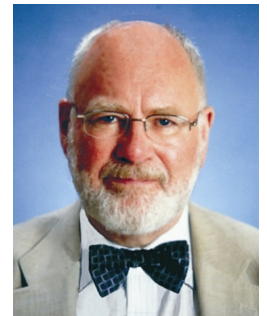


**HEALITE II With Photosequencing Technology:
A NOVEL PHOTOTHERAPEUTIC APPROACH INVOLV-
ING PHOTOSEQUENCING WITH 590 nm AND 830 nm LED
ENERGY: PRECONDITIONING 590 nm MICRO-LOW
LEVEL LIGHT THERAPY (μ -LLLT) IN COMBINATION
WITH 830 nm LED PHOTOTHERAPY**



R Glen Calderhead PhD, DrMedSci, FRSM

VP, Clinical Affairs, Lutronic Corporation, Goyang, Korea

1. OVERVIEW

The very high efficacy of phototherapy with low incident levels of 830 nm radiation in the near infrared waveband with appropriate intensities and doses has now been well-reported in a large variety of pan-specialty indications. Originally the domain of gallium aluminum arsenide (GaAlAs) laser diode-based systems, a new generation of 830 nm LEDs based on NASA Space Medicine technology has added LED phototherapy to the clinician's armamentarium. Although LEDs are not coherent and thus not monochromatic like laser diodes (LDs), they are quasimonochromatic with almost all of their photons at the rated wavelength. In addition LEDs have two main advantages over laser diodes: they can be mounted in large planar arrays thus enabling hands-free treatment of large areas of tissue; and they are much less expensive.

It may be possible to improve the already proven cellular, tissue and systemic response to 830 nm through a novel photosequencing technology in which another wavelength of LED energy is used to precondition the target cells before irradiation with the main 830 nm LED energy. This novel technology is discussed, which delivers sequential doses of very low intensity 590 nm light (visible yellow) in the microwatt ($\mu\text{W}/\text{cm}^2$) domain followed by appropriate clinically useful levels of near-infrared 830 nm light (in the milliwatt [mW/cm^2] range).

1.1. The Device

The HEALITE II system consists of planar LED-based arrays with two rows of double-wavelength 590/830 nm LEDs sandwiched between multiple rows of 830 nm LEDs per single planar array unit, 5 units making up the total planar array area [Figure 1]. When the device is activated, it first delivers 1s of only 590 nm energy at extremely low incident levels of photon intensity from both rows of 590 nm LEDs in each panel, working sequentially along the planar arrays from left to right, and returning to the first array. With 10 rows of yellow LEDs per total array area of 5 panels, 2 rows per panel, this takes 5 sec. This is repeated 12 times for a total of 1 minute. While the yellow photosequencing then continues, the 830 nm LEDs are then activated in continuous wave at the set intensity (20, 40, 60, 80 or 100 mW/cm^2) until the desired fluence, also called the energy density or dose, of 830 nm energy has been delivered.

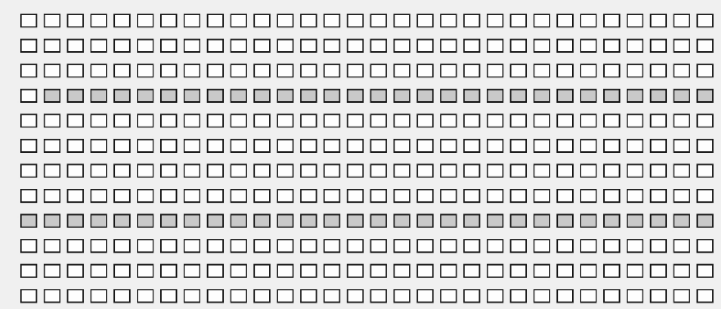
1.2. Potential Applications

The device is intended for multiple pan-specialty applications in the area of phototherapy. These include, but are not restricted to, the following:

- Wound healing of traumatic or iatrogenic wounds in both hard and soft tissues, including the donor and recipient sites of skin grafts
- Healing of recalcitrant wounds, for example torpid or nonhealing crural ulcers of all etiologies

- Osseointegration of osseous implants and prostheses in dentistry, maxillofacial and orthopedic surgery
- Attenuation of pain in chronic and acute pain entities
- Increased cerebral blood flow in simple senile dementia patients (currently not trialed for Alzheimer's or other clinically diagnosed forms of dementia, Parkinson's or Huntington's disease)
- Photorejuvenation of extrinsically-aged and intrinsically-aged skin
- Photoactivation of transepidermally delivered cosmetics and other agents including stem-cell-derived materials and platelet-rich plasma (PRP)
- Adjunctive therapy with any other treatment modalities from mild epidermal peels all the way through to full face ablative laser resurfacing
- Hair re-growth for hair loss due to alopecia areata, stress alopecia and so on

□ 830 nm LEDs ■ 590 nm / 830 nm LEDs



Scale 1:1.25



[Figure 1] (*Upper panel*): Schematic of a single LED array panel showing the arrangement of the 830 nm and the 830/590 nm LEDs on their printed circuit board (PCB). (*Lower panel*): View of the actual HEALITE II treatment head showing the 5 articulated and adjustable panels

2. RATIONALE

The use of phototherapy at the near-infrared wavelength of 830 nm has already been extensively reported in the literature for almost 3 decades. Efficacy has been proven for the healing of recalcitrant ulcers,⁽¹⁾ treatment of accidental and iatrogenic wounds including burns,^(2,3) for osseointegration of osseous implants⁽⁴⁾ and in successful photorejuvenation of age-damaged skin.^(5,6) As underlying mechanisms for these successful and effective applications, light energy at 830 nm has been shown to selectively and significantly upregulate the action potential of the main skin cells involved in wound healing, including macrophages,⁽⁷⁾ neutrophils,⁽⁸⁾ mast cells⁽⁹⁾ keratinocytes⁽¹⁰⁾ and fibroblasts (personal communication, Prof Kyoung Chan Park, Seoul National University, as yet unpublished data). 830 nm increases blood flow in axial pattern flaps, with the elevated blood flow and volume still evident on laser speckle Doppler flowmetry 90 min after a single irradiation, and significantly better flap survival in the 830 nm-treated animals.⁽¹¹⁾ At a genetic level, 830 nm has been proved to upregulate the genetic mechanisms responsible for osteoblast activation.^(4,12)

Yellow light at 590 nm has mostly been used for the pulsed dye laser photothermal treatment of vascular lesions, but sub-purpuric doses at the skin surface have produced interesting extravasation of wound-healing mediators in the dermis at sub-mW levels of energy at the dermal blood vessels,^(13,14) and LED phototherapy with yellow light has been well-reported as effective in a number of applications.⁽¹⁵⁻¹⁷⁾

3. PRECONDITIONING

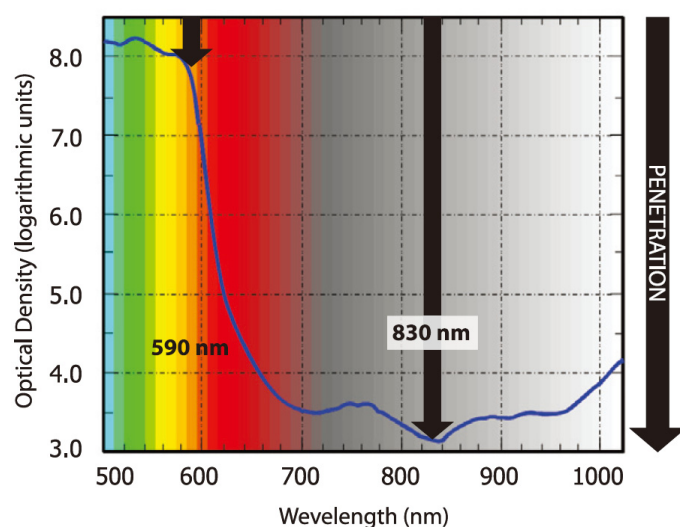
This subsection will examine the scientific basis for the preconditioning component of the HEALITE II photo-sequencing technology embodied in the proprietary photo-sequencing technology.

3.1. Why Preconditioning with 590 nm?

The major photobiological difference between 590 nm and 830 nm is the depth to which these wavelengths will inherently penetrate into living human skin at equalized levels of photon intensity, with 830 nm penetrating around 5 orders of magnitude greater than 590 nm [Figure 2].⁽¹⁸⁾ This is due to the presence of competing chromophores for 590 nm, namely hemoglobin and melanin, compared with 830 nm, whose main chromophores are ester chains and subcellular components located in the membranes of target cells and their subcellular organelles. In addition, 830 nm is right at the bottom of the water absorption curve, and in the middle of the so-called 'tissue window' described by Karu and others.⁽¹⁹⁾

An alternative for 590 nm as the preconditioning wavelength might have been 633 nm, as a great deal of the literature since the beginning of phototherapy in the late

1960's was based on the helium neon (HeNe) laser, with the wavelength of 632.8 nm. However, referring again to Figure 2, the reader will see that 633 nm penetrates almost 3.5 orders of magnitude better into tissue than 590 nm, due to its comparatively low absorption in epidermal melanin and dermal blood, so it was feared that some dissonance would occur in deeper dermal cells due to the combination of 633 nm and 830 nm being absorbed simultaneously after the initial preconditioning stage. Although blue light will penetrate even less than yellow, blue light is known to over-stimulate melanocytes with potential melanogenesis and is furthermore not associated very well with mitochondrial cytochrome c oxidase absorption, the key to cell photoactivation and induced ATP synthesis by visible light.



[Figure 2] Schematic representation of the comparative penetration depths of LED energy at 590 nm and 830 nm in a photospectrogram of regulated 'white light' through a human hand *in vivo*. Wavelength is shown along the x-axis and optical density, in logarithmic units, on the y-axis. The higher the OD, the poorer the penetration. (Adapted from Ref 17)

For these reasons, when we considered the optimum preconditioning wavelength, 590 nm was the first choice because its excellent photoactivative qualities are already known *in vivo* and *in vitro* from previous studies as mentioned above. Furthermore, 590 nm LED energy has been demonstrated *in vitro* by Karu to be right at the absorption peak of mitochondrial cytochrome c oxidase⁽²⁰⁾ the importance of which will be discussed below.

3.2. What is Preconditioning?

The entire concept of 'preconditioning' is underpinned by restriction of the absorption of the preconditioning wavelength photon energy to the living cells of the epidermal stratum basale, the basal layer, including components of the basement membrane and possibly some extremely superficial parts of the papillary dermis, as will be discussed in detail below. In Figure 2 above, the intensity across all wavelengths was equalized, so the comparatively shallow penetration of 590 nm light will be further compounded by the ultra-low incident intensity of around 300 $\mu\text{W}/\text{cm}^2$

for HEALITE II 590 nm energy compared with the 40, 60, 80 03 100 mW/cm² delivered by the 830 nm LEDs. This is combined with the 1 s exposure per panel to give an incident radiant fluence or dose per exposure to 590 nm of 300 µJ/cm² compared with the usual recommended dose of around 60 J/cm² for the 830 nm wavelength. Thus we class this as micro-low level light therapy, or µ-LLLT.

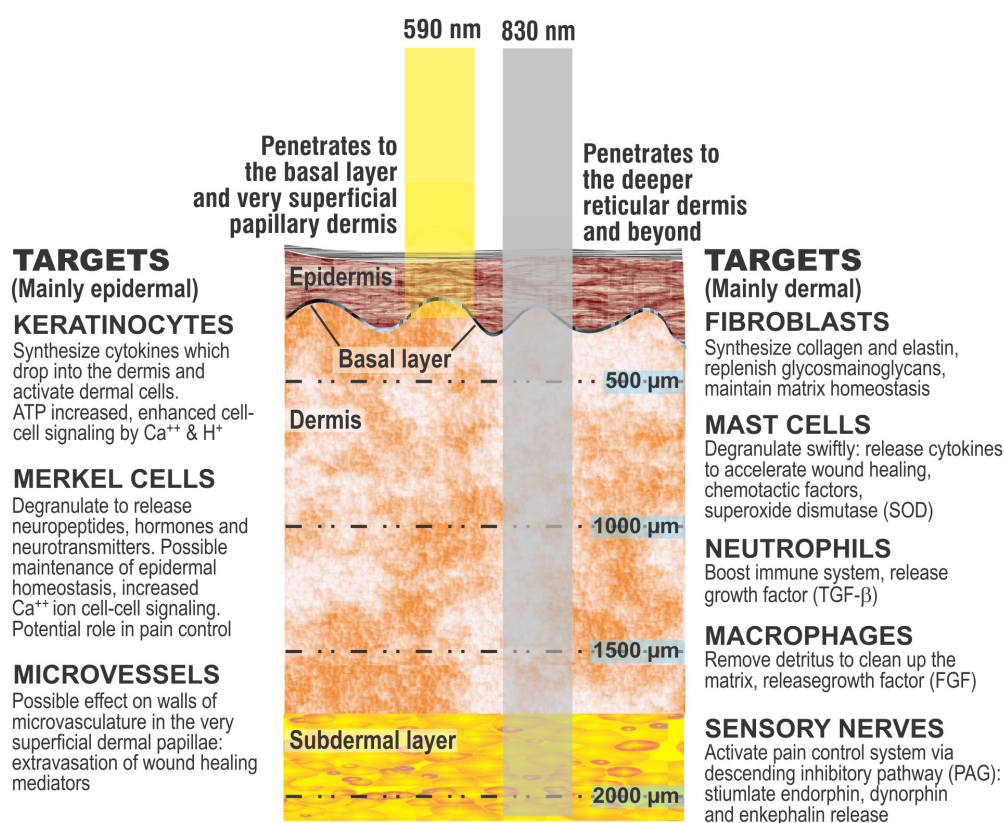
The model is based on the theory that the 590 nm µ-LLLT delivered sequentially over that first minute will ‘precondition’ the epidermal cells to start to manufacture cytokines, and may also induce the formation of neuropeptides, neurotransmitters and hormones from the epidermal Merkel cells. These photoproducts will then in turn ‘precondition’ the dermal target cells for the main 830 nm treatment.

3.3. What are the Targets for 590 nm µ-LLLT?

With the extremely low incident fluence delivered by the 590 nm component of the HEALITE II system, namely around 300 µJ/cm² (0.3 mJ/cm²), coupled with its already low penetrative characteristics, the penetration of the 590 nm light into the target skin will be curtailed even further and the targets will therefore all be epidermal, particularly the living cells in the basal layer and immediately adjacent tissues, as mentioned above. The main putative target cells will therefore be the basal layer keratinocytes and the little-discussed but very interesting Merkel cells, as already mentioned briefly above. Figure 3 compares schematically

the absorption depth of 590 nm and 830 nm, and lists the main targets.

The extremely low incident intensity selected for the HEALITE II 590 nm preconditioning µ-LLLT component was mainly based on the large body of evidence which has accrued over the past decade and recently reviewed comprehensively in *Lasers in Medical Science* by Baratto and colleagues (with associated references).⁽²¹⁾ The authors presented their own empirical experimental findings for what they termed ultra-low level laser therapy (ULLLT) and backed up their hypotheses with a large series of published studies, showing that very low levels of incident visible light (around 150 µW/cm² at a dose of 450 µJ/cm²) had significant trophic effects on a variety of cells, including Merkel cells, as well as potent anti-inflammatory and anti-edematous actions.⁽²²⁾ Baratto and colleagues conclude; “The striking biological response to [ULLLT] represents a puzzling issue for biologists and, at the same time, an opportunity for clinicians.” We believe that HEALITE II µ-LLLT preconditioning with 590 nm will take advantage of this opportunity in a practical manner with clinical utility. Even though the Baratto paper examined visible red light, the intracellular absorption characteristics of yellow 590 nm are known to be similar, and in common with all visible light, 590 nm induces a purely photochemical cascade within the cell with the cytochrome c oxidase terminal of the mitochondrial respiratory chain as the principle target.^(15,16)



[Figure 3] Schematic representation of the comparative penetration depths of LED energy at 590 nm (300 µW/cm²) and 830 nm (100 mW/cm²) into skin (typical skin from a female cheek), and the main targets of these wavelengths and their potential effect.

The primary role of the Merkel cell is as a mechanoreceptor. There is controversy as to its fetal origin: originally thought to have developed from the neural crest, similar to melanocytes, more recent mammalian studies have suggested that Merkel cells are the result of a neuronal-independent differentiation process with their final morphology more akin to the keratinocyte than the melanocyte. Merkel cells are located in the basal layer with abundant mitochondria in their cytosol, and are therefore a potential phototherapy target for visible light such as 590 nm, for which the mitochondrial cytochrome c oxidase is the starting point of the ATP synthesis cascade. Merkel cells also contain dense-core granules, on the degranulation of which neuropeptides and hormones can be re-released. In this respect they are similar to the mast cell.

The role of the Merkel cell has recently been attracting attention as a part of the neuroendocrine system and a potential mediator of epidermal homeostasis, in addition to its basic sensory function, and the part that these cells can play in the pain attenuation process through the release of neuropeptides and neurotransmitters.⁽²³⁻²⁵⁾ Of note is Calcitonine gene-related peptide (CGRP), significantly associated with the antiinflammatory action of acupuncture.⁽²⁶⁾ Because Merkel cells have been extremely difficult to culture, *in vitro* studies are sparse. However, with the large numbers of mitochondria and also the presence of dense granules, it can be hypothesized that Merkel cells will respond to photostimuli in the same manner as other similarly constructed cells. 590 nm μ LLLT may well photoactivate these interesting cells to enhance ATP production with the corresponding increase in intercellular Ca^{++} signaling, and the potential for enhanced degranulation of their dense

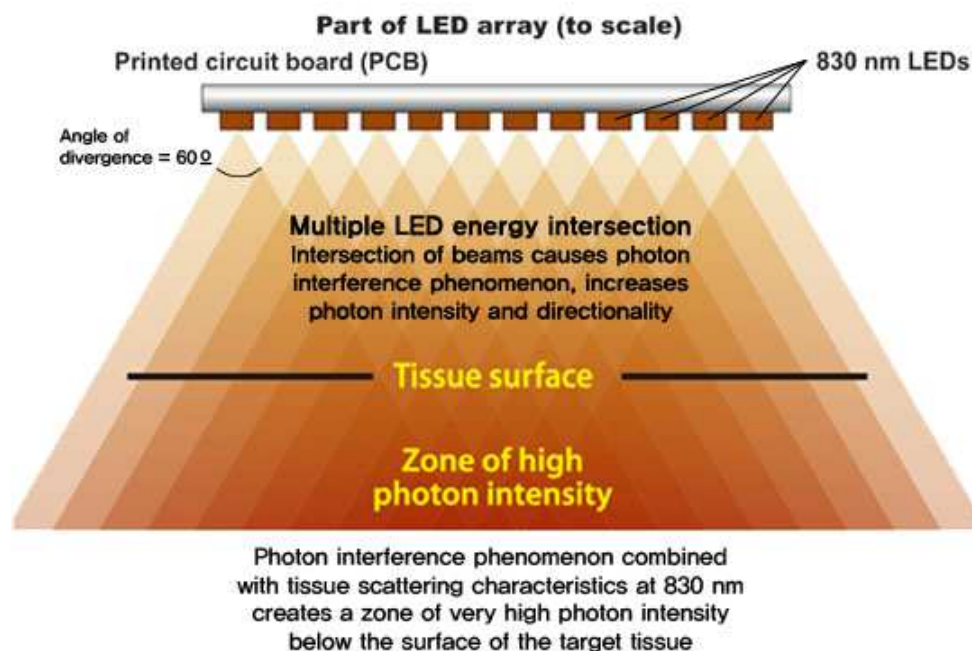
core granules. This is a subject worthy of further research to prove the hypothesis.

As for the keratinocyte as a target, low levels of visible light, including the yellow waveband, have been shown by Karu^(19,20) and Zhevago and Samoilova⁽²⁷⁾ to work extremely well in stimulating keratinocytes to release a large number of interleukins and other cytokines, some of which will drop down into the dermis to precondition the target cells for 830 nm, and where they can have effects on and be picked up by the microcirculation. Furthermore, increased ATP production from photoactivated keratinocytes will most probably result in higher levels of Ca^{++} ions in and outside of the photoactivated cells. Ca^{++} ions are recognized as mediators for potent cell-cell signaling.⁽²⁸⁾

In addition to epidermal cellular involvement, some 590 nm energy may reach the microcapillaries feeding the dermoepidermal junction (DEJ) in the very superficial layer of the dermis, thereby potentially mediating extravasation of the wound-healing mediators already mentioned above.⁽¹³⁾

4. PHOTOSEQUENCING

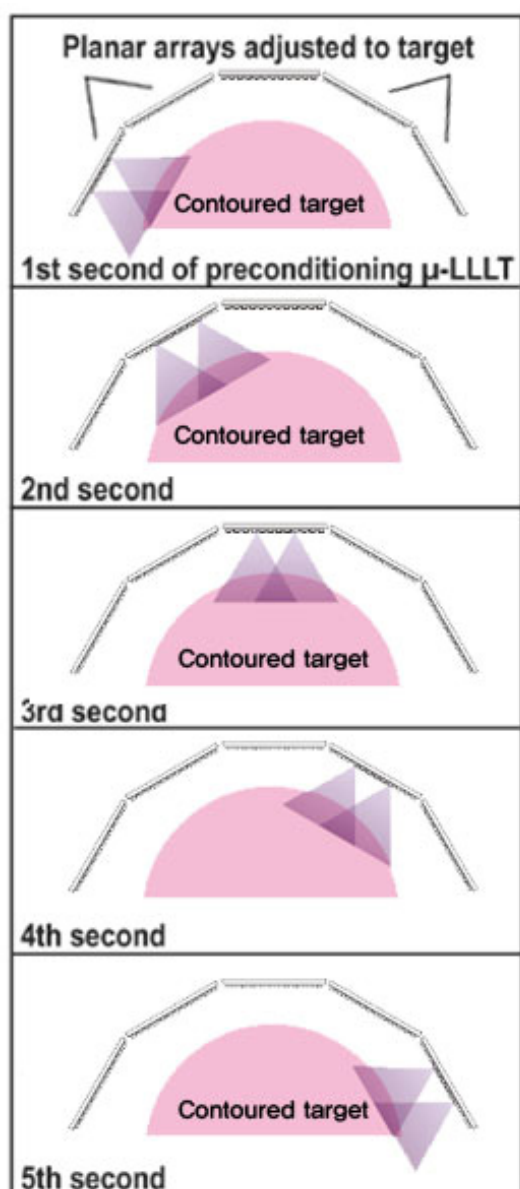
The concept of starting with the scanned 590 nm LED energy at μ -LLLT preconditioning energy levels followed by the continuous wave 830 nm LED energy at recognized clinically useful photon and energy densities is referred to as 'photosequencing'. The full name given to the process is '590/830 nm photosequencing with preconditioning μ -LLLT'.



[Figure 4] Scale section of a HEALITE II planar array of 830 nm LEDs illustrated schematically. Although individual LEDs have a much lower photon intensity than a laser diode, when LEDs are mounted precisely in arrays to allow multiple beam intersection, the phenomenon of photon interference finds synergy with the excellent and powerful tissue scattering characteristics of 830 nm to create a zone of extremely high, almost laser-like photon intensity beneath the surface of the target tissue.

The levels of delivered photon intensity are so low that no 590 nm-mediated photoeffect is expected in the dermis, with the exception of the microvasculature in the extreme superficial papillary dermal zone under the DEJ. Also, because the 590 nm energy is scanned sequentially and affects only the epidermis, it will have no contradictory or interference effect with the much deeper-penetrating and continuous wave 830 nm energy, but the dermal cells will have been preconditioned to interact with the 830 nm LED energy once it starts irradiating the target tissues.

The intersection characteristics of the 830 LEDs create powerful photonic reactions, allowing for the creation of photon interference where the beams intersect which, coupled with the excellent scattering characteristics of 830 nm energy in living tissue, creates a zone of very high photon intensity beneath the surface of the target tissue as illustrated in Figure 4.



[Figure 5] Schematic showing the first 5-second sequence of the 590 nm LEDs during the initial 1-min preconditioning μ -LLLT component of the HEALITE II photosequencing technology. This will be repeated for another 11 sequences before the 830 nm LEDs are illuminated.

On the other hand, only two rows of 590 nm LEDs per panel are incident on the target tissue during the 1 s for which they are illuminated, and the distance between the rows allows for poor intersection characteristics (Figure 5) but covers the entire area treated by the head. Even allowing for the intersection among the LEDs in each row, the intensity in the target tissue is very low, around $300 \mu\text{J}/\text{cm}^2$, as discussed above. This is however enough to begin photoactivation of the epidermal target cells, particularly the keratinocytes and Merkel cells, which is repeated for each 'strip' of tissue every 5 sec during the first 1 min preconditioning period. The system then enters the full treatment mode with the much more powerful 830 nm LEDs activated in continuous wave, but the 590 nm LEDs continue to operate in sequence during the remainder of the set treatment period. This is photosequencing in a nutshell.

Another basic but important aspect of photosequencing is the enhanced safety of the treatment, and therefore of HEALITE II. Light energy at 830 nm is in the near-infrared waveband and is therefore invisible to the naked human eye. At the higher intensity settings of the HEALITE II system, although perfectly harmless, and indeed beneficial, in skin and other tissues, ocular overexposure to the energy could cause damage to the unaccommodated and unprotected eye. However, because of the highly visible 590 nm component of the photosequencing process, the 'blink reflex' will be triggered in the eyes of anyone accidentally being irradiated with the system, thus protecting the eyes from damage.

CONCLUSIONS

This latest advance in LED phototherapy based on 590/830 nm photosequencing with preconditioning μ -LLLT, embodied in the 830/590 nm module of the HEALITE II system from Lutronic Corporation, offers clinicians a unique and highly efficient method of irradiating target tissue for a variety of phototherapeutic purposes and achieving excellent results faster and better. HEALITE II may be used on its own for the applications already listed in the Introduction section, and an increasing number of others, but it also offers a powerful adjunctive tool to enhance the already good results obtained from any other kind of surgical or aesthetic procedure which alters the architecture of the epidermis and dermis. It is proposed that the preconditioning with the μ -LLLT 590 nm light will work together with the already proven efficacy of the 830 nm energy in many clinical fields to achieve even more efficient results.

REFERENCES

- Kubota J: Defocused diode laser therapy (830 nm) in the treatment of unresponsive skin ulcers: a preliminary trial. *J Cosmet Laser Ther*, 2004. 6: 96-102.
- Trelles MA and Allones I: Red light-emitting diode (LED) therapy accelerates wound healing post-blepharoplasty and periocular laser ablative resurfacing. *J Cosmet Laser Ther*, 2006. 8:39-42.
- Kim JW and Lee JO: Low Level Laser Therapy and Phototherapy Assisted Hydrogel Dressing in Burn Wound Healing: Light Guided Epithelial Stem Cell Biomodulation. In Eisenmann-Klein M and Neuhann-Lorenz C (Eds) *Innovations in Plastic and Aesthetic Surgery*. 2007. Springer, Berlin & Heidelberg. pp 36-42.
- Kim YD, Kim SS, Hwang DS, Kim SG, Kwon YH, Shin SH et al. (2007): Effect of low-level laser treatment after installation of dental titanium implant-immunohistochemical study of RANKL, RANK, OPG: an experimental study in rats. *Lasers Surg Med*, 39: 441-450.
- Goldberg DJ, Amin S, Russell BA, Phelps R et al. (2006): Combined 633-nm and 830-nm LED treatment of photoaging skin. *J Drugs Dermatol*, 5: 748-753.
- Lee SY, Park KH, Choi JW, Kwon JK, et al.(2007): A prospective, randomized, placebo controlled, double-blinded, and split-face clinical study on LED phototherapy for skin rejuvenation: Clinical, profilometric, histologic, ultrastructural, and biochemical evaluations and comparison of three different treatment settings. *J Photochem Photobiol (B)*, 88: 51-67 (available online as Epub ahead of print).
- Young S, Bolton P, Dyson M, Harvey W and Diamantopoulos C. Macrophage responsiveness to light therapy. *Lasers Surg Med*, 1989. 9: 497-505.
- Osanai T, Shiroto C, Mikami Y, Kudou E et al.: Measurement of GaAlAs diode laser action on phagocytic activity of human neutrophils as a possible therapeutic dosimetry determinant. *Laser Therapy*, 1990. 2: 123-134.
- Calderhead RG, Kubota J, Trelles MA and Ohshiro T (2008): One mechanism behind led phototherapy for wound healing and skin rejuvenation: key role of the mast cell. *Laser Therapy*, 17: 141-148.
- Samoilova KA, Bogacheva ON, Obolenskaya KD, Blinova MI, et al.: Enhancement of the blood growth promoting activity after exposure of volunteers to visible and infrared polarized light. Part I: stimulation of human keratinocyte proliferation in vitro. *Photochem Photobiol Sci*. 2004 Jan;3(1):96-101. Epub 2003 Sep 1.
- Kubota J: Effects of diode laser therapy on blood flow in axial pattern flaps in the rat model. *Lasers Med Sci*, 2002.17: 146-153.
- Tamura K, Hosoya S, and Hiratsuka K (1998): Enhancement of mouse CDC46 gene expression in osteoblasts by laser irradiation. *Laser Therapy*, 10: 25-32.
- Bering P, Clement M, Heickendorf L, Egevis H and Kieran M (2000): Selective non- ablative wrinkle reduction by laser. *J Cutan Las Ther*, 2: 9-15.
- Omi T, Kawana S, Sato S, Takezaki S, Honda M, Igarashi T, et al.(2005): Cutaneous immunological activation elicited by a low-fluence pulsed dye laser. *Br J Dermatol*, 153 Suppl 2: 57-62.
- Weiss RA, McDaniel DH, Geronemus RG, Weiss MA, Beasley KL et al. (2005): Clinical experience with light-emitting diode (LED) photomodulation. *Dermatol Surg*, 31:1199-1205.
- Weiss RA, McDaniel DH, Geronemus RG, Weiss MA (2005): Clinical trial of a novel nonthermal LED array for reversal of photoaging: clinical, histologic, and surface profilometric results. *Lasers Surg Med*, 36: 85-91.
- DeLand MM, Weiss RA, McDaniel DH, Geronemus RG (2007): Treatment of radiation-induced dermatitis with light-emitting diode (LED) photomodulation. *Lasers Surg Med*, 39:164-168.Rationale of Photosequencing Page 9 of 6
- Smith KC: *The Science of Photobiology*. 1977. Plenum Press, New York, USA.
- Karu, T: Primary and secondary mechanisms of action of visible to near-IR radiation on cells. *J Photochem Photobiol B*, 1999. 49: 1-17.
- Karu T: *Ten Lectures on Basic Science of Laser Phototherapy*. 2007, Prima Books AB, Grängesberg, Sweden.
- Baratto L, Calza L, Capra R, Gallamini M, Giardino L et al. (2011): Ultra-low level laser therapy. *Lasers in Medical Science*, 26: 103-112.
- Giuliani A, Fernandez M, Farinelli M, Baratto L, Capra R et al. (2004): very low level laser therapy attenuates edema and pain in experimental models. *International Journal of Tissue Reaction*, XXVI: 29-37.
- Moll I, Roessler M, Brandner JM, Eispert AC, Houdek P, Moll R (2005): Human Merkel cells – aspects of cell biology, distribution and functions. *Eur J Cell Biol*, 84:259-271.
- Boulais N, Misery L (2007): Merkel cells. *J Am Acad Dermatol*, 57:147-165. Epub 2007 Apr 6
- Boulais N, Pereira U, Lebonvallet N, Gobin E, Dorange G et al. (2009): Merkel cells as putative regulatory cells in skin disorders: an *in vitro* study. *PLoS One*. 4: e6528.
- Zijlstra F, van den Berg-de LI, Huygen FJPM, Klein J (2003) Anti-inflammatory actions of acupuncture. *Mediators of Inflammation*, 12: 59–69
- Zhevago NA, Samoilova KA (2006): Pro- and anti-inflammatory cytokine content in human peripheral blood after its transcutaneous (in vivo) irradiation with polychromatic visible and infrared light. *Photomed Laser Surg*, 24: 129-39.
- Lavi R, Shainberg A, Friedmann H, Shneyvays V, Rickover O et al. (2003): Low energy visible light induces reactive oxygen species generation and stimulates an increase of intracellular calcium concentration in cardiac cells. *J Biol Chem*, 278: 40917-4022. Epub 2003 Jul 7.



Low Level Light Therapy (LLLT) with HEALITE II is a medical treatment that uses low levels of incident light energy, delivered with photosequencing technology, to alter cellular function through atraumatic and athermal photoactivation.