Ultrasound in Med. & Biol., Vol. 00, No. 00, pp. 1–7, 2018

0301-5629/$ - see front matter

https://doi.org/10.1016/j.ultrasmedbio.2018.05.011

● *Clinical Note*

EFFECTS OF NON-THERMAL, NON-CAVITATIONAL ULTRASOUND EXPOSURE ON HUMAN DIABETIC ULCER HEALING AND INFLAMMATORY GENE EXPRESSION IN A PILOT STUDY

ANAMIKA BAJPAI* SUMATI NADKARNI* MICHAEL NEIDRAUER* MICHAEL S. WEINGARTEN†
PETER A. LEWIN* and KARA L. SPILLER*

* School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, Pennsylvania, USA; and
† Department of Surgery, College of Medicine, Drexel University, Philadelphia, Pennsylvania, USA.

(*Received* 16 *January* 2018; *revised* 24 *April* 2018; *in final from* 10 *May* 2018)

Abstract—The purpose of this clinical study was to assess, in a limited patient population, the potential for a novel advanced wound care treatment based on low-frequency (20 kHz) low-intensity (spatial peak temporal peak intensity <100 mW/cm^2; *i.e.,* pressure amplitude of 55 kPa) ultrasound (LFLI-US), to affect wound closure rate in human diabetic foot ulcers (DFUs) and to effect changes in the relative expression of pro-inflammatory and anti-inflammatory genes. The ratio of expression of these genes, termed the M1/M2 score because it was inspired by the transition of macrophages from pro-inflammatory (M1) to anti-inflammatory (M2) phenotypes as wound healing progresses, was previously presented as a potential healing indicator for DFUs treated with the standard of care. We previously found that non-cavitational, non-thermal LFLI-US delivered with a pulse repetition frequency of 25 Hz was effective at improving wound healing in a pilot study of 20 patients with chronic venous ulcers. In this study, we assessed the potential for weekly LFLI-US exposures to affect wound healing in patients with diabetic ulcers, and we analyzed temporal changes in the M1/M2 score in debrided diabetic wound tissue. Although this was a limited patient population of only 8 patients, wounds treated with LFLI-US exhibited a significantly faster reduction in wound size compared with sham-treated patients ($p < 0.001$). In addition, the value of the M1/M2 score decreased for all healing diabetic ulcers and increased for all non-healing diabetic ulcers, suggesting that the M1/M2 score could be useful as an indicator of treatment efficacy for advanced DFU treatments. Such an indicator would facilitate clinical decision making, ensuring optimal wound management and thus contributing to reduction of health care expenses. Moreover, the results presented may contribute to an understanding of the mechanisms underlying ultrasonically assisted chronic wound healing. Knowledge of these mechanisms could lead to personalized or patient-tailored treatment. (E-mail: Spiller@drexel.edu)

Key Words: **Diabetic ulcers, Low-frequency, low-intensity ultrasound, Inflammation.**

INTRODUCTION

The purpose of this brief clinical study in a limited patient population was to evaluate the potential effects of low frequency, low intensity ultrasound (LFLI-US) on diabetic ulcer healing and the temporal changes in the relative expression of genes associated with pro-inflammatory (M1) and anti-inflammatory/pro-healing (M2) macrophage phenotypes, which may have potential as a healing indicator to aid in clinical decision making to improve wound care.

Chronic ulcers represent a substantial clinical burden; 30.3 million people in the United States alone have diabetes (American Diabetes Association), and it is estimated that 15% of diabetic patients will develop an ulcer at some time during their disease course (Reiber et al. 1998). The standard wound care practice involves debridement of necrotic tissue, application of moist dressings and offloading (Powers et al. 2013). However, these passive treatments have limited success (Frykberg and Banks 2015), with 5-y amputation rates as high as 29% (Moulik et al. 2003). Compounding the problem is the lack of an effective diagnostic method to determine if the wound is healing or not. The Wound Healing Society recommends using a 40% reduction in wound size as

Address correspondence to: Kara L. Spiller, School of Biomedical Engineering, Science and Health Systems, 3141 Chestnut Street, Drexel University Philadelphia, PA 19104. E-mail: Spiller@drexel.edu

an indicator that a wound is on a healing trajectory, but this method has only 50%−70% positive predictive value (Cardinal et al. 2008; Sheehan et al. 2003). It has been estimated that switching to a more effective treatment after 4 wk of unsuccessful treatment would save approximately $12,000 per patient (Weingarten et al. 2012). At the same time, the most effective advanced wound care treatments have only a ~60% success rate (Johnson et al. 2017). Thus, there is a well-defined need for more effective therapies that actively promote wound healing and for innovative diagnostics that aid in clinical decision making.

We recently reported the results of a pilot clinical study indicating that a novel LFLI-US contact-based applicator actively promoted healing of chronic venous ulcers (Samuels et al. 2013). In that study, patients were exposed to a 55-kPa-pressure-amplitude ultrasound wave corresponding to 100 mW/cm^2, spatial peak temporal peak intensity (I_{SPTP}), for 15 or 45 min weekly. The weekly treatment frequency was intentionally chosen to minimize patients' travel burden associated with clinical visits. All exposures were delivered at 20 or 100 kHz with a pulse repetition frequency of 25 Hz. Such field parameters are considered tolerable because they exclude thermal and cavitation effects, even for prolonged exposure times (>250 min). Wounds that were exposed to 20-kHz ultrasound for 15 min per week healed at the fastest rate. The purpose of this study was to determine the potential for the same treatment modality to promote healing of diabetic foot ulcers. In addition, we evaluated the potential of a novel healing indicator based on the behavior of macrophages as healing progresses.

Macrophages, the primary cells of the innate immune system, regulate all stages of the healing process by transitioning from the M1 to the M2 phenotype over time as healing progresses (Mirza and Koh 2011; Spiller et al. 2014). The M1 phenotype is associated with early processes in wound healing, such as the initiation of angiogenesis, whereas the M2 phenotype is associated with late processes in wound healing, such as blood vessel stabilization (Spiller and Koh 2017; Spiller et al. 2014). The M1-to-M2 transition is known to be defective in chronic wounds, characterized by prolonged M1 activation, leading to impaired healing (Mirza and Koh 2011; Mirza et al. 2013). Previously, we reported that expression of individual genes associated with the M1 and M2 phenotypes did not change appreciably over time in human diabetic ulcers; however, processing the data into a novel ratio of four M1-associated genes (VEGF, CCR7, CD80 and IL1β) to three M2-associated genes (PDGFβ, TIMP3 and MRC1) accurately predicted healing or non-healing in all 10 patients in the pilot study. More specifically, the M1/M2 score decreased for healing patients and increased for non-healing patients (Nassiri et al. 2015). By processing the gene expression data into the M1/M2 score, the relative levels of M1 and M2 gene expression were magnified and the data were normalized such that they were not affected by the size of the sample. It is important, however, to note, that although the selected genes are associated with macrophage phenotype, they are not specific to macrophages and may be expressed at different levels by other cells present in the wound tissue, especially fibroblasts, keratinocytes and endothelial cells. Thus, the M1/M2 score can be considered an indicator of wound inflammation, as opposed to macrophage phenotype *per se* (Nassiri et al. 2015).

In this study, we treated diabetic foot ulcers with the same LFLI-US treatment parameters that were successful in our pilot study of venous ulcers (Samuels et al. 2013). In addition, we analyzed changes in inflammation *via* the M1/M2 score in tissue samples debrided from LFLI-treated and sham-treated wounds to assess the potential of the novel M1/M2 score as a healing indicator when used with advanced wound care treatments such as LFLI-US.

METHODS

Patient enrollment and ethical considerations

The study was conducted in accordance with the ethical guidelines set forth by the 1975 Declaration of Helsinki and in compliance with the study protocol approved by Drexel University institutional review board. All patients were recruited from the Drexel University Wound Healing Center and had at least one diabetic ulcer that had not healed for 2 mo at the time of enrollment. Patients who exhibited signs and symptoms of untreated vascular disease or invasive infection such as cellulitis or abscess in the tissue with systemic manifestation were excluded from the study. The study was double blind (neither the patient nor the physician knew whether the patient was being given the treatment or sham, although the assisting researcher did know; an expanded double-blind, National Institutes of Health-sponsored, randomized controlled study is currently underway; see Acknowledgments). After providing informed consent, the patients were randomly assigned to the LFLI-US treatment or sham-treatment group. The sham group was treated in the exact same way as the ultrasound-treated group, except that the device was not activated. During the study, all participants in both treatment groups underwent standard ulcer care procedures determined by the attending physician, including weekly or biweekly wound debridement, prescribed topical dressings to maintain a moist wound environment and offloading. Patients whose wounds fully closed within 12 wk of study enrollment were considered healing, whereas patients whose wounds did not heal within 12 wk of study enrollment were considered non-healing