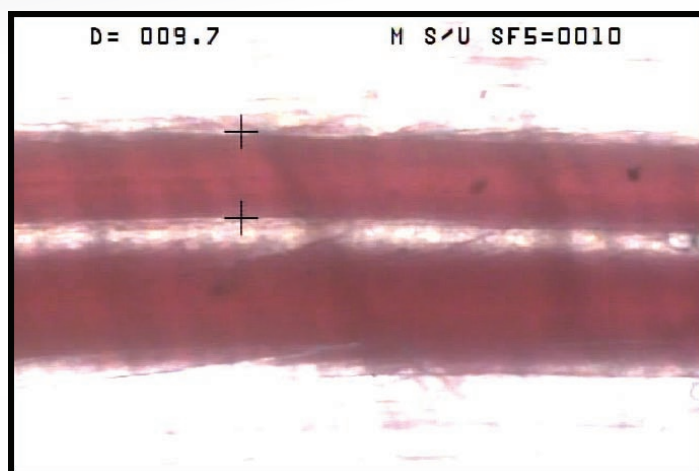


Pulsed Acoustic Cellular Expression (PACE™) Technology Supports Chronic Wound Healing



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Background

Patients with diabetes are susceptible to co-morbidities including chronic foot ulcerations, known as a diabetic foot ulcer (DFU), due to the affects of the diabetic's systemic disease. Diabetes not only renders the patient susceptible to sustaining a wound, but the disease can halt the wound healing process causing a chronic wound condition. Pulsed Acoustic Cellular Expression (PACE™) is a new modality currently being evaluated for use in treating DFU in a multi-center, randomized clinical trial under the guidelines of an FDA approved Investigation Device exemption (IDE) protocol, utilizing the dermaPACE® device (SANUWAVE, Alpharetta, GA). This new modality is based on extracorporeal shock wave technology (ESWT), first introduced to medical practice over 30 years ago for use in lithotripsy. Research has shown that ESWT also has bone and soft tissue healing effects.^{1,2} A shock wave is characterized by an extremely rapid increase in pressure. In the dermaPACE device this impulse is created by the formation and collapse of a vapor bubble following the discharge of a high voltage spark in a fluid medium, somewhat similar to the effect of lightning and its resultant thunder. Reflecting these impulse waves with a semi-ellipsoid reflector allows the energy generated to be focused to a target tissue site. Pulsed Acoustic Cellular Expression (PACE) Technology is a specific set of specific shock wave variables and a unique proprietary protocol utilizing shock wave impulses to achieve healing. This study investigated the affect that PACE has on microcirculation, immune system and inflammatory response and angiogenic growth factor up-regulation.

Introduction

Trauma caused by mechanical, surgical, biological, or chemical means initiates the ulceration of the foot in a diabetic patient. In addition, specific diabetic complications such as diabetic neuropathy, ischemia or peripheral vascular disease (PVD), and immune deficiency may contribute to and perpetuate the ulceration. The wound healing cascade that begins in response to ulceration is a complex synchronization of molecular events that typically initiates and completes wound closure, however, many diabetic patients are unable to heal with the current standard of care resulting in only 30-45% of wounds achieve closure using current standard of care.³

Diabetic Complications and Foot Ulcers

The coordinated recruitment of multiple cellular and molecular events must occur to initiate the growth factor-mediated extracellular matrix (ECM) homeostasis of wound healing. Healing stages include inflammation, mitosis, angiogenesis, synthesis, contraction, and ECM remodeling.⁴ Non-healing ulcers occur when this process is interrupted or out of sequence, often the case in diabetics with the complications described above. At a molecular level, chronic healing failure may result either from deficient supply or functional inhibition of growth factors.⁵ Growth factor deficiency has been shown to be due to increased protease levels that degrade growth factors and ECM components at the wound site.⁴ Fibroblasts can become senescent, due to damaged DNA, and will not divide. Increasing growth factor levels, inhibiting protease activity, or recreating conditions of initial wound response to trauma will be necessary to activate healing in a persistent diabetic foot ulcer.

Methodology: PACE Mechanism of Action Model

The PACE mechanism of action was investigated during this study using a rat cremaster model (Figure 1). Previous experimental work on microcirculatory responses in a cremaster model in rats has been proven to be a well established study tool for the microcirculatory system and leukocyte-endothelial interactions.^{6,7,8,9} Ischemia was induced in the model to simulate a chronic wound condition. While the rat was under anesthesia, the cremaster flap was isolated and mounted to a Plexiglas tissue bath, irrigated with Ringers and covered with an impermeable plastic film to allow for in vivo measurements. This model was used to establish the mechanisms of PACE by monitoring microcirculatory hemodynamics, leukocyte-endothelial interactions and the production of pro-angiogenic growth factors. The same processes that are affected in chronic wound healing conditions as described above.

Following cremaster muscle dissection, the Lewis rats were divided into 2 groups.

- 1) 5 hour ischemia control
- 2) PACE after 5 hour ischemia

All PACE procedures were conducted using a prototype dermaPACE® device at the E2 setting. Doses of 500 impulses were used.

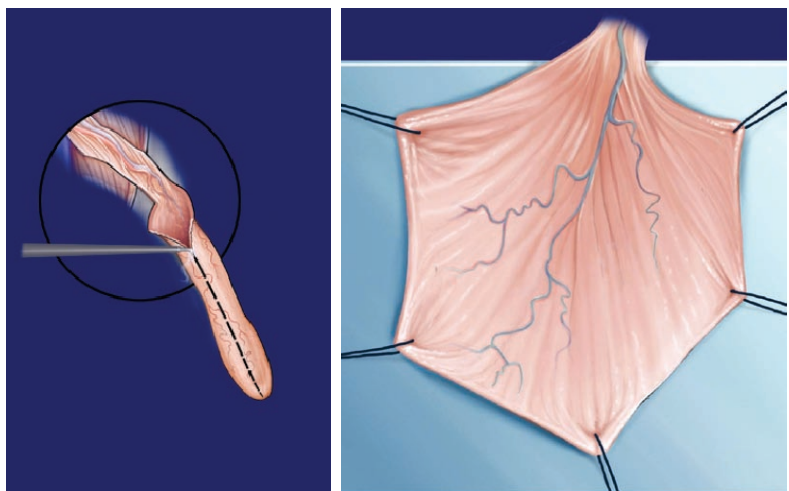
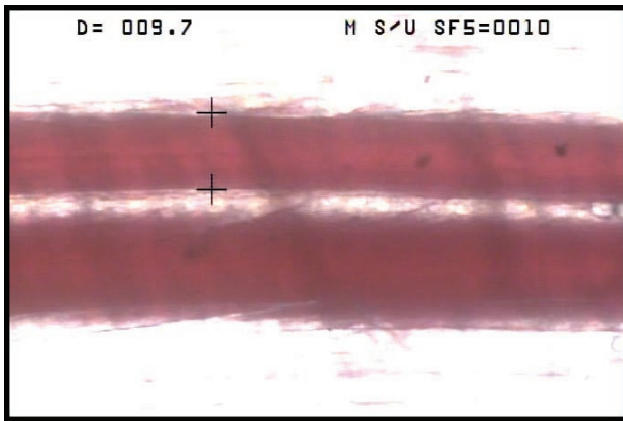


Figure 1. Cremaster dissection in a rat microcirculation model

Microcirculatory Reaction to PACE Treatment

In vivo microcirculatory recordings were made using an intravital microscope (Nikon Optiphot-2, Japan) equipped with an optical Doppler flow velocimeter (customer made, Texas A&M, College Station, TX) a 19-inch monitor (Sony Trinitron, Japan) and a color digital camera (Carl Zeiss AxioCam MR and Carl Zeiss AxioVision Rel.4.6, Carl Zeiss, Goettingen, Germany). Final magnification on the monitor was 1800x (Figure 2).

The microcirculatory analysis determined that there was an immediate acute microcirculatory response to PACE treatment. Vessel diameters of the first and second order arteriole increased by 14% after PACE treatment (103.9um and 80.04um respectively) compared to the control group (91.48um and 70.29 respectively) ($p < 0.05$). Red blood cell velocity was observed to increase in response to PACE



treatment although the difference was not statistically significant. The average functional capillary density was also measured. The group treated with PACE after 5 hours of ischemia had a higher capillary density when compared to the ischemic control group. The increase in vessel diameter, red blood cell velocity and average functional capillary density indicate a marked improvement in vascularization as a result of PACE treatment. This observation is in agreement with other studies that have investigated the vascular component of the shock wave mechanism of action.

Figure 2. Microcirculation Recording

Immune System and Inflammatory Response to PACE Treatment

Leukocyte-endothelial interaction assessment also revealed a reaction to PACE treatment. The numbers of rolling and sticking leukocytes were elevated in the ischemic control group but decreased in the PACE treatment group. However, the number of transmigrating leukocytes were slightly elevated in the PACE treatment group. The transmigrating leukocyte increase assists in the inflammatory phase of wound healing by triggering vessel endothelial cells immune response and initiating proangiogenic factor production.

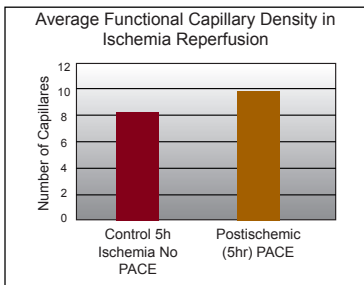


Figure 3. Average Functional Capillary Density

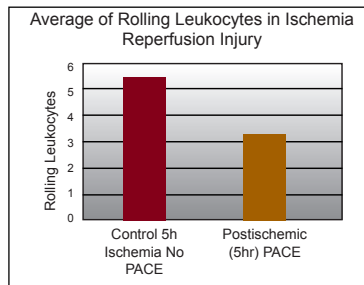


Figure 4. Average of Rolling Leukocytes

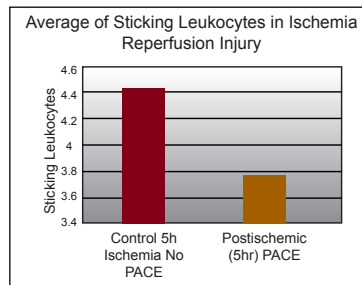


Figure 5. Average of Sticking Leukocytes

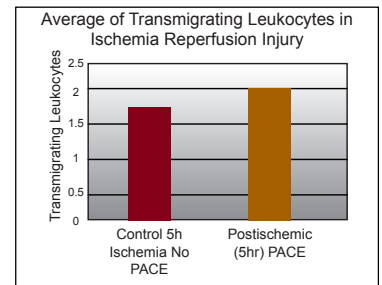


Figure 6. Average of Transmigrating Leukocytes

Growth Factor Production

At the end of the experiment animals were euthanized, and the cremaster muscle flaps were harvested and fixed in 10% formalin for standard hematoxylin and eosin (H+E) staining. Histology was conducted to determine if endothelial cells were producing vascular endothelial growth factor (VEGF) and von Willebrand factor (vWF). These proangiogenic growth factors have been described in multiple studies to be evidence of neovascularization. Compared to control ischemic groups, post-ischemic PACE treatment induced expression of the pro-angiogenic proteins (VEGF, vWF). These proangiogenic factors were expressed at the gene level and finally at a protein level indicating cellular responses to the PACE treatment. The expression of VEGF and vWF up-regulation leads to increase cellular proliferation, neovascularization and tissue regeneration.

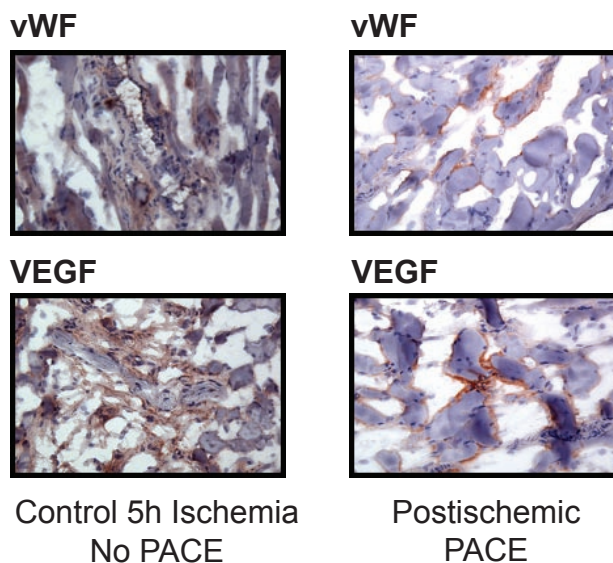


Figure 7. Histological examination of ischemic groups (control and PACE treatment)

Conclusions

The results of this study indicate that PACE Technology has a direct effect on microcirculation, leukocyte activity (immune and inflammatory response) and tissue regeneration through pro-angiogenic growth factors. These effects would induce a favorable wound healing environment for a patient with a chronic wound, such as a DFU. The increase in vessel diameters, red blood cell velocity and functional capillary density will provide a better blood supply to the chronic wound tissues including better tissue oxygenation and nutrient flow. Activation of the leukocyte-endothelial interactions after PACE treatment indicates an increased presence of transmigrating leukocytes into the surrounding chronic wound tissues. This is evidence of an immune system response in reaction to PACE treatment. Leukocyte involvement would enable a chronic wound to reinitiate acute healing stages. Finally, the upregulation of VEGF and vWF expression in the endothelial cells after PACE treatment represents a critical step in tissue regeneration. In this study it was confirmed that PACE increased synergistic expression of VEGF and vWF locally on the endothelial cells of established blood vessels and this may suggest potential for angiogenesis. New vascularization pathways may indicative of not only initial healing but of long-term sustainability of the tissues after healing is complete or a 'curing' of the conditions which led to the formation and perseverance of the chronic wound. In conclusion, the PACE Technology mechanism of action is a multi-stage, multi-system coordination of processes that all work together toward the creation of a wound healing environment.

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