SFDI derived chromophore data may be useful for quantitative non-invasive assessment of post-burn tissue health. Here we have demonstrated that it can be used to monitor heterogeneous changes in oxygen saturation and water concentration over a large (as opposed to microscopic) field-of-view, thereby allowing researchers to identify burn areas that are at risk of further vascular damage or edema progression. Future work will include a longer-term study to look at the predicative capability of SFDI related burn parameters.

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