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Utility of spatial frequency domain imaging (SFDI) and laser speckle imaging (LSI) to non-invasively diagnose burn depth in a porcine model☆

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ABSTRACT

Surgical intervention of second degree burns is often delayed because of the difficulty in visual diagnosis, which increases the risk of scarring and infection. Non-invasive metrics have shown promise in accurately assessing burn depth. Here, we examine the use of spatial frequency domain imaging (SFDI) and laser speckle imaging (LSI) for predicting burn depth. Contact burn wounds of increasing severity were created on the dorsum of a Yorkshire pig, and wounds were imaged with SFDI/LSI starting immediately after-burn and then daily for the next 4 days. In addition, on each day the burn wounds were biopsied for histological analysis of burn depth, defined by collagen coagulation, apoptosis, and adnexal/vascular necrosis. Histological results show that collagen coagulation progressed from day 0 to day 1, and then stabilized. Results of burn wound imaging using non-invasive techniques were able to produce metrics that correlate to different predictors of burn depth. Collagen coagulation and apoptosis correlated with SFDI scattering coefficient parameter (μ_s') and adnexal/vascular necrosis on the day of burn correlated with blood flow determined by LSI. Therefore, incorporation of SFDI scattering coefficient and blood flow determined by LSI may provide an algorithm for accurate assessment of the severity of burn wounds in real time.

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Abbreviations: SFDI, spatial frequency domain imaging; LSI, laser speckle imaging; LDI, laser Doppler imaging.

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1. Introduction

Over a half million people seek treatment annually for burns [1]. Excision and grafting is the most common surgical procedure performed after burn, with estimates as high as 66% of all burns undergoing surgery [2,3]. Categorically, first degree burns will heal spontaneously and do not benefit from surgical intervention. Third degree burns are the most severe and benefit most from excision/debridement because they will not heal spontaneously; additionally, first and third degree burns are easily identified based on visual inspection. Intermediate of these two extremes are second degree wounds which present a diagnostic challenge using visual inspection alone. The accuracy of diagnosis of second degree burn severity has been reported to be only 60–80% and is further reduced if the diagnosis is made within the first 48 h after-burn [4–8].

The treatment strategy for second degree burns depends on the actual burn severity. Superficial second degree burns, which are similar to first degree burns, are treated conservatively (i.e. covered and monitored) and will normally heal within 1–2 weeks. Deeper second degree burns, are similar to third degree burns and benefit from debridement/escharotomy and grafting as early as possible. Early excision has been shown to reduce the occurrence of infection and wound healing complications, thus shortening the duration of hospital stays [9–11]. Therefore, inaccurate burn severity diagnosis can have drastic consequences for the patient. If burn depth is overestimated, unnecessary surgeries may be performed. If burn depth is underestimated, the increased delay in treatment time can lead to the morbidities mentioned above, and result in impaired cosmesis and function (e.g. limited range of motion) due to scar formation. Clearly, an objective and quantifiable measure that would accurately assess burn severity would improve patient outcomes.

To this end, a number of non-invasive imaging techniques have been investigated for their use in determining burn depth, including terahertz imaging, infrared spectroscopy, and reflectance mode confocal microscopy [12–17]. The most successful of these techniques implement some aspect of examining blood flow, exploiting readouts such as temperature changes or vascular patency. Perhaps the most promising of these techniques studied thus far are laser Doppler imaging (LDI) and indocyanine green angiography (ICG), however both of these have caveats to their use [5]. To perform ICG it is necessary to inject a fluorescent dye intravenously; this procedure is associated with several side effects, from mild headaches and pruritus to the potential for a severe anaphylactic response [18]. LDI has been shown to accurately assess burn severity [19], however, LDI has several limitations including long scan times, and superficial resolution. Moreover, it has been shown that LDI is only superior to visual assessment after 48 h after-burn [8].

Two emerging technologies, laser speckle imaging (LSI) and spatial frequency domain imaging (SFDI) have recently been shown to provide accurate assessments of burn depth. LSI measures blood flow, with similar consistency as LDI [20]. LSI, however, is easier, less expensive, and allows improved patient comfort than LDI. Additionally, LSI provides real time perfusion maps that allow for evaluation of blood flow in relation to the patient's anatomy. SFDI is a wide-field imaging modality that non-invasively yields quantitative spatial maps of tissue optical properties and biochemical composition. These include concentration of chromophores, such as oxy- and deoxyhemoglobin, as well as structural tissue matrix integrity via scattering coefficients (μ_s) [21–24]. Recently, SFDI has been shown to be able to predict burn severity in a rodent comb burn wound model [25].

While a variety of techniques have been employed in burn research, histopathology is still used because of its accuracy. Masson's trichrome staining is the most often used histological stain, however other immunohistochemical stains have recently been shown to be superior in determining burn depth [26–28]. Specifically, caspase 3 is a protease that is involved with the activation phase of cell apoptosis. Staining tissue early after burn wounding with caspase 3 antibodies has been shown to stain distinct bands of apoptotic cells, delineating burn depth as early as 1 day after-burn. Additionally, high mobility group box protein 1 (HMGB1) has recently been identified as being able to define the zone of stasis, identifying initially viable tissue that eventually becomes necrotic [27]. Moreover, both of these markers correlated with each other, and with the eventual level of tissue necrosis seen at 7 days [27]. Histopathology has been proposed for use clinically, but is seldom used due to the invasiveness of the procedure, which could be circumvented if other non-invasive techniques were available to measure apoptotic or necrotic cell death.

The current study was designed to examine whether LSI and/or SFDI have the ability to non-invasively measure burn depth in a porcine model. It is well accepted that porcine skin closely resembles humans in terms of structure and wound healing [29,30]. We used a contact burn procedure with brass probes to create burn wounds spanning from superficial to full thickness. The current clinical standard of histopathology was used to define burn depth using three main methods; collagen coagulation (trichrome staining), cellular apoptosis (caspase 3), and vascular/adnexal necrosis (HMGB1). The results show that LSI and SFDI are able to non-invasively predict different burn which may aid the clinician in diagnosing burn depth.

2. Materials and methods

2.1. Animals

Two female Yorkshire swine (Midwest Research Swine) weighing 49 and 50 kg at the time of burn were used in this study. Animals were singly housed, with ad libitum access to water, and were allowed to acclimate to the facilities for at least 7 days prior to any procedures. This protocol (A13-018) was approved by the Animal Care and Use Committee, Institute of Surgical Research. This study has been conducted in compliance with the Animal Welfare Act, the implementing Animal Welfare Regulations, and the principles of the Guide for the Care and Use of Laboratory Animals.

2.2. Anesthesia

Animals were fasted and a transdermal fentanyl patch (100 μg/h) was placed on the ear of the animals on the night