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Preliminary Report

The Use of a Proprietary Near-Infrared Laser to Enhance Wound Healing: A Preliminary Preclinical and Clinical Study

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Abstract

Background: Nonthermal light energy has been used to enhance wound healing. This is known as photobiomodulation. Although preclinical evidence is largely based on laser light, light-emitting diodes (LEDs) form the mainstay of clinical studies owing to the lack of available lasers for nonclinical use. However, it is speculated the 2 technologies exhibit dissimilar biological responses.

Objectives: The influence of a new, commercially available near-infrared laser device on the gene expression profile of human skin relative to an equivalent, near-infrared LED device was evaluated. Additionally, the wound healing potential of the device was examined in practice.

Methods: Defatted human skin was exposed to the laser (3), LED (3), or negative control (3) for 5 days. On Day 6, skin samples were biopsied for ribonucleic acid extraction and gene expression assays run for 107 genes of interest. Twenty patients with chronic wounds were randomized to receive standard wound care ± laser therapy 3 times weekly for 4 weeks, and wounds were analyzed for healing.

Results: The laser altered expression of 45 genes. Highly up-regulated genes (>5-fold change) included those implicated in wound healing and antiaging, whereas highly down-regulated genes included those implicated in inflammation and extracellular matrix integrity. The LED device altered expression of only 1 gene relative to negative controls. The laser reduced mean wound area by 78% and healed 4 of 10 wounds completely. In contrast, 8 of 10 of those receiving standard care exhibited no change.

Conclusions: A proprietary near-infrared laser exhibited superior ability to influence gene expression in healthy skin than an equivalent LED device and induced the healing of chronic wounds.

Level of Evidence: 2 (Therapeutic)

Photobiomodulation (PBM) is a phenomenon characterized by nonthermal epigenetic manipulation of the target cell.[1] This is achieved by inducing the cell to a high-energy state through the absorption of red and near infrared (NIR) light energy by cytochrome c oxidase of the oxygen transport chain and the subsequent enhancement of ATP synthesis in cellular mitochondria[2-5] and mitochondrial signal transduction.[6] Modulation of the intracellular stress response by metabolism of reactive oxygen species is also believed to play a role.[5,7]

The phenomenon was first observed by Mester et al at Semmelweis University, Hungary, in the 1960s.[8] Subsequently, the ability of red laser light to enhance wound healing in a rat model was observed.[9] It was many years, however, before the mechanisms explaining this phenomenon were unraveled and work continues refining the theory to this day.[10] Presently, the ability of low level red and NIR laser therapy (sometimes known as LLLT) to enhance wound healing in experimental models is well established.[11] However, even though much of the preclinical evidence for PBM is based on laser light, light-emitting

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diode (LED)-based light forms the mainstay of clinical studies owing to the lack of commercially available lasers for use in nonclinical settings.[12] LED technology was developed around the same time as the discovery of PBM and served as substitute for laser light in clinical studies as the technology could produce quasi-monochromatic light cheaply, safely and over a wide surface area simultaneously.[13] The theoretical basis for substituting laser light with LED was the view that because cytochrome *c* oxidase was a chromophore for red/NIR light, then by inference the key determinant of the efficacy in PBM therapy was the wavelength of the light source.[14] The reality is more complex. Monochromaticity is only one of several unique features of electromagnetic waves produced by lasers. In addition to wavelength, power, irradiance (power density), and pulse structure are variables shared between laser and LED light, whereas narrow spectral distribution, collimation, temporal and spatial coherence, and polarization are features unique to laser light. When electromagnetic waves travel through a medium, they encounter molecules and they can be reflected, transmitted, refracted, absorbed, diffracted, or scattered.[15] It has been argued that some properties unique to laser light contribute to the photobiomodulatory response in target tissue, either directly because of the effect of absorption of electromagnetic energy on the target or indirectly, because of different properties of reflection, transmission, refraction, diffraction, and scatter.[16,17] This debate continues. What is clear is that in the PBM literature, there remains a dichotomy, with preclinical evidence of enhanced wound healing generated mainly using red and NIR laser light, and clinical studies of aesthetic skin rejuvenation and the treatment of radiotherapy-induced oral mucositis generated mainly using red and NIR LED.[11,12] With the recent development of a proprietary, safe NIR laser for nonclinical use, there is now an urgent, unmet need to establish the clinical role of lasers in PBM and to examine the relative efficacy compared with commercially available LED devices.

A proprietary NIR laser has been developed for use in both nonclinical and clinic settings (LYMA Life Ltd, London, United Kingdom). This device delivers light at 808 nm and 500 mW (a 1500 mW version is also available) and has been rendered safe by utilizing the scatter phenomenon to diffuse the beam without altering coherence. The purpose of this study was to evaluate the effect of this NIR laser device on human skin by evaluating gene expression profile relative to a leading commercially available LED device and to bridge the evidence gap between the theoretical and clinical applications of this technology by examining the wound-healing potential of the device in practice.

METHODS

Preclinical Study

Discarded postabdominoplasty human skin from a consented 43-year-old female (Fitzpatrick Type III) underwent excision of the subcutaneous fat within 4 h of surgery. The full thickness skin was excised to nine 4 × 4 cm^2 samples and transferred to individual petri dishes containing 10 mL cornification medium with antibiotics but without steroids. Tissues were incubated at 37 °C in 5% CO_2, 95% humidity. At time zero, at 24 h and each 24 h thereafter to Day 5, the samples were washed in phosphate-buffered saline, dried, then exposed to the following protocol:

1. NIR LED (810 ± 3 nm, 5 W [approximate], exposure time 180 s, approximate total energy 56 J/cm^2) × 3
2. NIR laser (808 nm, 500 mW, exposure time 180 s, approximate total energy 5.6 J/cm^2) × 3
3. Negative control (laser not switched on) × 3

The relative properties of the laser and LED light sources used in this study are compared in Table 1. Because the laser is recommended for use with a proprietary skincare formulation (LYMA Life Ltd), Cohort 2 was preprepared using this formulation. After each exposure, the incubating medium was changed, and the samples returned to the incubator. On Day 6, a 10 mm punch biopsy was harvested from the center of the skin sample and stored at −80 °C until ribonucleic acid (RNA) extraction was performed. All RNA extractions were undertaken using a RNeasy Mini Kit (Qiagen Diagnostics GmbH, Hilden, Germany) following the manufacturer's instructions. Briefly, RNA concentration and purity were determined using a nanodrop 2000 spectrophotometer confirming sample purity (Supplemental Table 1). Copy deoxyribonucleic acid (cDNA) was generated from 100 ng of RNA using a superscript Vilo RT kit and a custom primer pool (Thermo Fisher Scientific Inc., Waltham, MA) as per manufacturer's instructions. cDNA samples were preamplified for 12 cycles and diluted 1:20 with 1×TE buffer for quantitative polymerase chain reaction (qPCR) processing. qPCR was run in the Open Assay format using validated Taqman gene assays in a Life Technologies QuantStudio 12K flex instrument (Thermo Fisher). Each gene was assayed in duplicate. Endogenous control genes selected included glyceraldehyde-3-phosphate dehydrogenase, β-glucuronidase, hypoxanthine phosphoribosyltransferase-1, peptidylprolyl isomerase-A (PPIA), and ubiquitin-C. Based on the stability scores, PPIA was chosen as the control gene (Supplemental Table 2). Data quality and statistical analyses were performed using Thermo Fisher Connect software. Microsoft Excel (Microsoft Corporation Inc., Redmond, WA) was used to analyze these data. Unpaired *t*-tests were performed (relative to PPIA) to establish *P*-values, whereas linear respiratory quotient values and linear fold change values were also calculated. Additionally, 3 genes on the panel showed poor quality amplification (tropoelastin, interleukin [IL]-23A, and colony stimulating factor-2), and these were excluded from further analyses. A 1.5 times fold-change in gene expression was considered significant as this is the industry standard.

Clinical Study

Participants

The study was conducted at the Hungarian Military Hospital, Budapest, Hungary, European Union. For this Phase 1 clinical trial, a total of 20 consecutive patients with nonhealing wounds were recruited over a period of 6 months between June and December 2023. A nonhealing wound was defined as a wound which had failed to exhibit a predictable pattern of healing within an acceptable time frame and had thus become chronic. All wounds were at least 6 weeks old. Nonhealing wounds included pressure ulcers, diabetic foot ulcers, and stumps following amputation for peripheral vascular disease. All co-morbidities were permissible. There were no exclusions. Patients were randomized (by simple coin toss) to receive standard wound care by way of 3 times weekly dressings alone (Cohort 1) or standard wound care by way of 3 times weekly dressings with NIR laser therapy for 3 min at each dressing change (for a total of 12 NIR laser exposures). At each dressing change, the wounds were gently cleaned with saline, evaluated by way of measurements of the dimensions of the wound as well as assessments of the clinical features of inflammation at the wound edge and wound depth, then dressed with a paraffin-impregnated nonadherent mesh dressing or silicone mesh dressing, followed by dressing gauze and crepe or an adherent

Table 1. A Comparison of the Properties of Laser vs LED Light Used in the Study

	Laser	LED	Implication
Power (mW)	500	5000	Laser requires less power than LED as energy transfer is more efficient
Fluence (energy density J/cm^2)	5.6	56	In line with clinical recommendations
Wavelength	808 nm	810 ± 3 nm	Chromophore is cytochrome *c* oxidase
Collimation	Yes	No	Safety diffuser in proprietary laser device de-collimates laser beam rendering it safe
Coherence (temporal)	Yes	No	Laser: *constructive* interference amplifies the energy carried by the wave LED: *destructive* interference diminishes the energy carried by the wave
Coherence (spatial)	Yes	No	Laser: retention of potential for *constructive* interference as light passes through reflective medium (speckle phenomenon) LED: no potential for *constructive* interference as light passes through reflective medium (no speckle phenomenon)
Directionality	Unidirectional	Multidirectional	Energy transfer more focused, where light energy is unidirectional
Polarization	Yes	No	Polarization may be significant in photobiomodulation but clinical implications uncertain at present

LED, light-emitting diode.

Table 2. Gene Expression Profile Changes Following Skin Sample Exposure to Near-Infrared Laser (808 nm) vs Near-Infrared (810 ± 3 nm) LED

Gene	Function	LED		Laser	
		FC	%C	FC	%C
TXNRD1	Antioxidant/stress response	n.s.	n.s.	32.33	3133[a]
HBEGF	Growth factor/wound healing	n.s.	n.s.	31.63[a]	3063[a]
AHR	Antioxidant/stress response	n.s.	n.s.	6.17[a]	517[a]
SIRT1	Antiaging	n.s.	n.s.	5.97[a]	497[a]
VEGFA	Growth factor/wound healing	n.s.	n.s.	5.46[a]	445[a]
ITGB1	Epidermal barrier	n.s.	n.s.	5.07[a]	407[a]
PCNA	Cell replication	n.s.	n.s.	5.02[a]	402[a]
PANK4	Antiaging	n.s.	n.s.	4.97	397
KITLG	Growth factor/cell migration	n.s.	n.s.	4.42	342
HSPG2	Antiaging	n.s.	n.s.	3.95	295
OCLN	Epidermal barrier	n.s.	n.s.	3.54	254
MMP-1	Extracellular matrix breakdown	n.s.	n.s.	3.45	245
TXN	Antioxidant/stress response	n.s.	n.s.	3.45	245
ADAM17	Immune modulation	n.s.	n.s.	3.33	233
POLG1/MDP1	Antiaging	n.s.	n.s.	2.55	155
GSK3B	Cell replication	n.s.	n.s.	2.46	146
NFE2L2	Antioxidant/stress response	n.s.	n.s.	2.27	127
DSG3	Stability of dermoepidermal junction	1.53	53	2.00	100
KRT14	Keratinocyte stability	n.s.	n.s.	1.81	81

Table 2. Continued

Gene	Function	LED		Laser	
		FC	%C	FC	%C
MFN2	Mitochondrial function	n.s.	n.s.	1.5	50
TNF	Proinflammatory cytokine	−1.36	−26	n.s.	n.s.
SERPINH1	Fibrosis/scarring	n.s.	n.s.	−1.63	−39
IL-1B	Proinflammatory cytokine	n.s.	n.s.	−1.79	−44
COL17A1	Extracellular matrix integrity	n.s.	n.s.	−2.04	−51
COL7A1	Extracellular matrix integrity	n.s.	n.s.	−2.68	−63
PTGS2/COX-2	Inflammation	n.s.	n.s.	−2.76	−64
ICAM1	Immune modulation	n.s.	n.s.	−3.02	67
NMRK1	Antiaging	n.s.	n.s.	−3.62	−72
SOD2	Antioxidant/stress response	n.s.	n.s.	−3.61	−72
HAS2	Glycosaminoglycans of extracellular matrix	n.s.	n.s.	−3.82	−74
COL3A1	Extracellular matrix integrity	n.s.	n.s.	−4.61	−78
MMP-2	Extracellular matrix breakdown	n.s.	n.s.	−4.85	−79
PKP1	Intracellular function	n.s.	n.s.	−4.72	−79
KRT5	Epidermal keratin	n.s.	n.s.	−5.32[a]	−81[a]
PTGS1/COX-1	Inflammation	n.s.	n.s.	−5.41[a]	−82[a]
SPINK5	Extracellular matrix breakdown	n.s.	n.s.	−6.76[a]	−85[a]
FBN1	Extracellular matrix integrity	n.s.	n.s.	−7.75[a]	−87[a]
IL-6	Proinflammatory cytokine	n.s.	n.s.	−8.00[a]	−88[a]
COL4A2	Extracellular matrix integrity	n.s.	n.s.	−8.93[a]	−89[a]
TNC	Extracellular matrix integrity	n.s.	n.s.	−10.42[a]	−90[a]
VCAN	Extracellular matrix integrity	n.s.	n.s.	−10.00[a]	−90[a]
SERPINB3	Extracellular matrix breakdown	n.s.	n.s.	−13.51[a]	−93[a]
KRT10	Epidermal barrier	n.s.	n.s.	−25.00[a]	−96[a]
DSC1	Extracellular matrix integrity	n.s.	n.s.	−33.33[a]	−97[a]
FN1	Extracellular matrix integrity	n.s.	n.s.	−28.57[a]	−97[a]
KRT1	Epidermal barrier	n.s.	n.s.	−52.63[a]	−98[a]

The results are stratified based on the fold change as follows: 1.5 to 3.0: up-regulated; 3.0 to 5.0: highly up-regulated; >5.0: very highly up-regulated; −1.5 to 3.0: down-regulated; −3.0 to 5.0 highly down-regulated; >5.0: very highly down-regulated. %C, percentage change; FC, fold change; IL, interleukin; LED, light-emitting diode; n.s., not significant; TNF, tumor necrosis factor.

[a]Highly unregulated or highly down regulated.

dressing with absorbable pad as appropriate given the site and dimensions of the wound. The single assessor was blinded as to the treatment cohort. Any safety issues or side effects were noted as per Phase 1 trial methodology. The trial duration was 4 weeks, based on an a priori assessment of the likelihood of observing a difference in the cohorts. Beyond 4 weeks, we surmised that, as each patient may have been better served by further surgery (and thus dressings alone were not necessarily the standard of care), it may not have been appropriate to continue beyond this point.

Ethics

Institutional review board approval from obtained from the Budapest health science council on March 28, 2023, and was registered with

the National Center for Public Health and Pharmacy (Budapest, Hungary; no. OGYEI/8363/2020). All participants gave informed consent for recruitment to the trial.

RESULTS

The preclinical study revealed that exposure of the defatted skin samples to the NIR laser for 180 s/day over 5 consecutive days influenced the expression of 45 of 107 genes tested at Day 6 relative to the negative control samples. Twenty genes were up-regulated, and 25 genes were down-regulated. In contrast, when defatted skin samples were exposed to LED using the same protocol, the differential expression of only one gene relative to the negative control was noted. At this single time point, the magnitude of up-regulation noted ranged from 1.5 × baseline (a 50% change) to over 32 × baseline (over 3000% change). The maximum magnitude of down-regulation was over 50-fold (a reduction in over 98% of baseline). Largely, the genes up-regulated and highly up-regulated were genes encoding for proteins implicated in tissue growth and wound healing, cell replication, antiaging, antioxidation/stress response modulation, and strengthening of the epidermal barrier and dermoepidermal junction. In contrast, the genes down-regulated and highly down-regulated were genes encoding for proteins implicated in the proinflammatory response and extracellular matrix (ECM) maintenance and stability. The results are summarized in Table 2.

In the clinical study, 10 patients were randomized to receive standard wound care, and 10 patients were randomized to receive standard wound care and exposure and NIR laser therapy during dressing changes. The mean age of standard treatment cohort was 72.8 years (range, 57-93 years) and was 73.1 years (range, 54-89 years) for the laser cohort. The wound healing results are shown in Figure 1 and Table 3. In the cohort treated with the laser, there was a mean reduction in wound area of 78%, with 4 of 10 having completely healed by 4 weeks. The poorest response was seen in a patient with a lower extremity vascular ulcer who exhibited no inflammation, reduced wound depth and a 10% reduction in total wound area at 4 weeks. Seven of 10 wounds treated with the laser were inflammation-free at the cessation of study (4 weeks). Five of 10 were completely flat, and the remaining 5 exhibited reduced wound depth.

In contrast, only 1 of 10 wounds treated with standard dressings exhibited reduced inflammation and the wound depth remained unchanged in all 10 cases. There was no change in the wound area in 8 of the 10 wounds. In 1 case, wound area reduced by 30% at 4 weeks. This was also the single case that exhibited reduced inflammation at the wound edge. In 1 further case, the wound area increased by 20% over the 4 week study period. No adverse events were reported in either cohort during the study period.

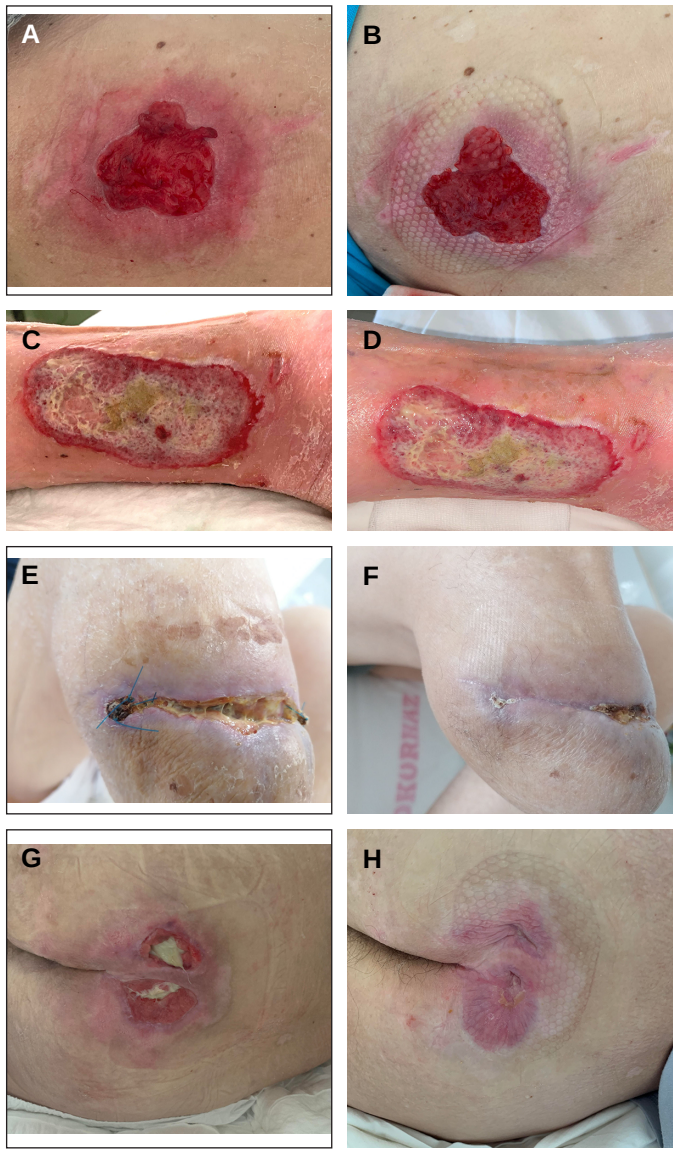

Figure 1. A comparison of wound healing in chronic wounds with and without exposure to NIR laser. Patient 2 (74-year-old female; trochanteric pressure sore) (A) before and (B) after 4 weeks of standard dressings only. Patient 9 (69-year-old male; lower extremity vascular ulcer) (C) before and (D) after 4 weeks of standard dressings only. Patient 11 (67-year-old male; below-knee amputation stump) (E) before and (F) after 4 weeks of standard dressings with NIR laser therapy at dressing changes. Patient 12 (71-year-old female; sacral pressure sore) (G) before and (H) after 4 weeks of standard dressings with NIR laser therapy at dressing changes. NIR, near infrared.

DISCUSSION

This study has demonstrated that, following 5 consecutive days of exposure to a proprietary safe NIR laser light, human skin exhibits differential gene expression. In the panel of 107 genes tested, 20 genes were up-regulated, and 25 genes were down-regulated. Genes up-regulated encode for proteins involved in tissue growth and wound healing, cell replication, antiaging, antioxidation/stress response modulation, and strengthening of the epidermal barrier and dermoepidermal junction, whereas genes down-regulated encode for proinflammatory cytokines and proteins involved in ECM maintenance and stability. The clinical implications of these findings were confirmed by a study of nonhealing wounds which suggested that exposure to the laser reduced inflammation and induced wound healing.

Other studies have observed that red and NIR laser light enhances cell proliferation and differentiation and down-regulates genes associated with apoptosis.[18-22] Modulation of redox-sensitive signal transduction pathways is believed to play a role in NIR laser-associated cell proliferation.[23] Thus, although the gene panel used in the current study was selected specifically to observe epigenetic associations

Table 3. Results From a Single Blinded, Randomized Controlled Preliminary (Phase 1) Trial of the Management of 20 Chronic Wounds Using Standard Dressings with or Without Exposure to NIR Laser 3 Times Weekly for 4 Weeks

Cohort	Patient	Wound	Wound edge inflammation	Wound depth	Wound dimensions before (mm)	Wound dimensions after (mm)	Wound area change (%)
Standard wound care	1	Decubitus ulcer (sacrum)	Inflamed	No change	50 × 30	50 × 30	0
	2	Trochanteric pressure sore	Reduced inflammation	No change	50 × 40	40 × 35	−30
	3	Lower extremity ulcer (vascular)	Inflamed	No change	40 × 40	45 × 43	+20
	4	Decubitus ulcer (ischium)	Inflamed	No change	45 × 35	45 × 35	0
	5	Lower extremity ulcer (vascular)	Inflamed	No change	63 × 35	63 × 35	0
	6	Lower extremity amputation stump (vascular)	Inflamed	No change	95 × 10	95 × 10	0
	7	Lower extremity ulcer (vascular)	Inflamed	No change	43 × 27	43 × 27	0
	8	Lower extremity ulcer (vascular)	Inflamed	No change	40 × 35	40 × 35	0
	9	Lower extremity ulcer (vascular)	Inflamed	No change	120 × 55	120 × 55	0
	10	Lower extremity ulcer (vascular)	Inflamed	No change	50 × 35	50 × 35	0
Standard wound care + laser	11	Lower extremity amputation stump (vascular)	No inflammation	Flat	85 × 12	34 × 3	−90
	12	Decubitus ulcer (sacrum)	No inflammation	Flat	95 × 65	0	−100 (healed)
	13	Lower extremity amputation stump (vascular)	Reduce inflammation	Reduced	53 × 37	38 × 26	−50
	14	Lower extremity amputation stump (vascular)	No inflammation	Reduced	74 × 14	51 × 2	−90
	15	Lower extremity amputation stump (vascular)	No inflammation	Flat	38 × 35	0	−100 (healed)
	16	Lower extremity ulcer (vascular)	No inflammation	Flat	43 × 32	0	−100 (healed)
	17	Lower extremity ulcer (vascular)	No inflammation	Reduced	75 × 14	51 × 2	−10
	18	Lower extremity ulcer (vascular)	Reduced inflammation	Reduced	68 × 45	47 × 32	−50
	19	Lower extremity ulcer (vascular)	Reduced inflammation	Reduced	40 × 40	14 × 12	−90
	20	Lower extremity ulcer (vascular)	No inflammation	Flat	48 × 30	0	−100 (healed)

NIR, near infrared.

with wound healing, this is only part of a much broader picture of laser light-induced epigenetic modulation.

The laser was generally anti-inflammatory, as evidenced by the down-regulation of expression of IL-1B, IL-6, COX-1, and COX-2. The anti-inflammatory effect of NIR laser light on stromal cells and macrophages in culture is already well established[24-28] and may also be altered by wave pulsatility which was not examined in this study.[29] It was therefore surprising that it appeared to have no effect on tumor necrosis factor alpha (TNF-α). The reason for this is probably on account of the single time point chosen for the analysis. Temporal expression profiles are nuanced, especially in relation to the inflammatory response. It has been demonstrated elsewhere that, unlike IL-1B and IL-6, TNF-α exhibits a biphasic response, initiating an inflammatory response early before receding to baseline

levels. It is probable that active TNF-α down-regulation had ceased by Day 6.[30-33]

The up-regulation of MMP-1 and down-regulation of MMP-2 is also an interesting finding. It is widely understood that changes in the relative balance of MMP-1 and MMP-2 are observed in different scar phenotypes, with a relative increase in MMP-1 observed in experimental scarless (and fetal) wound healing. Danno et al reported that a polychromatic light source within the NIR spectrum enhanced MMP-2 expression in cultured human fibroblasts over 48 h so, once again, temporal expression is an important variable to consider.[34] Many of the genes down-regulated and highly down-regulated encode for proteins involved in maintenance of the ECM. At first glance, this is curious and, perhaps, even puzzling until we appreciate the complex interplay between ECM turnover and tissue rejuvenation.[35]

The clinical study mirrors findings from some experimental models. A number of preclinical studies have reported enhanced wound healing when wounds were exposed to a single or intermittent therapeutic regimen of NIR laser light.[36-38] The influence of red and NIR laser light on collagens, vascular endothelial growth factor (VEGF), and alpha smooth muscle actin expression in fibroblasts and myofibroblasts are likely important here.[39-41] So too, is the role of reactive oxygen species, with some studies highlighting NIR laser-activation of redox-sensitive signaling pathways, thereby enhancing cell survival, migration, and proliferation.[23,42] Rarely, some experiments have not observed enhanced wound healing with a NIR laser when the dose regimen chosen was likely too low.[43] Others have posited that delivering NIR laser light in a femtosecond pulsatile structure may also contribute to wound healing independent of any other light feature.[44]

Inevitably, some studies have sought to compare NIR laser with LED light to establish whether the light source results in an observable difference in wound healing. The results are mixed. Keshri et al observed that both laser and LED (at 810 and 808 ± 3 nm, respectively) equally enhanced wound healing in an experimental burn wound.[45] Investigating human wound healing using a NIR laser has been performed rarely. In a study of 5 patients, Halevy et al observed that cutaneous wounds ("fissures") irradiated with a diode laser at 780 nm appeared to heal faster with less pain than control lesions on the same patient. The results were not statistically significant owing to the small numbers involved. Moreover, using nonirradiated wounds from the same patient as control assumes that the laser exerts only localized effects,[46] whereas some evidence supports the conclusion that the healing benefits of laser irradiation are systemic.[47] Carvalho et al demonstrated that NIR laser light can improve scar quality after hernia surgery.[48] Red laser light has also been shown to accelerate healing of oral mucosa.[49,50] The healing potential of NIR LED therapy has also been explored.[51]

There are limitations to both the preclinical and clinical arms of this preliminary study. The preclinical arm examined only one human skin sample. Repeating the protocol using several human skin samples from different donors would improve the validity and generalizability of the results. Moreover, the experiment was terminated at a single time point (Day 6) and thus the gene expression profile data tells us what was happening at Day 6 but does not yield important data about the temporal expression profiles of the relevant genes in question. The third important point is the laser and LED light-exposure protocols. It has already been established that, to a certain extent, PBM is dose dependent and thus a series of optimization experiments to investigate this would be desirable. This requires a great deal of time and resources, including many skin samples which is why the single protocol was designed to replicate the clinical application. For the same reason, the skin samples in the laser arm of the study were also exposed to the skincare products, as this most accurately replicates the clinical recommendations, albeit at the expense of introducing a potential confounding variable.

The limitations of the clinical study are 3-fold. Firstly, there is heterogeneity among the nonhealing wounds. Although all wounds exhibited, by definition, chronicity, they may have each exhibited different healing potential which was not captured by the investigative protocol. Secondly, the study is only single blinded, because the patients themselves were aware of which arm of the study they were randomized to. Although this was unlikely to make a difference, it would have been preferable, were it possible, to have conducted the study in a double-blinded fashion. Thirdly, as with the preclinical study, the exposure protocol remains underoptimized. To minimize the inconvenience to the patients, the decision was made to expose the wounds to the laser therapy during standard dressing changes, which were typically thrice weekly but, as is often the case in clinical practice, sometimes practical necessity requires a more flexible approach. To complicate the issue of protocol optimization, there is evidence to suggest that PBM exhibits a biphasic dose response (Arndt–Shultz curve) where supraoptimal exposure diminishes the positive biological effect.[52,53] This has been demonstrated in in vitro cell culture studies.[54] The optimal frequency and duration of exposure remains uncertain, and thus, there is valuable information to be gained by a much larger study where frequency and duration of exposure are variables investigated by the experimental algorithm.

Further research should focus on expanding the preclinical study to include as many human skin samples as possible, obtaining daily skin samples to record temporal expression profiles of the relevant genes, altering the light exposure protocols to establish the extent to which dosage influences gene expression and decoupling the skincare protocol from the light exposure to minimize confounding variables. Having demonstrated the safety and potential efficacy of the laser in a Phase 1 study of nonhealing wounds, a Phase 2 study is now justified, to confirm the efficacy and optimize the exposure protocol.

CONCLUSIONS

The proprietary NIR laser induced up-regulation of genes associated with antiaging, cell replication, wound healing, and the stress response, whereas down-regulating genes associated with inflammation and ECM integrity. This implicates the laser in tissue rejuvenation by manipulation of both the cellular component of skin and the ECM. The clinical trial confirms the efficacy of the laser as a means of enhancing wound healing, with no reported adverse effects. A further preclinical study is needed to elucidate temporal gene expression profiles, and a Phase 2 clinical trial is needed to optimize the clinical application of this emerging therapy.

Supplemental Material

This article contains supplemental material located online at https://doi.org/10.1093/asjof/ojaf009.

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Disclosures

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