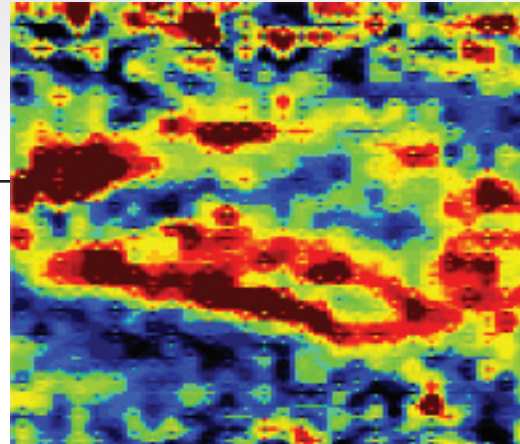
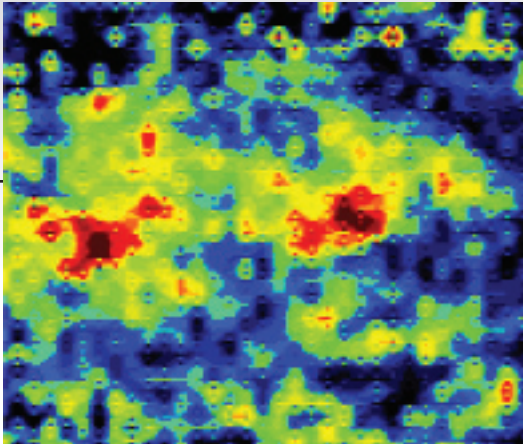


## Experimental Review of the Mechanisms of Action for Pulsed Acoustic Cellular Expression (PACE™) Technology



Before and after effects of PACE™ treatment on perfusion demonstrated through Doppler imaging.

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Investigational Device. Not for Sale in the U.S.

## Introduction

### Introduction

Extracorporeal Shock Wave Technology (ESWT) has been utilized medically for lithotripsy and orthopaedic indications for over 30 years prior to wound care indications. Clinical publications have also reported the initiation and acceleration of wound healing in burns,<sup>1</sup> traumatic wounds and reconstructive skin flaps,<sup>2</sup> as well as diabetic wounds.<sup>3</sup>

Different mechanisms of action have been researched to understand how shock wave interacts with the body during different applications. Lithotripsy, a procedure used for non-invasive fragmentation of kidney stones, utilizes shock wave pressure pulses that result in direct mechanical forces, as well as the formation and collapse of cavitation bubbles surrounding the stone to break the stones into smaller pieces which can then easily pass through the ureters.<sup>4</sup> The mechanism of shock wave in lithotripsy is mechanical in nature and the results, disintegration of the stone, are immediate.

The mechanism of action utilized by ESWT in the promotion of orthopaedic healing is considered to be largely vascular in nature. Shock waves cause mechanical stresses at a cellular level inducing an inflammatory response that promotes blood flow into the treated area. This immediate reaction is followed by a longer term healing response that includes increased capillary formation (neovascularization) resulting in increased perfusion and tissue regeneration that can take weeks or months to have full effect.<sup>5,6</sup>

PACE Technology is a unique proprietary protocol utilizing shock wave impulses to achieve healing. The dermaPACE is the first device to be developed based on the PACE Technology platform. It has been engineered to provide an effective surface treatment allowing focused acoustic pressure waves to directly treat the wound bed and peri-wound tissues. A dermaPACE Applicator gently glides over the wound surface as the acoustic pressure impulse is applied into the tissue surface by direct contact through sterile conductive gel. These acoustic pressure waves cause stresses within the wound area that result in a biological response. The mechanism of action that PACE utilizes to promote chronic wound healing is thought to have vascular, inflammatory, antibacterial, and tissue regeneration components. This study was designed to explore each of these mechanisms in human patients with chronic ulcerations through the analysis of clinical results as well as histomorphological, immunohistochemical, bacteriological and blood flow visualization methodologies.

### Protocol

Patients received a total of 4 to 6 PACE treatments using a dermaPACE device. PACE treatments were conducted at the E2 setting using 500 impulses over 2 minutes.

## Mechanisms of PACE™ Treatment

### Profusion & Microcirculation

The local blood flow perfusion before and after PACE treatment was measured using the PeriScan PIM II Laser Doppler Perfusion Imager (Perimed AB, Stockholm, Sweden) with LDPIwin software Windows 95/98/2000. The treatment resulted in a statistically significant increase in perfusion from an average of 0.56±0.21 to 0.74±0.19 (p<0.001). Increased perfusion will have a direct effect on ischemic wound conditions that are causative or effect co-morbidities in chronic wound disease states. The increased perfusion response is an immediate inflammatory reaction to the high-energy pressure waves that put stress (mechanical forces) on the microcirculatory system. By elevating the perfusion acutely in the wound area the ischemic component of the chronic wound disease state is decreased immediately encouraging a wound healing environment (Table 1).

	Before treatment	After treatment	P-value
<b>Mean ± SD</b>	<b>0.56±0.21</b>	<b>0.74±0.19</b>	<b>&lt;0.001</b>
<b>(Range)</b>	<b>(0.2 – 0.93)</b>	<b>(0.33 – 1.13)</b>	<b>N/A</b>

Table 1. Results of Doppler imaging on PACE™ and HBO patients.

### Effect on Bacteria

Bacterial analysis was conducted by determining the bacterial levels of each wound before and after treatment using a culture taken from the most contaminated portion of the wound. The bacterial scale was 0 for no growth, I slight growth, II moderate growth, III heavy growth, and IV for extremely heavy growth (Table 2). There was a significant decrease in bacteria counts after PACE treatment (p=0.018).

Bacteria Growth		0	I	II	III	IV	p-value
PACE™	<i>Before</i>	2	2	4	21	2	N/A
	<i>After</i>	12	0	1	18	0	0.018

Table 2. Bacterial results before and after advanced modality treatment.

## Histomorphological & Immunochemical Results

The cellular activity levels of all wounds were determined by obtaining a biopsy from the periphery of the ulcer. Hematoxylin-eosin (HE) staining was reviewed under 40x magnification to determine levels of viable cells within samples. After treatment the PACE group had statistically increased cellular activity and viability levels directly attributable to the dermaPACE application (Figure 1).

Levels of other histomorphological and immunochemical markers, eNOS, VEGF, PCNA and TUNEL (apoptosis indicator), were measured before and after the advanced modality treatments (Table 3). VEGF, eNOS, and PCNA significantly increased from baseline measurement following PACE treatment (Figures 2-4). These growth factors and cytokines have essential roles in a wound healing environment. An increase in VEGF is an indication of increased vascular permeability and microvascular activity including angiogenic growth of new blood vessels. eNOS, an immune system transmitter and vasodilator is also associated with wound closure. An increase in PCNA, a precursor to DNA synthesis and repair, indicates an elevation in cellular proliferation assisting wound healing. TUNEL technique detects the rate of cellular apoptosis, or cell death. Apoptosis is increased in chronic wound beds and necrotic tissues. TUNEL technique showed a decrease in apoptosis after PACE treatment (Figure 5) indicating an increase in cellular viability and decrease in the rate of cell death.

Mean±SD (Range)	Before treatment	After treatment	P-value
<b>eNOS</b>			
dermaPACE	22.53±12.42 (8-50)	55.12±20.74 (11-80)	<0.001
<b>VEGF</b>			
dermaPACE	37.77±7.58 (32-59)	66.63±13.52 (40-98)	<0.001
<b>PCNA</b>			
dermaPACE	27.21±12.68 (10-45)	59.63±20.7 (27-98)	<0.001
<b>TUNEL</b>			
dermaPACE	65.9±21.25 (22-96)	28.64±17.06 (10-64)	<0.001

Table 3. Histomorphological analysis of wound healing markers in PACE treatment groups.

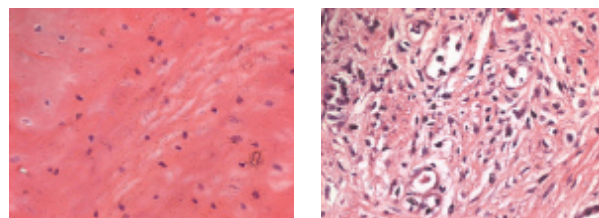


Figure 1. Before and after histology (Hematoxylin-Eosin stain x40)

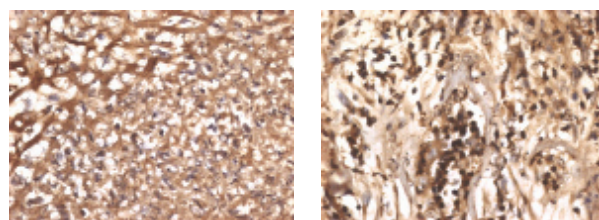


Figure 2. Before and after (VEGF: Vessel endothelial growth factor)

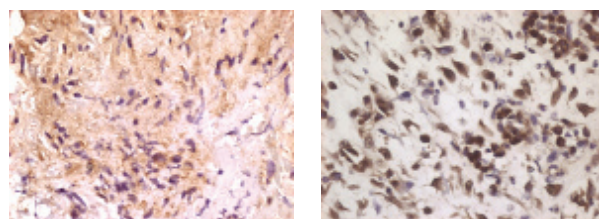


Figure 3. Before and after histology (eNOS: Endothelial nitric oxide synthase)

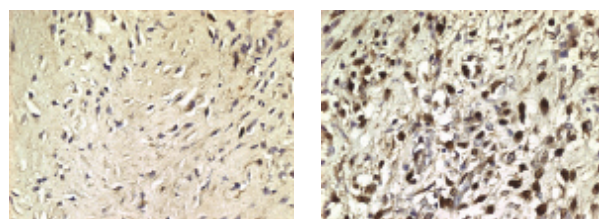


Figure 4. Before and after (PCNA: proliferation cell nuclear antigen)

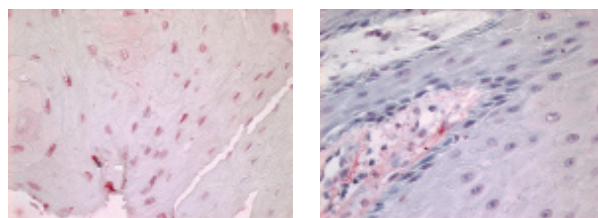


Figure 5. Before and after (TUNEL: transference-mediated digoxigenin-deoxy-UTP nick end labeling)

## Discussion & Conclusion

The coordination and 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.<sup>7</sup> Non-healing wounds occur when this process is interrupted or out of sequence, often the case with diabetes, PVD, infection, etc. At a molecular level, chronic healing failure may result either from deficient supply or functional inhibition of growth factors such as those investigated during this study.<sup>8</sup>

Growth factor deficiency may result from decreased expression due to low metabolic activity in cells or from increased protease levels causing degradation of growth factors and ECM components at the wound site.<sup>4</sup> Fibroblasts can become quiescent, due to lack of nutrients or oxygen or 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 chronic wound.

Shock waves, such as those used in PACE Technology, have been shown to cause mechanical forces within tissues that initiate a biological response at a cellular level producing angiogenic growth factors, including eNOS, VEGF, and PCNA, which are known to be present during normal wound healing.<sup>9</sup> These observations were supported during this study. Further this study demonstrated that PACE treatment also effects healing by acutely elevating perfusion and decreasing bacteria counts.

Understanding the underlying mechanisms of wound healing that are initialized and propagated by PACE Technology is essential to optimizing the clinical outcomes of this advanced modality. The stresses that are applied to the tissues by the high-energy acoustic pressure waves cause an apparent biological response in cells within and adjacent to the wound bed. The results of this study suggest that PACE Technology has the ability to improve the wound environment by increasing perfusion, decreasing bacterial count, normalizing the rate of apoptosis, and making positive changes to growth factor and cytokine levels.

This study provides evidence of the mechanism of action initiated by PACE Technology and supports the use of this advanced modality in wound healing treatment.

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