Light-Emitting Diode Phototherapy in Dermatological Practice

R. Glen Calderhead

Introduction

- Phototherapy is not new! It was being used more than 4,000 years ago
- Light-emitting diodes have attracted interest as a phototherapeutic source
- LEDs are solid state and robust
- LEDs are comparatively inexpensive

History of Phototherapy

Phototherapy in its broadest sense means any kind of treatment (from the Greek *therapeia* 'curing, healing,' from *therapeuein* 'to cure, treat.') with any kind of light (from the Greek *phos, photos* 'light'). The modern accepted definition of phototherapy, however, has become accepted as: "the use of low incident levels of light energy to achieve an athermal and atraumatic, but clinically useful, effect in tissue". Under its basic original definition, phototherapy is an ancient art because the oldest light source in the world is the sun, and therapy with sunlight, or heliotherapy, has been in use for over 4,000 years with the earliest recorded use being by the Ancient Egyptians.[1] They would treat what was probably vitiligo by rubbing the affected area with a crushed herb similar to parsley, then expose the treated area to sunlight. The photosensitizing properties of the parsley caused an intense photoreaction in the skin leading to a very nasty sunburn, which in turn hopefully led to the appearance of postinflammatory secondary hyperpigmentation, or 'suntan' thereby repigmenting the depigmented area. In their turn the Ancient Greeks and Romans used the healing power of the sun, and it was still being actively used in Europe in the eighteenth, nineteenth and early twentieth century, particularly red light therapy carried out with the patient placed in a room with red-tinted windows. One famous patient was King George III of Great Britain and Northern Ireland who ruled from 1760 to 1801, popularly though erroneously known as 'Mad King George'. We now strongly suspect that he was actually suffering from the blood disease porphyria, so being shut in a room with red-draped walls and red tinted windows to treat his depression probably only served to make him even more mad, since porphyria is often associated with severe photosensitivity! Entities treated this way included the eruptive skin lesions of rubella and rubeola, and even 'melancholia', as was the case with King George III, now recognised as clinical depression. Hippocrates, the Father of Medicine, certainly concurred with the latter application some two millennia before King George: Hippocrates prescribed sunlight for depressive patients and believed that the Greeks were more naturally cheerier than their northern neighbors because of the greater exposure to the sun.

In the field of wavelength-specific phototherapy research, red light therapy was examined at a cellular level under the newly-invented microscope by Fubini and colleagues in the late eighteenth century,[2] who were able to show that visible red light, provided *via* lenses and filters from sunlight, selectively activated the respiratory component of cellular mitochondria. There is nothing new under the sun. However, the sun is a fickle medical tool, particularly in northern Europe, and modern phototherapy as we know it started around the turn of the last century with Finsen's electric arc lamp-based system, giving phototherapy at the turn of a switch, independent of the sun.[3] However, apart from the use of blue light therapy for neonatal bilirubinemia which continues to the present day, phototherapy was, in the majority of its applications, overtaken in the first part of the twentieth century by better medication or improved treatment techniques.

The development of the first laser systems, a race which was narrowly won by Theodore Maiman in 1960 with his flashlamp-pumped ruby-based laser, next gave clinicians and researchers a completely different and unique light source to play with. In the 4 years between 1960 and 1964, the ruby laser

R.G. Calderhead
Japan Phototherapy Laboratory, Tsugamachi, Tochigi-ken, Japan
e-mail: docrgc@cc9.ne.jp

K. Nouri (ed.), *Lasers in Dermatology and Medicine*,
DOI: 10.1007/978-0-85729-281-0_19,

was followed by the argon, helium-neon (HeNe), neodymium: yttrium-aluminum-garnet (Nd:YAG) and carbon dioxide (CO_2) lasers all of which have remained as workhorses in the medical field, and the HeNe laser (632.8 nm) has in fact provided a large bulk of the phototherapy literature over the last three decades. As for light-emitting diodes (LEDs), the first light from a semiconductor was produced in 1907 by the British experimenter H. J. Round. Independently in the mid 1920s, noncoherent infrared light was produced from a semiconductor (diode) by O-V Losev in Russia. These studies were published in Russia, Germany and the UK, but their work was completely ignored in the USA.[4] It was not till 1962 that the first practical and commercially-available visible-spectrum (633 nm, red) LED was developed in the USA by Holonyak, regarded as the 'Father of the LED' while working with the General Electric Company. In the next few years, LEDs delivering other visible wavelengths were produced, with powers ten times or more that of Holonyak's original LED. For reasons which will be discussed later, these LEDs were really inappropriate as therapeutic sources, although they were extremely bright and very cheap compared with laser diodes, and it was not till the late 1990s that a new generation of extremely powerful, quasimonochromatic LEDs was developed by Whelan and colleagues as a spin-off from the National Aeronautic and Space Administration (NASA) Space Medicine Program.[5] Unlike their cheap and cheerful predecessors, the so-called 'NASA LEDs' finally offered clinicians and researchers a new and truly practical therapeutic tool.[6]

The What and Why of LEDs

What Is an LED?

Light-emitting diodes belong to the solid state device family known as semiconductors. These are devices which fall somewhere between an electrical conductor and an insulator, although when no electrical current is applied to a semiconductor, it has almost the same properties as an insulator. Simply explained, light-emitting semiconductors or diodes consist of negative (N-type) and positive (P-type) materials, which are 'doped' with specific impurities to produce the desired wavelength. The n-area contains electrons in their ground or resting state, and the p-area contains positively charged 'holes', both of which remain more or less stationary (Fig. 1a–c). When a direct current electric potential with the correct polarity is applied to an LED, the electrons in the N-area are boosted to a higher energy state, and they and the holes in the P-area start to move towards each other (Fig. 1d), meeting at the N/P junction where the negatively-charged electrons are attracted into the positively-charged holes. The electrons then return to their resting energy state and, in doing so, emit their stored energy in the form of a photon, a particle of light energy (Fig. 1e). The wavelength emitted is noncoherent, ideally very narrow-band, and depends on both the materials from which the LED is constructed, the substrates, and the p-n junction gap. Table 1 shows a list of the main substrates and associated colors. Figure 2 shows the anatomy of a typical dome-type LED. These can be mounted on circuit boards at regular and precise distances from each other to provide an LED array, part of which is shown in Fig. 3. However, the latest generation of LEDs actually form part of the board (so-called 'on-board' chips) which are much more compact than the dome-type LED and more efficient.

What Is the Difference Between LEDs and Lasers or IPLs?

The laser is a unique form of light energy, possessing the three qualities of monochromaticity, collimation and phase which make up the overall property of 'coherence'. Monochromaticity means all the photons are of exactly the same wavelength or color; collimation means the built-in parallel quality of the beam superimposed by the conditions of the laser resonator; and phase means that all of the photons march along together exactly equidistant from each other in time and in space. Laser diodes do not have inherent collimation, but because they are still true lasers, and therefore a so-called point source, the light can be gathered and optically collimated: the humble but ubiquitous laser pointer works on this principle. Intense pulsed light is, on the other hand, totally noncoherent, with a very large range of polychromatic (multicolored) light from near infrared all the way down to blue; has no possibility of collimation with extreme divergence; and has its vast variety of photons totally out of phase. The new generation of LEDs, on the other hand, has an output plus or minus a few nanometers of the rated wavelength, and so are classed as quasimonochromatic; some form of optical collimation can be imposed on the photons which are divergent but do have some directionality; but they are not in phase. Laser energy can easily produce high photon intensity per unit area, IPLs much less so, but provided LEDs are correctly arrayed, they are capable of almost laser-like incident intensities. Figure 4 schematically illustrates the differences between lasers, IPLs and LEDs. In short, LEDs for therapeutic applications must be quasimonochromatic, be capable of targeting wavelength-specific cells or materials, have stable output, and be able to deliver clinically useful photon intensities.

Why Use LEDs?

There are many excellent laser and intense pulsed light (IPL) systems available to the dermatologist. Why should LEDs be

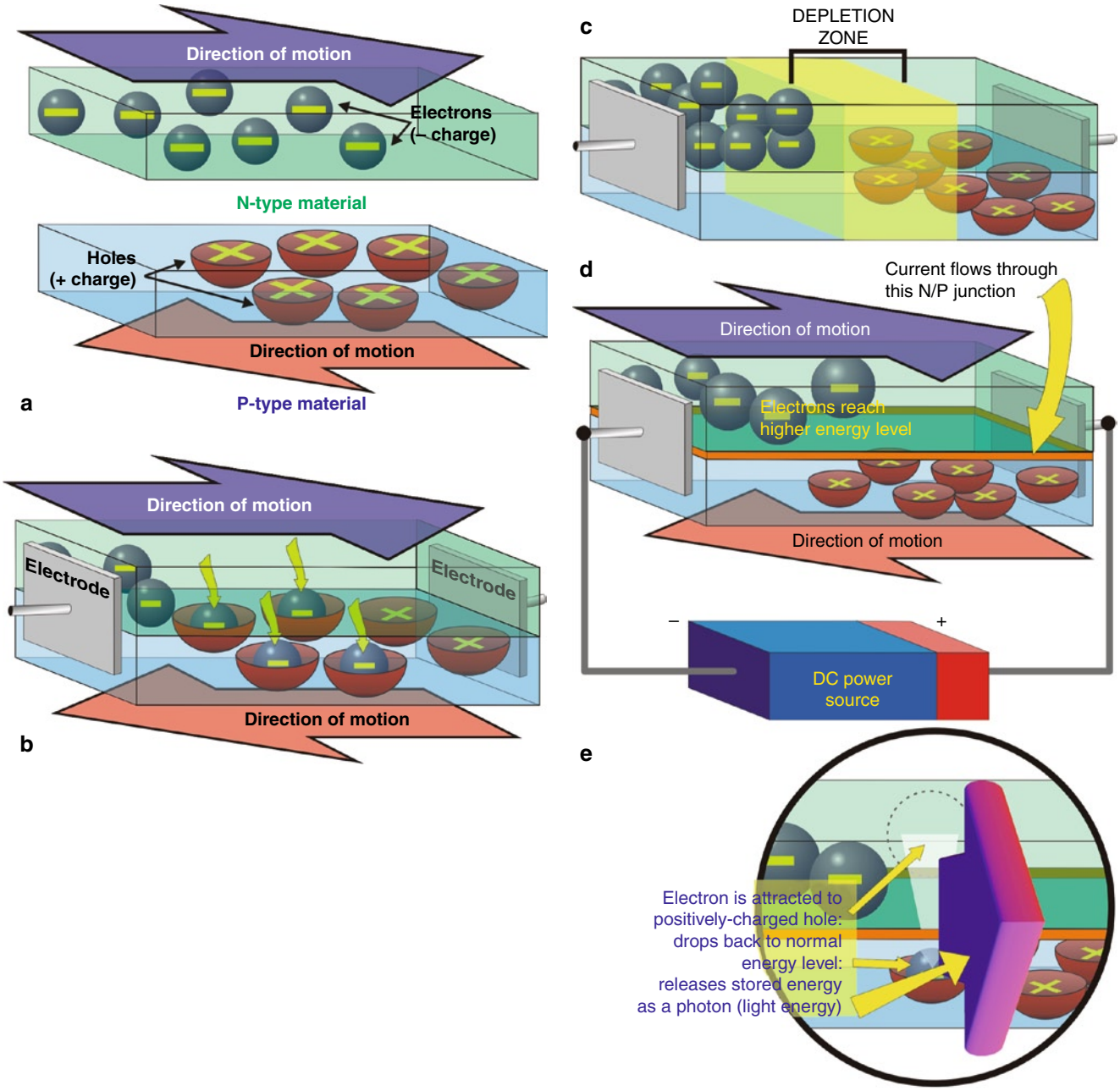

Fig. 1 What is an LED and how can it produce light? (**a**) An LED is basically composed of two materials, the N-type or negative material and the P-type or positive material. The N-material contains negatively charged electrons which move as shown, and the P-material contains positively charged holes, which move in the opposite direction. When the materials are apart and not connected to any power source, movement continues, so both materials are conductors. (**b**) When the materials are sandwiched together, however, without any power applied to the electrodes attached to opposite ends, the negatively charged electrons in the center of the chip are attracted to the holes, and form an area called the depletion layer as seen in (**c**) and all movement ceases in both the N- and P-materials: the chip is now an insulator. (**d**) Power is applied to the electrodes, with the positive electrode or anode at the origin of movement of the holes and the negative electrode or cathode at the origin of movement of the electrons. Observing the polarity when connecting a direct current (DC) power source is extremely important. Power flows through the junction between the materials, called the N/P junction, and movement of both electrons and holes starts again, but with power applied the electrons move to a higher energy level from their ground or resting state. (**e**) As in 1b above, the N-electrons are attracted to the P-holes, but in moving down through the N/P junction they must return to their ground energy level, and lose their extra stored energy in the form of a photon, the smallest packet of light energy. Unlike the situation in 1b, however, when power is applied this action continues endlessly and no depletion layer is formed. The N- and P-materials are 'doped' with other materials which determine the distance of the 'fall' between electrons and holes: the greater the distance the electrons have to fall, the higher is the energy level of the photons emitted. Photons with high energy levels have shorter wavelengths than those with lower energy levels, thus the wavelengths of the emitted light are determined by the materials and their doping. High quality N- and P-materials and pure doping substances will give photons of very nearly the same wavelength, i.e., quasimonochromatic light

Table 1 Most common substrate combinations and the colors they are capable of producing

Substrates	Formula	Colors produced
Aluminum gallium arsenide	(AlGaAs)	Red, infrared
Aluminum gallium phosphide	(AlGaP)	Green
Aluminum gallium indium phosphide	(AlGaInP)	Green, yellow, orange, orange-red(all high-intensity)
Gallium arsenide phosphide	(GaAsP)	Yellow, orange, orange-red, red
Gallium phosphide	(GaP)	Green, yellow, red
Gallium nitride	(GaN)	Blue, green, pure green (emerald green): also white (if it has an AlGaN Quantum Barrier, so-called 'white light' LED)
Indium gallium nitride	(InGaN)	Near ultraviolet, blue, bluish-green

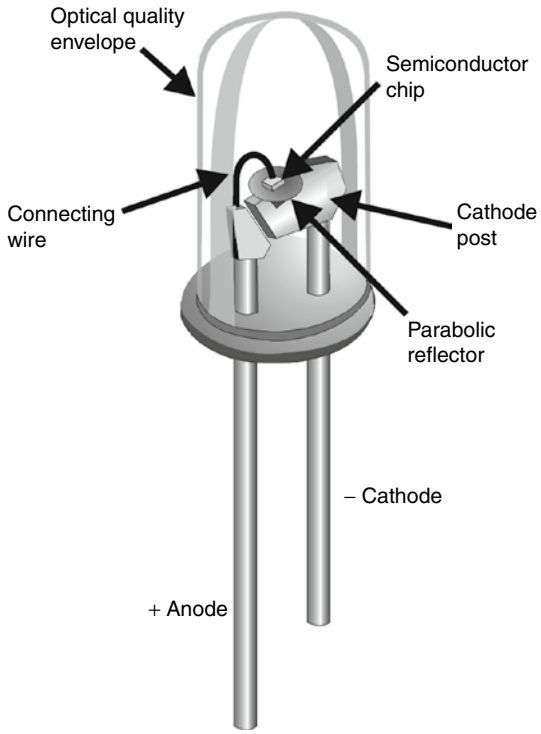

Fig. 2 Anatomy of a typical high-quality dome-type LED. The cathode is always shorter than the anode and there is a flat surface in the base of the LED by the cathode so polarity is clearly determined when connecting to a DC power source. On top of the cathode post and forming part of the negative electrode of the LED chip is a parabolic reflector in which the chip itself is mounted thus ensuring as much light as possible is directed forwards, with a consistent angle of divergence, typically 60° steradian or less depending on the specifications of the LED. A fine wire connects the positive electrode of the chip to the anode post, thus completing the circuit. The entire assembly is encapsulated in an optical quality clear plastic envelope, giving the final assembly its robust nature

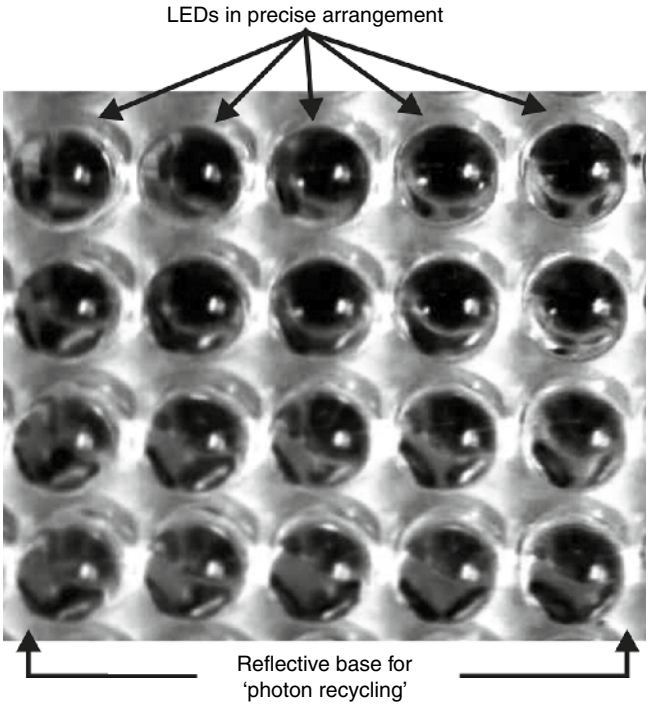

Fig. 3 Close-up view of dome-type LEDs mounted in an actual array from a therapeutic system. Note the precise x-y spacing of the LEDs, (*cf* Fig. 6 and associated text), and the reflective backing into which they are mounted. When light is incident on living skin, a certain amount will be reflected from the outer layer of skin, the stratum corneum. The longer the wavelength, the greater this reflection will be. In addition, some light is always back-scattered out of skin. The purpose of the reflective backing of the array is to capture these photons and reflect them back into the skin, known as 'photon recycling'

considered as a viable alternative phototherapy source? The main reasons are efficiency and price. The electricity-light conversion ratio of a typical laser is very low, requiring 100 or even 1,000 of watts in to give an output of a few watts. The same applies to IPL systems, where the flashlamp has to be pumped with enormous amounts of energy to provide polychromatic light, which may however be filtered (cut-on or cut-off). Even when filtered, IPL energy is delivered over a waveband rather than at a specific wavelength. In the case of LEDs, which are quasimonochromatic and require no filtering, the conversion efficiency is very high so that very few watts at a low voltage are required to produce a clinically useful output. LEDs are much less expensive than even laser diodes. Depending on quality and wavelength, anywhere from 300 new-generation LEDs can be purchased for the cost of a single laser diode. The cost of laser and IPL systems

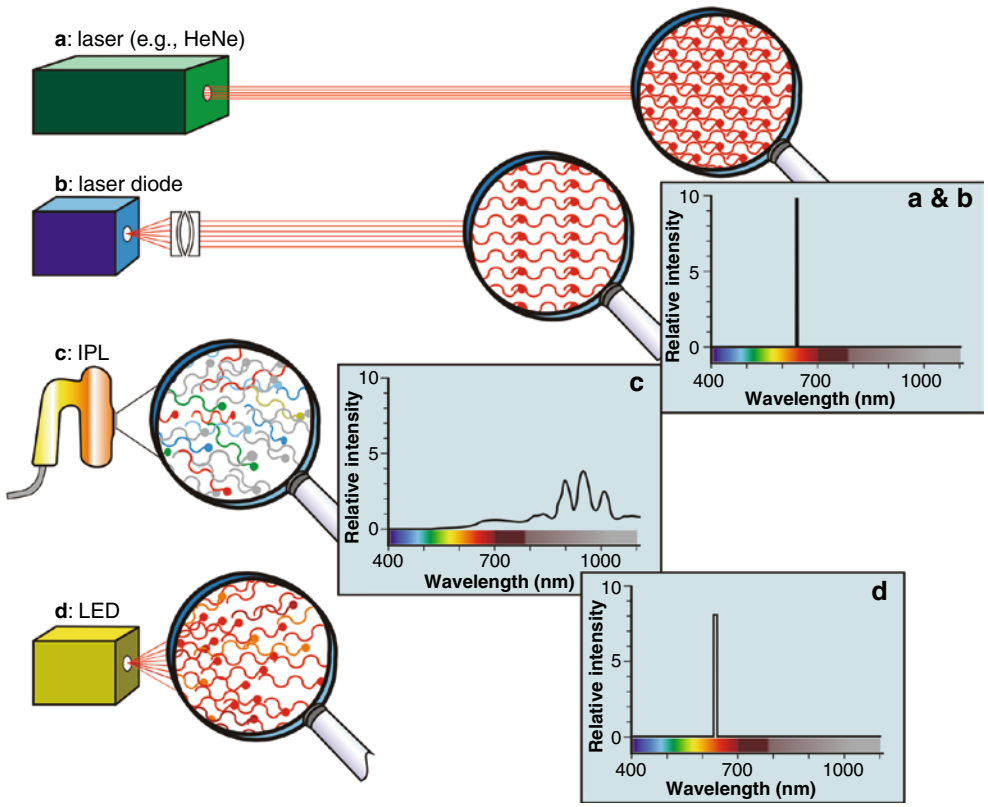

Fig. 4 Comparison among the output characteristics of a laser, laser diode, intense pulsed light system and a new generation LED. (**a**) A laser emits all of its energy at one precise wavelength, in a coherent beam, i.e., monochromatic, collimated and with the photons all in phase both temporally and spatially. If a 'special magnifying glass' could view the beam, it would show the picture as seen in the figure. All of the energy is delivered at a precise wavelength, as illustrated in the spectrogram, so the relative intensity of the beam is extremely high. (**b**) A laser diode has all the characteristics of a laser, except that the beam is divergent, without collimation. However, because it is a point source the beam can be collimated with condensing optics. The magnified view of the beam shows a lower photon intensity than the laser, but the relative intensity is still very high. (**c**) An IPL system emits a pulse of broad-band polychromatic noncoherent light, so the 'magnifying glass' would show a plethora of widely divergent photons of many different wavelengths, but with the majority in the near infrared as seen from the spectrogram. Because of the very broad waveband, the relative intensity at any given wavelength is low to very low. (**d**) The LED is somewhat similar to the laser diode, but the light is noncoherent, highly divergent and quasi-monochromatic. The 'magnifying glass' shows plenty of photons, mostly the same color, with some degree of directionality but without the true collimation and phase associated with the laser diode. The relative intensity is still very high, however, because the vast majority of the photons are being delivered at the nominal wavelength with a very narrow waveband of plus or minus a very few nanometers

is very high, so a much cheaper LED-based system offers the possibility to halt the ever-upward spiralling costs of health care for both the clinicians and their patients. A further advantage is the solid state nature of LEDs. There are no filaments to be heated up, and no flashlamps are required to produce light or to pump the laser medium: LEDs thus run much cooler than their extremely higher-powered cousins, so less is required in the way of dedicated cooling systems, again helping to reduce the cost. However, some cooling of LEDs is still required, especially when LEDs are mounted in multiple arrays, because as the temperature of an LED increases, its output will move away from the rated wavelength. When wavelength cell- or target-specificity is required, this could be a major problem. The solid state nature of LEDs also makes them much more robust than either lasers or IPL systems, so they tend to be able to take the sometimes not-so-gentle handling which is part of a busy clinical practice without causing either output power loss or alignment problems. Finally, LEDs can be mounted in flat panel arrays, which may in turn be joined together in a treatment head which can be adjustable to fit the contour of the large area of tissue being treated, whether it is the face, an arm, the chest or back, or a leg. Compare this potentially very large treatment area of some 100 of square centimeters with that of a laser, usually a very few millimeters in diameter, or that of an IPL treatment head, typically 1 cm × 3 cm, and the clinician-intensive nature of the latter two is quickly evident when large areas are to be treated such as the entire face. Multiple shots are required, and the handpiece has to be manually applied and controlled by the user. The LED-based treatment head can be attached to an articulated arm to make individual adjustment even easier. Heads with different wavelengths

can be designed to be easily interchangeable, controlled by the same base unit. With 'set-it-and-forget-it' microprocessor-controlled technology, the clinician simply sets the head up over the area to be treated, following the manufacturer's recommendations, turns the system on, and he or she can then leave the patient for the requisite treatment time and attend to other patients or tasks. Moreover, in many cases a suitably trained nurse can carry out the treatment once the clinician has prescribed it, because LED systems are much more inherently safe for the patient than lasers or IPLs.

Basics of Light-Tissue Interaction

- Light-emitting diodes provide athermal and atraumatic photoactivation
- LEDs are a viable and valuable phototherapeutic tool
- LEDs are capable of interesting light-tissue interactions, provided certain criteria are met. The most important criteria are:
  - Wavelength.
    Determines both the target and the depth at which the target can be reached
    Quasimonochromaticity is essential
    Wavelengths should be applied separately and sequentially, and not combined at the same time
  - Photon density.
    Gives suitably high intensity at all levels of target cells or materials
    Ensures sufficient athermal energy transfer to raise targets' action potentials
  - Dosimetry.
    Provided the wavelength and intensity are appropriate, correct dosage obtains the optimum effect with the shortest irradiation time
  - Temporal beam profile.
    Continuous wave would appear to be more efficient for most cell types *in vivo*, compared with 'pulsed' (frequency modulated) light

The main purpose of using phototherapy is to achieve some kind of clinical reaction in the target tissue through the use of light energy. If the incident power is high, heat will be the end product as with the surgical laser. If a too-low photon intensity is delivered, there will be very little or no reaction. The trick in LED phototherapy is to deliver just the right amount of photon intensity to achieve the desired clinical effect but in an athermal and atraumatic manner.

Photothermal and Athermal Reactions

Despite their very different output powers, lasers, IPLs and LEDs all depend on the 'L' which is found in all their names, standing for 'light'. It could be said that they are all different facets of the same coin, but even in photosurgery, phototherapy plays a very important role. If we consider the typical beam pattern of a surgical CO_2 laser in tissue, we see the range of temperature-dependent bioeffects as illustrated schematically in Fig. 5, ranging from carbonization above 200°C, vaporization above 100°C, through coagulation around 60–85°C, all the way down to photobiomodulation, which occurs atraumatically when there is no appreciable rise in the tissue temperature at the very perimeter of the treated area. These effects occur virtually simultaneously as the light energy propagates into the target tissue with photon intensity decreasing with depth, and can be divided as shown into varying degrees of photosurgical destruction and reversible photodamage, and athermal, atraumatic photobiomodulation. The zones are also shown in a typical CO_2 laser specimen stained with hematoxylin and eosin (Fig. 5).

Laser surgery involves all zones, but the importance of the photobiomodulative zone cannot be stressed enough. It is the existence of this zone which sets laser surgery apart from any other thermally-dependent treatment, such as electrosurgery, or even athermal incision with the conventional scalpel, and it is the photoactivated cells in this zone which provided the results that interested the early adopters of the surgical laser compared with the cold scalpel or electrosurgery, namely better healing with less inflammation and much less postoperative pain. IPL systems, and so-called nonablative lasers, produce areas of deliberate but controlled coagulative damage beneath a cooled and intact epidermis (Fig. 6), however they also produce the photobiomodulative zone to help achieve the desired effect of neocollagenesis and neoelastinogenesis through the wound healing process in the dermal extracellular matrix (ECM). LED-based phototherapy systems, on the other hand, deliver only athermal and atraumatic effects, but are still capable of inducing the wound healing process almost as efficiently as IPLs and nonablative lasers, as will be shown in detail in a later section.

Wavelength and Its Importance

The first law of photobiology, the Grotthus-Draper Law, states that only energy which is absorbed in a target can produce a photochemical or photophysical reaction. However, any such reaction is not an automatic consequence of energy absorption. It may be simply converted into heat, as in the surgical and non-ablative lasers or IPL systems, or re-emitted at a different wavelength (fluorescence). The prime arbitrator of this

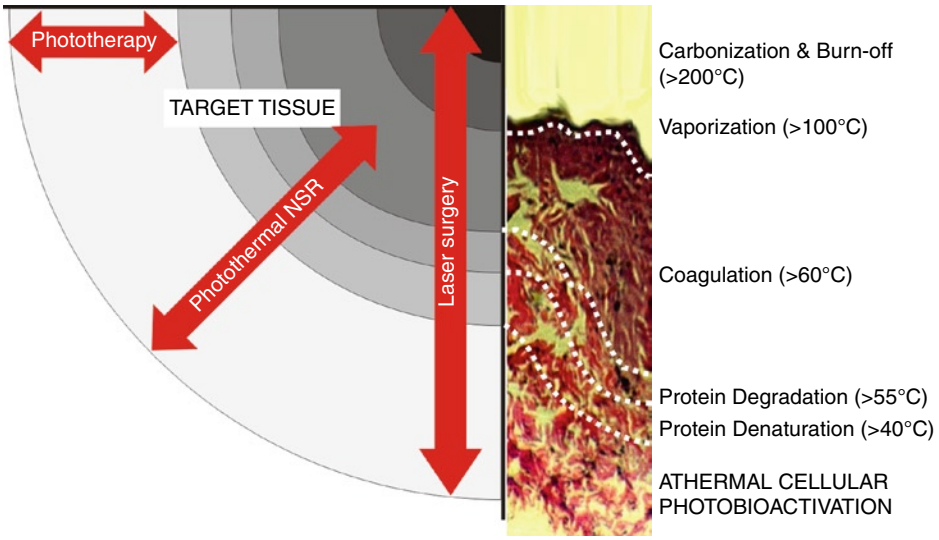

Fig. 5 Range of photothermal and athermal photobioreactions in tissue following a typical surgical laser impact, e.g., a CO_2 laser. A hematoxylin and eosin stained specimen of actual CO_2 laser treated skin is also included to show the typical histopathological changes for each of the bioreactions: the epidermis has been totally vaporized leaving a layer of carbon char above the coagulated dermis. The outermost layer, the photobioactivation layer, shows normal tissue architecture, even though some photons will have reached this layer and transferred their energy to the cells in an athermal and atraumatic manner. Laser surgery involves all levels of bioreactions. Photothermal nonablative skin rejuvenation (NSR) delivers controlled coagulative photothermal damage, with all the subsequent layers, whereas phototherapy only delivers athermal and atraumatic photobioactivation

'no absorption-no reaction' precept is not the output power of the incident photons, but their *wavelength*, and this comprises two important considerations: wavelength specificity of the target, or the target chromophore; and the depth of the target. Based on these two considerations, the wavelength must not only be appropriate for the chosen chromophore, but it must also penetrate deeply enough to reach enough of the target chromophores with a high enough photon density to induce the desired reaction. In theory, a single photon can activate a cell, but in actual practice multiple photon absorption is required to achieve the desired degree of reaction.

Phototherapy is athermal and atraumatic, hence achieving selective photothermolysis is of no concern as it would be for surgical or other photothermal applications. On the contrary, penetration of light into living tissue is, however, extremely important in phototherapy, and very frequently displays characteristics which are often in discord with results produced by mathematical models, a point often totally ignored by some researchers. A favorite, but photobiologically false, axiom beloved of phototherapy opponents, is that 'all light is absorbed within the first millimeter of tissue'. Anyone who has shone a red laser pointer through their finger, transilluminating the entire fingertip and completely visible on the other side, has already disproved that statement. A totally different finding is seen with green or yellow laser pointers, however. Figure 7 is based on a transmission photospectrogram of a human hand captured *in vivo* over the waveband from 500 nm (visible blue/green) to 1,100 nm in the near infrared.[7] The photospectrometer generator was positioned above the hand, delivering a 'flat spectrum' of 'white light', and the recorder placed beneath it. The wavelength is shown along the *x*-axis, and the calculated optical density (OD) is on the *y*-axis, from lower ODs to higher. The higher the OD, the greater is the absorption of incident light, and hence the lower the transmission, or penetration depth into the tissue. It must also be remembered that the OD is not an arithmetic but a logarithmic progression, so that the difference between an OD of 4 and one of 6 is not simply 2, but 2 *orders of magnitude*, i.e. a factor of 100.

From 500 to 595 nm (blue-green to yellow), the OD was from 8.2 to approximately 7.6, respectively, resulting in poor penetration. At 633 nm, the approximate wavelength of the HeNe laser, the photobiological efficacy of which is well recorded, the OD was approximately 4.5. In other words, red light at 633 nm penetrated living human tissue by 3 orders of magnitude better than yellow at 595 nm, because of the pigment-specific absorption characteristics of the two wavelengths. Visible yellow at 595 nm is at the peak of the oxyhemoglobin absorption curve, and is also much higher absorbed in epidermal melanin than 633 nm, which is why the yellow light in the spectrogram did not penetrate at all well into the tissue due to competing chromophores of epidermal melanin and superficial dermal blood. Accordingly, cellular and other targets in the mid to deep reticular dermis are inaccessible to yellow light with sufficient photon intensities to achieve multiple photon absorption in the target cells. The deepest penetration was achieved at 820–840 nm

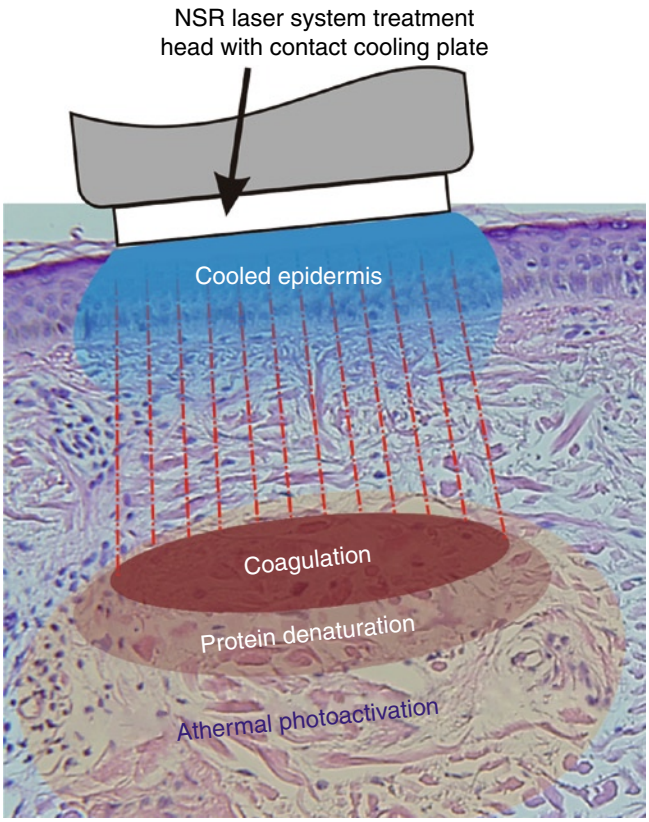

Fig. 6 Theory behind photothermal nonablative skin rejuvenation: the laser energy passes through the cooled epidermis without harming it, and delivers a controlled area of coagulation in the typically elastotic dermis associated with photoaged skin. However, the photons do not stop there, and there are zones of protein denaturation and, most importantly for the good result, athermal and atraumatic photoactivation around and beyond the controlled thermal damage. The photoactivated cells in the last of these three zones will assist with the wound healing process

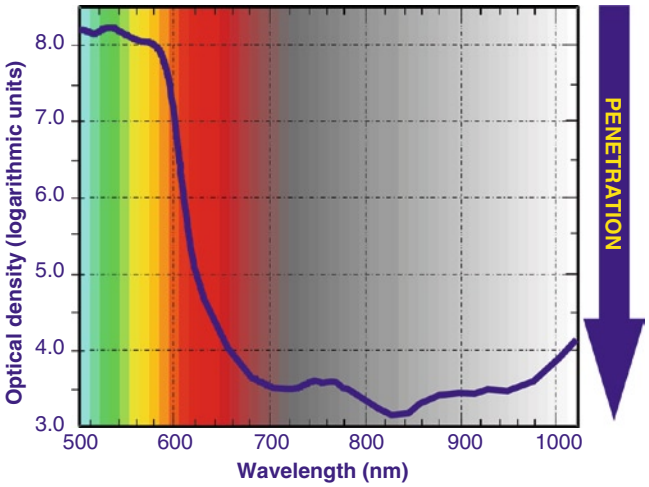

Fig. 7 Photospectrogram of a human hand *in vivo*. The generator, delivering uniform 'white light' at the waveband shown on the x-axis, was placed above the hand and the sensor below the hand. Optical density on the y-axis is shown in logarithmic units. Penetration is shown on the right-hand axis. The further down the curve reaches, the better the penetration at that wavelength into living human tissue (Adapted from Smith[7])

in the near infrared. At this waveband, pigment is not a primary chromophore with proteinous targets as the major chromophore, and this 820–830 nm waveband coincides with the bottom of the water absorption curve. The most successful of the laser diode systems used in laser therapy as distinct to laser surgery, or low level laser therapy (LLLT) as it was also known, delivered a wavelength of 830 nm for this very reason,[8] and was shown to penetrate living hands, and even bone, very successfully.[9] After around 1,000 nm, water absorption once again starts to play a significant role, and in the curve in Fig. 2 the OD was seen to increase thereafter. In general, shorter visible wavelengths penetrate less than longer visible and near IR wavelengths, up to a given waveband, depending on the absorbing chromophore.

Following these findings, it made a great deal of sense to source LEDs for LED systems at wavelengths already tried, tested and proven in the more than three decades of laser therapy application and research, so LED systems delivering 633 nm or thereabout in the visible red and 830 nm in the near infrared, and at high enough photon densities, have been reported as having significant effects on their target tissues at a good range of depths well into the mid and even the deep reticular dermis. The usefulness of visible red and near IR LED phototherapy has already been reported in a wide range of medical specialties, including dermatology. Yellow light at 590–595 nm has also attracted attention, but the penetration properties of yellow light must be carefully considered, as illustrated *in vivo* in Fig. 7. From the standpoint of photobiological theory, yellow light has very good potential specificity in a number of subcellular and haematological targets, such as cytochrome-c oxidase, however its poor penetration into the intermediate and deeper dermis, where cellular targets such as fibroblasts lie, limits the practical efficacy of yellow light in living tissue. Blue light at around 415 nm has very interesting properties regarding the eradication of the bacterium *Propionibacterium acnes* (*P. acnes*) although the photoreaction is different and will be discussed later in the chapter. LED systems with many other wavelengths have been produced, but basically they have very little or no published work to back up the claims of the manufacturers. 'Any old LED will not do' is an axiom which must be borne in mind by the dermatologist wishing to incorporate LED phototherapy into his or her practice.

Finally, the different wavebands, visible light and invisible infrared light, have different primary mechanisms. Absorption of visible light photons at appropriate levels induces a photochemical reaction, and a primary photochemical cascade occurs within the cell, usually instigated by specific components in the respiratory chain of the mitochondria, the adenosine triphosphate-producing power-houses of the cell.[10] Infrared photons, on the other hand, are primarily involved in

photophysical reactions which occur in the cell membrane, changing the rotational and vibrational characteristics of the membrane molecules. Through subsequent activation of the various membrane-located transport mechanisms, such as the Na^{++}/Ca^{++} and Na^{++}/K^{++} pumps and changes in the cell permeability, changes occur in the chemical and osmotic balance in the cytosol, finally resulting in the induction of a secondary chemical cascade which gives more or less the same endpoint as the visible light photons, namely cellular activation or proliferation.[11] These photoreactions are illustrated schematically in Fig. 8.

To sum up, the wavelength of a therapeutic source therefore has a double importance, namely to ensure absorption of the incident photons in the target chromophores, and to be able to do so at the depths at which these chromophores exist. The waveband in which the wavelength of the incident photons is located determines not only which part of the cell is the target, but also the primary photoaction. Wavelength is thus probably the single most important consideration in LED phototherapy, because without absorption, there can be no reaction.

Photon Density

Light energy travels in the form of photons. It is obvious that the more photons are incident per unit area of tissue, the greater will be the bioeffect. This incident photon intensity is called the power density, or irradiance, of a beam of light. The power density (PD) is an extremely important factor, following wavelength, and is calculated using the following formula:

$$PD = \frac{OP}{TA} \ (W/cm^2)$$

where OP is the output power incident on the target in watts (W) and TA is the irradiated area in square centimeters (cm^2). PD is usually expressed in watts per square centimeter (W/cm^2) or milliwatts ($mW)/cm^2$. It is the power density of a beam that will determine more than anything else (apart from wavelength) the magnitude of the bioeffect in the target tissue. Consider Table 2, where a laser with a constant incident output power of

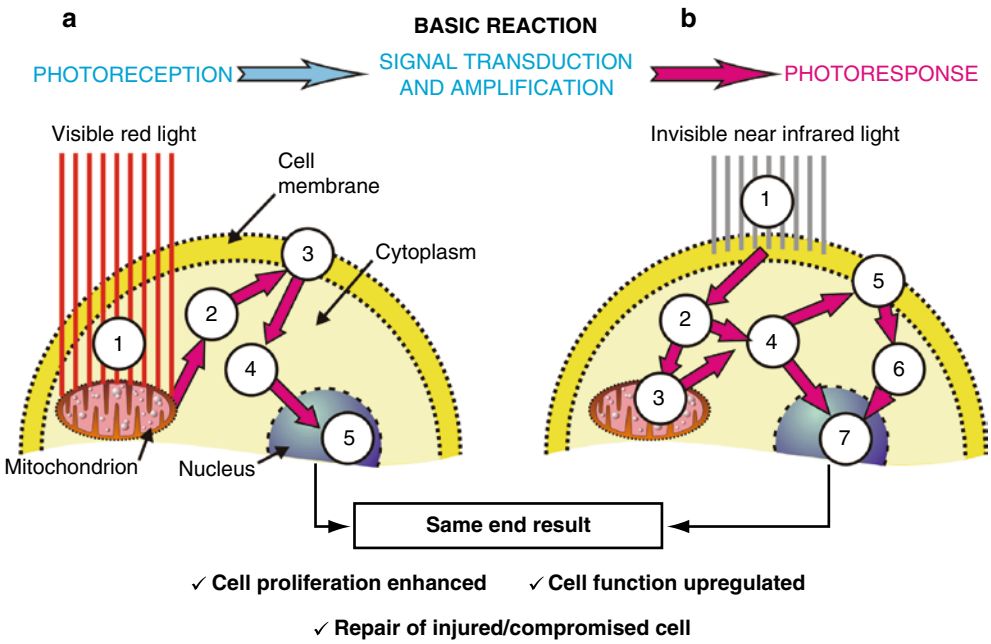

Fig. 8 Schematic depicting photoreception (absorption) of light in a cell, and the subsequent wavelength-specific response. The basic reaction as defined by Karu is absorption, which is followed by signal transduction and amplification within the cytosol, and leads to the photoresponse. (**a**) (1): Visible red light induces a primary photochemical cascade initiated in the mitochondrion, the energy factory and cell power house, which results in increased levels of nicotinamide adenine dinucleotide (NAD) extremely important in a wide range of redox (reduction-oxidation) reactions, one of the results of which is the generation of adenosine triphosphate (ATP) which is the 'gasoline' for the cell. (2): The increased levels of cytoplasmic ATP fuel the membrane transport pumps, the $Na^{++}K^{++}$ and $Ca^{++}K^{++}$ pumps (3) which induce extra- and intracellulation of messenger Ca^{++} ions and protons (H^+) which are elementary particles carrying a positive electric charge, the flow of which is used to generate energy from ATP via ATPase. Cytoplasmic levels of Ca^{++} ions and H^+ dramatically increase. (4) This in turn upregulates intracellular signaling including mRNA production from ribosomes on the rough endoplasmic reticulum, and finally (5) nuclear activity is also up regulated. (**b**) In the case of near infrared light, the primary mechanism of absorption is completely different (1) resulting in a photophysical reaction which changes the energy levels of the cell membrane, in which near IR energy is absorbed. This kick-starts the $Na^{++}K^{++}$ and $Ca^{++}K^{++}$ pumps so that cytoplasmic levels of Ca^{++} and H^+ dramatically increase (2) and (4), prompting the mitochondrion to manufacture more ATP to fuel the increased energy requirement (3), thereby raising cytoplasmic levels of ATP (4) which again impacts on the transport mechanisms of the membrane not affected by the near IR light. Despite the totally different pathways, the end result is however the same as in the case of visible light, namely further cyclic increased energy levels in the cytoplasm (6) and upregulation of nuclear activity (7)

Table 2 Illustration of the importance of altering the power density to achieve a complete range of bioeffects from incision to photobiomodulation with a constant incident output power

Incident power (W)	Spot size (Ø, units as given)	Power density (W/cm²)	Bioeffect
2	100 μm	25,000	Incision; excision
2	200 μm	6,250	Vaporization; deep coagulation
2	1 mm	250	Mild coagulation; protein denaturation
2	1 cm	2.5	Athermal, atraumatic photobiomodulation

2 W targets tissue with a range of spot sizes. Simply changing the spot size, and thus the power density, can have dramatically different effects on the target tissue. Because the power density is worked out per unit area, calculated by the formula πr^2, where π is the constant pi, 3.142, and r is the radius (half the diameter) of the irradiated area, we have to remember that there is an inverse square ratio between spot size and power density for a constant output power. Doubling the spot size will not cut the power density by one-half, but by one quarter: increasing the spot size by a factor of 10 will cut the power density by one-hundredth, and vice-versa.

In LED phototherapy, it is therefore necessary to achieve a high enough incident photon intensity to achieve the desired degree of multiple absorption in the target cells, but not so high as to cause any degree of photothermally-mediated changes in the tissue architecture, in other words ideal LED phototherapy should achieve athermal and atraumatic photobiomodulation. The Arndt-Schultz law, first appearing in the mid-nineteenth century, states that weak stimuli excite biologic behavior, stronger ones favor it, powerful ones arrest it and very powerful ones retard it. This was adapted by Ohshiro and Calderhead in 1988 into the Arndt-Schultz curve to explain the efficacy of LLLT (Fig. 9)[8,12] from which it is clear that photon intensity should not be too weak (no reaction) or too strong (retardation or cell death) but must be adjusted to achieve maximum optimum photobiomodulation of the target cells or materials.

A final note on intensity: one single LED, even one of the new generation of LEDs, when used on its own, will not achieve anywhere near a clinically useful photon intensity in the target tissue (Fig. 10a). When multiple LED's are mounted close together in a planar array, however, (*cf* Fig. 3) and precisely positioned positioned according to the angle of divergence of the beam, the interaction where the beams impact with each other gives an extremely intense photon density due to the phenomenon of interference, particularly with the physical forward- and backward scattering characteristics of red and near IR light which result in the greatest photon intensity being beneath the surface of the skin, exactly

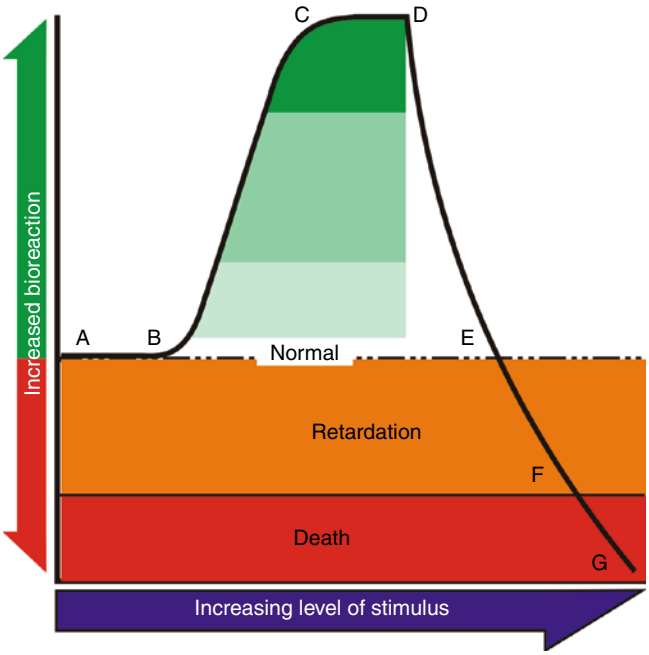

Fig. 9 Ohshiro and Calderhead's Arndt-Schultz curve (1988, [8]), based on the Arnd-Schultz law. From stimulus strength A to B, no reaction occurs: the stimulus is too weak. From B to C there is a sharp rise in bioeffect, plateauing at C-D. This curve is based mostly on incident power density (photon intensity), but the ideal combination of intensity and dose in phototherapy must therefore be attained to reach the effect shown by the dark green shaded area, preferably as much as possible at the C-D effect plateau. From point D onwards there is a sharp drop in the effect, although it is still higher than normal until point E. Strength B-E corresponds to the zone of athermal photobioactivation in Fig. 5 above. At stimulus strength E-F the bioeffect is gradually retarded, corresponding to the protein denaturation/ degradation zones in Fig. 5, and target death results from strength F-G corresponding to the coagulation and vaporization zones in Fig. 5

where it should be to achieve the optimum therapeutic effect (Fig. 10b). If the distance between the LEDs is too great, however, then the intensity will drop off dramatically because of the lack of interaction between the individual LED beams (Fig. 10c).

Dosimetry

So far, the time for which light of a given irradiance or power density is incident on a target has not been mentioned. When treatment time comes into the therapeutic equation it quantifies the dose of light delivered. When 1 W of power is incident on target tissue for 1 s, the energy delivered is 1 J. The joule in itself is a particularly useless therapeutic parameter since it expresses only power over time, and does not take into account the unit area of tissue being treated. The most important parameter for the therapeutic dose in

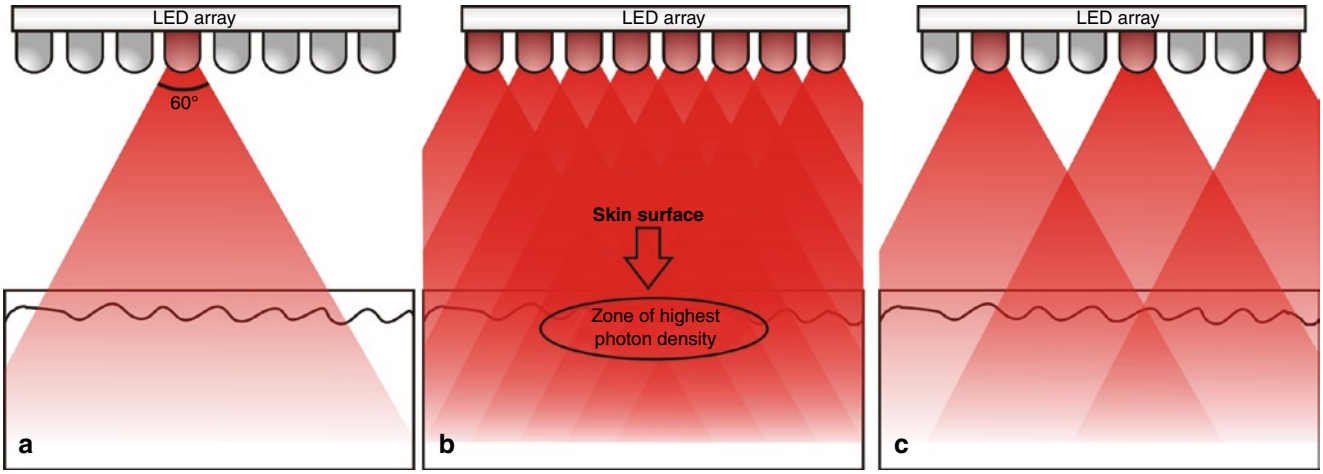

Fig. 10 Arrays of precisely spaced multiple LEDs are required to achieve clinically useful photon densities in tissue. This illustration is modeled on the actual LED array seen in Fig. 3 above, and is to scale. The distance from LEDs to the tissue is approximately 2.5 cm. (**a**) A single LED has insufficient photon intensity to achieve any recordable clinical effect. (**b**) On the other hand, when LEDs with similar output characteristics are mounted a precise distance apart to make use of the 60° divergence, the beams will interact where they cross each other to produce an extremely high photon intensity due to the phenomenon of photon interference. When this is coupled with the very strong forward and backward scattering characteristics of red light, which is even stronger for near IR energy, a zone of extremely high photon density, greater even than the intensity at the LEDs themselves, is created under the surface of the target tissue. (**c**) If LEDs are spaced too far apart, the photon intensity is sacrificed and is not clinically useful. This is the case in treatment heads with individual LEDs of different wavelengths

LED phototherapy is the energy density (ED), also known as the spectral fluence. ED is calculated as follows:

$$\mathrm{ED} = \frac{\mathrm{OP} \times t}{\mathrm{TA}} \ (\mathrm{J/cm^2})$$

where OP is the output power incident on the target in watts, t is the time in seconds and TA is the irradiated area in cm^2. ED is expressed in joules/cm^2 (J/cm^2). However, too much is often made of the dose as the most important parameter in phototherapy, and criticism has been leveled at an LED system that 'the dose is too high'. In fact, as stated in the previous subsection, it is the power density, rather than the energy density, which more than anything else determines the bioreaction, and this is illustrated in Table 3 where a constant dose of approximately 25 J/cm^2 can achieve the entire gamut of bioeffects from pure athermal photobiomodulation to severe photodestruction. The total uselessness of joules as a meaningful parameter is also illustrated: the greatest amount of energy in the table, 2,000 J, produced a phototherapeutic effect, whereas the smallest, 8 mJ (0.008 J), produced a photosurgical effect. In an experiment performed 20 years ago by the author of this chapter. The exposed rat knee joint, both encapsulated and unencapsulated, was irradiated with a GaAlAs diode laser giving an incident power density of 1 W/cm^2. A range of doses was applied from 20 J/cm^2 (20 s exposure) up to 1,800 J/cm^2 (30 min exposure). Tissue was examined macroscopically and microscopically immediately after irradiation for any signs of damage: none was found. The wounds were closed and followed up at different time points over 2 weeks. No differences were seen in coded specimens from

Table 3 This illustrates a variety of bioeffects (Δα) achieved with the same approximate energy density, or dose, of 25 J/cm^2. As can be seen from the table, the power density (PD) is the most important determinant of the bioeffect and the energy density given alone is therefore not a real determinant of effect

P	*SØ*	[*a*] (cm^2)	PD (W/cm^2)	*t*	*e*	ED (J/cm^2)	Δα
100 W	10.0 cm	78.6	1.3	20 s	2,000 J	25	–
50 W	3.5 mm	0.1	500	100 ms	5 J	25	+
10 W	1.0 mm	0.0008	1,250	20 ms	0.2 J	25	++
1 W	200 μm	0.0003	3,180	8 ms	8 mJ	25	+++
75 mW	3.0 mm	0.07	1.1	23 s	1.725 J	25	–

P incident power (units as shown), *SØ* spot size diameter (units as shown), [*a*] irradiated area, *PD* power density, *t* exposure time (units as shown), *E* energy (units as shown), *ED* energy density. Δα: graded bioeffects (+++, severe photodestruction; ++, medium photodestruction; +, mild and/or reversible photodestruction; –, bioactivation)

each group at all time points regarding morphological changes compared with the unirradiated control specimens. In pharmaceutical science, the medicine must be correct before adjusting the dosage. In phototherapy, the power density is analogous with the medicine, and the energy density is the dose. If the medicine is incorrect, i.e., a photon intensity which is too low (no effect) or too high (photothermal damage) no amount of playing around with the dose will achieve the optimum result.

Temporal Profile of the Beam

The temporal profile of a beam of light energy simply means the output mode in which the light is delivered to the target. There are two modes, continuous wave (CW) and pulsed. In CW, as the name suggests, when the light source is activated the power reaches its maximum level, from mW up to 100 W or so, and stays there till the system is switched off (Fig. 11a, *left pane*). An alternative to CW is when the beam is 'gated' to produce a train of square waves: this is often incorrectly referred to as 'pulsed' light (Fig. 11a, *right pane*). Gating can be accomplished by a mechanical shutter, or be achieved by simply switching the light source on and off. The correct name for this process is 'frequency modulation', because an exogenous frequency (the on-off sequence) is being superimposed on the inherent frequency of the beam which is predetermined by the wavelength, each wavelength having a fixed frequency. For example, near infrared at 830 nm, visible red at 633 nm and visible blue at 415 nm have 'built in' frequencies of approximately 3.6×10^8, 4.7×10^8, and 7.2×10^8 MHz. From this it can be seen that as the wavelength decreases, the frequency increases. Increased frequency is also positively associated with an increase in energy of the individual photons, expressed as electron volts (*ev*), and for the previous three wavelengths the respective photon energies are approximately 1.49, 1.96 and 2.99 *ev*. Photon energy determines the type of interaction between the incident light and skin cells. For 830 nm, as explained already, photophysical rotational and vibrational changes occur in the electrons making up the cell membrane, whereas for visible light there is a direct induction of an intracellular photochemical cascade. At very high *ev* values, such as those associated with ultrashort wavelengths, such as X- and γ-radiation, the very large photon energies result in molecular disassociation of cells with sufficient exposure, in other words the cells are literally blown apart.

In a true pulsed beam, from a high-powered laser, an extremely high peak power is reached in a spike-like waveform, with a very short pulsewidth, 1 ms or even in ns.

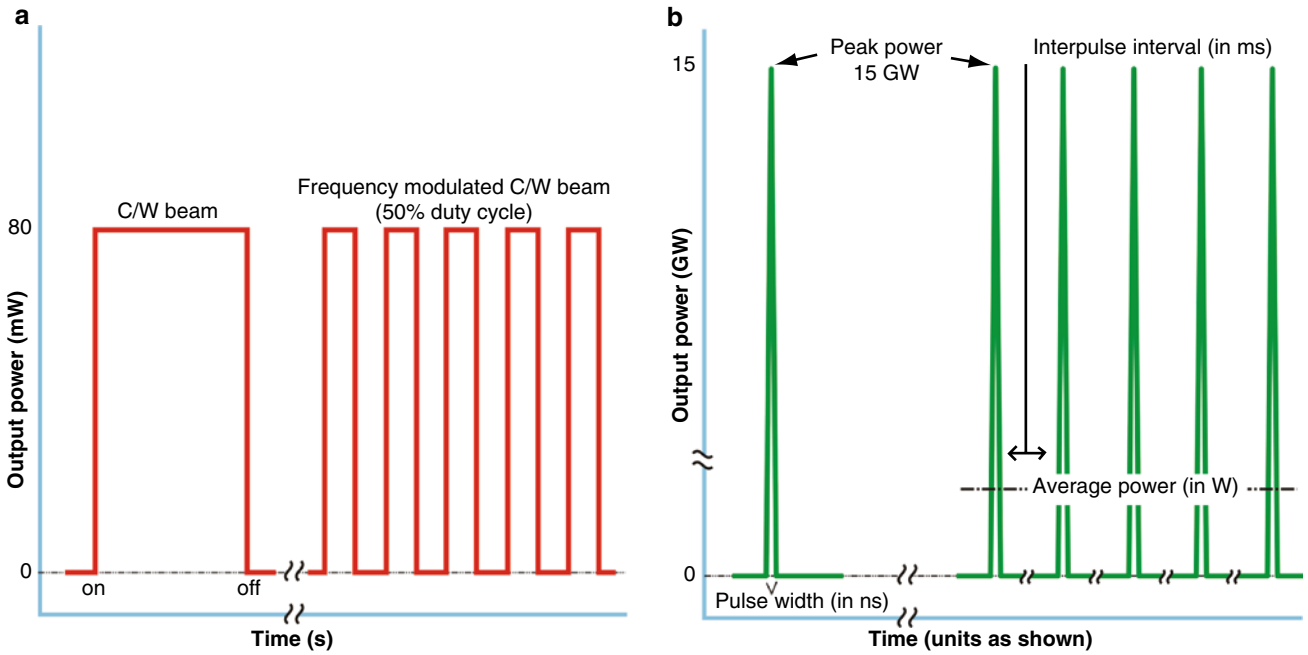

Fig. 11 Temporal profile of a beam of light. There are 2 basic profiles, continuous wave (CW) (**a**) or pulsed (**b**). In CW (**a**, *left pane*), the system is switched on, the light very rapidly reaches its maximum, and remains there till the system is switched off. This CW beam can be 'gated' mechanically or electrically, i.e. rapidly switched on and off (**a**, *right pane*), which is often incorrectly referred to a 'pulsing'. The correct name is frequency modulation. This gives a series of rectangular waveforms: a 50% duty cycle is illustrated. When a laser beam is truly pulsed, a tremendously high peak power, measured as high as gigawatts (*GW*) is released in an ultrashort pulse interval, measured in nanoseconds (ns) (**b**, *left pane*). If a train of these pulses is emitted with a comparatively long interpulse interval of milliseconds (ms) (**b**, *right pane*), then the target tissue 'sees' only the average power of the beam, measured in watts. This is called quasi-CW, also known as 'superpulsing' the beam

The peak power may be in mega- or even gigawatts.(Fig. 11b, *left pane*) If a train of such true pulses is delivered with a set interpulse interval often orders of magnitude longer than the pulse width, then the target tissue 'sees' only the average power of the beam, usually at CW output levels. This is also referred to as 'superpulsing', or more correctly, quasi-CW (Fig. 11b, *right pane*). No current LED system is capable of delivering a true pulsed beam.

LED Phototherapy: Mechanisms of Action

- LEDs are ideal for photon absorption therapy (PAT), an atraumatic and athermal direct exchange of energy raising the target's action potential
- LEDs can be successfully used in photodynamic therapy (PDT)
  - Exogenous PDT with 5-aminolevulinic acid (5-ALA) for treatment of non-melanoma skin cancers and severe photodamage.
  - Endogenous PDT in porphyrins endogenous to *Propionibacterium acnes*, for example, in the treatment of acne.

When light energy is incident on a target, the reaction in the target following absorption is known as the mechanism of action. In LED phototherapy, there are two main mechanisms of action: photodynamic therapy (PDT) and photon absorption therapy (PAT), which are totally different mechanisms of action.

Photodynamic Therapy (PDT)

PDT can be exogenous or endogenous, the better known form of which is exogenous.

Exogenous PDT

Exogenous PDT is typically defined as: "The use of a chemical, given orally, intravenously or topically (directly to the skin), that can be activated or energized by light to destroy a target tissue in which the chemical or substance has preferentially located. This activation causes the formation of new molecules and free radicals such as reactive oxygen species (ROS) which may also form other chemicals that, in turn, may destroy the targeted material to a varying extent, such as through ROS-mediated apoptosis of the photosensitized cells or closure of blood vessels feeding the target tissue." PDT is another arm of phototherapy, and whilst exogenous PDT is thus still an athermal reaction, it is not atraumatic as deliberate induction of apoptotic cell death is the main goal. The first main application for photodynamic therapy was in the treatment of certain cancers, with such photosensitizers as hematoporphyrin derivatives activated with low incident levels of laser light, particularly with visible red light such as from the HeNe laser due to this wavelength's better penetration than the shorter visible wavelengths in living human tissue.[13] This activated an oxygen-dependent phototoxic cytocidal action within the cells containing the agent, and the free radical singlet oxygen ($^1O_2$), a short-lived product from the reaction between an excited sensitizer molecule and oxygen, played a very important part in the induction of cell death (apoptosis) and destruction of the microvasculature feeding the tumor.

One of the first applications for LED phototherapy was in fact PDT for non-melanoma skin cancers (NMSCs), such as basal cell carcinomas and superficial squamous cell carcinomas, or severe sun damage such as actinic keratosis with the use of another exogenously-applied compound, 5-aminolevulinic acid or 5-ALA in any of its forms. This application continues to the present with good success and robust long-lasting results.[14,15] The topically applied 5-ALA penetrates into the dermis under an occlusive wrap, and is converted as part of the mitochondrial-based heme cycle into coproporphyrin III (Cp III), a member of the powerful porphyrin photosensitizing family. When the maximum amount of Cp III has been converted, the remainder of the 5-ALA is converted into another porphyrin, protoporphyrin IX (Pp IX). These two porphyrins become the specific targets of the LED energy at specific wavelengths, and, following photoactivation, non-selectively damage all of the superficial dermal tissue in which they exist, as illustrated schematically in Fig. 12.

When a photoreaction is desired such as in 5-ALA PDT for any purpose, an action spectrum has to be run to investigate the action potential of a range of wavelengths in the target compound. Figure 13 shows the absorption spectra of Pp IX and Cp III. There is a very large peak at 415 nm in the visible blue Soret band, but as will be remembered from the previous section on wavelength, blue light has very poor penetrative capability into the dermis, and so it would not cause deep enough damage to treat NMSCs successfully. Another much smaller peak is however seen at around 633 nm, which was used in the early days of hematoporphyrin derivative PDT for other cancer types as a much better-penetrating wavelength, thus giving a much deeper zone of porphyrin activation and hence a deeper zone and greater volume of controlled photodamage. Red 633 nm LED-activated 5-ALA has been successfully used for NMLCs and actinic keratoses, photorejuvenation and inflammatory acne vulgaris. These will be discussed in more detail in the appropriate subsection on LED phototherapy in clinical practice.

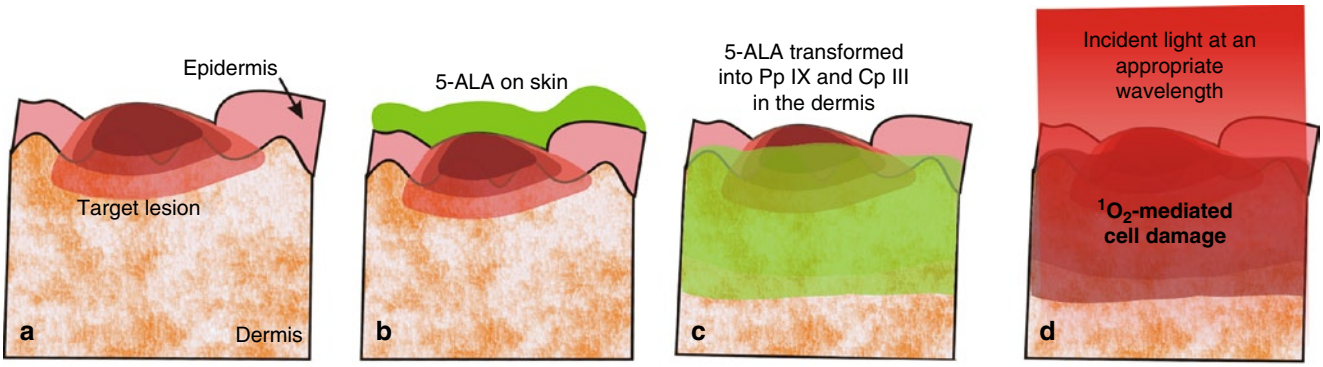

Fig. 12 Nonselective *en bloc* infiltration of skin by Pp IX and Cp III of 5-ALA origin illustrated schematically. (**a**) Target lesion in superficial dermis. (**b**) 5-ALA ointment applied topically to epidermis. (**c**) As 5-ALA penetrates *en bloc* into skin cells, it is transformed into Cp III and Pp IX, photosensitizing porphyrins. (**d**) Light at an appropriate wavelength activates the porphyrins to produce powerful but very short-acting reactive oxygen species, such as singlet oxygen, and the affected skin cells die through oxidative-stress mediated apoptosis (induced cellular destruction)

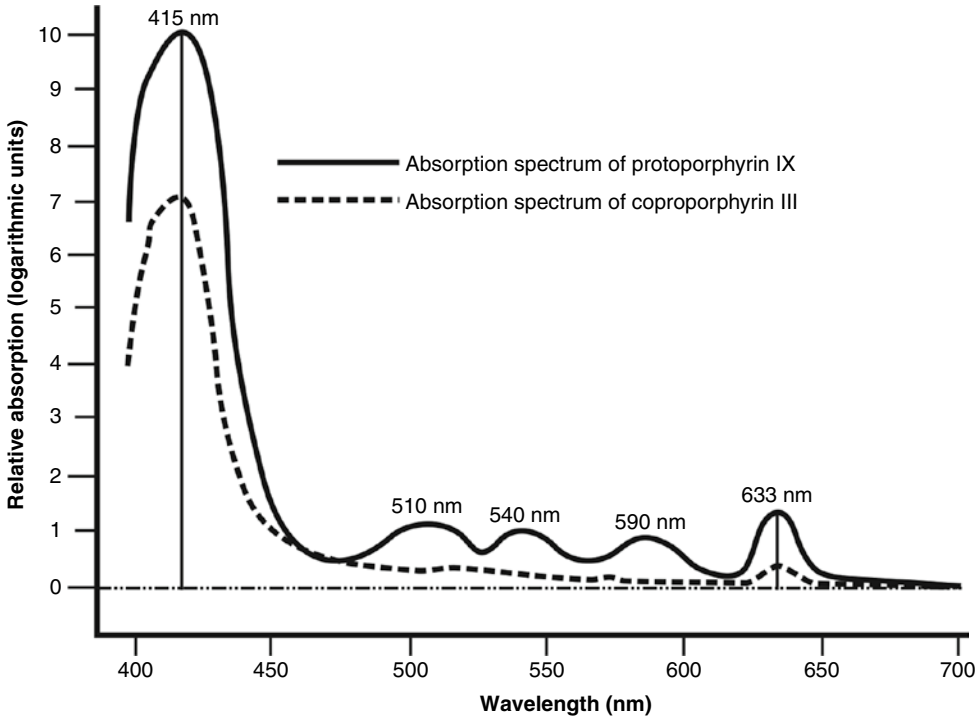

Fig. 13 Action spectra for coproporphyrin III and protoporphyrin IX. Note the extremely high peak at 415 nm, and the minor peak at 633 nm, visible red, particularly in Pp IX

Endogenous PDT

Exogenous PDT as discussed above depends on an external photosensitizer, such as 5-ALA. In endogenous PDT, the photosensitizer, or photosensitizing substances, can be found occurring naturally within the target cells or tissue. The exogenous application of 5-ALA induces the synthesis of the porphyrins Pp IX and CP III nonselectively in the epidermis and dermis under the area of application as already explained above. However, in the case of acne vulgaris the inflammatory acne lesions are associated with the presence of their causative bacterium, *Propionibacterium acnes* (*P. acnes*). It has been well demonstrated that both Pp IX and Cp III are endogenous to active *P. acnes*, and the more active is the bacterium, the higher the porphyrin concentration.[16-18] Referring again to Fig. 13, maximum photoactivation of both Pp IX and Cp III occurs at around 415 nm. Light at that wavelength, with a high enough photon intensity, could therefore achieve activation of the porphyrins within the *P. acnes*, thereby selectively destroying or at least severely damaging the *P. acnes* through

oxidative stress-induced apoptosis,[19] but without harming the surrounding skin cells. Endogenous PDT could therefore be applied in the light-only treatment of inflammatory *P. acnes* lesions without the need for any exogenous 5-ALA. This will be discussed in more detail in the appropriate part of the following section.

Photon Absorption Therapy (PAT)

Basically the majority of the information in subsections 1 and 2 has been based on the concept of *photon absorption therapy* (PAT) also known as photoactivation therapy, and this approach completely fulfills the definition of phototherapy, namely direct cellular activation in an athermal and atraumatic manner which was the umbrella mechanism of action long-associated with LLLT (low level laser therapy) over its more than 30 plus-year history. Atraumatic and athermal PAT thus differs from PDT which actively seeks to damage the target cells and tissues, although still in an athermal manner. As has already been discussed in subsection 2:2 on wavelength, near infrared and visible light have different absorption targets (cell membrane and subcellular organelles, respectively) but the end result is the same, and the energy level of the cell is raised by both near IR and visible light of appropriate wavelengths through direct absorption of the incoming photon energy, which is then transferred to the receptor cell with no loss through heat or luminescence. The main mechanism of action is connected with increased adenosine triphosphate (ATP) production and increased Ca^{++} ion intra- and intercellular signalling.[20]

Under PAT, three things can happen to the energized cell: if it is compromised or in some way damaged, the cell will heal much faster following PAT; if the cell is designed to perform some specific function, such as fibroblast collagenesis and elastinogenesis, then the PAT-treated cell will perform these functions better and faster; finally, if the cell is designed to replicate, then it will replicate faster.[10] These may happen singly, or in combination, and form the basis of the three decades of LLLT literature in which some, but not all, of the mechanisms under the umbrella of PAT have already been at least partly elucidated. In addition, in the past 3 years in particular, a good number of solid clinical and basic science papers have corroborated the previous LLLT findings for LED phototherapy, and some exciting new science on LED PAT was published in 2007. PAT can be used in combination with other conventional modalities to improve results and hasten healing time, and can also offer a very interesting combination with PDT in the treatment of inflammatory acne vulgaris. Once again, a detailed discussion will be found in the following section.

LED Phototherapy in Clinical Practice

- Combination LED therapy is the key to clinical efficacy.
- One wavelength will not target everything optimally.
- Based on the published peer-reviewed literature, a combination of wavelengths is necessary for effective LED phototherapy.
  - 633 nm has proved effective in 5-ALA PDT for non-melanoma skin cancers
  - Blue 415 nm endogenous PDT combined with red 633 nm PAT applied sequentially has been reported as a very effective light-only therapy for moderate to severe acne vulgaris.
  - Combined near infrared 833 nm and 633 nm red PAT, applied sequentially, has been reported as very effective in skin rejuvenation and all aspects of wound healing.
  - 595 nm yellow light has shown efficacy in collagen synthesis for skin rejuvenation and the treatment of rosacea.
  - Adjunctive LED phototherapy will complement any and all existing conventional modalities which alter in any way the architecture of the skin to achieve the desired clinical result.

Good basic science is of course extremely important to understand how LED phototherapy can be applied in current dermatological practice, to help bolster up evidence-based medicine from the practical clinical arena. However, a thorough understandings of the true capabilities, and indeed limitations, of LED phototherapy in clinical practice is even more essential to go about amassing the required evidence-based medicine in the most efficient manner. A large number of LED-based systems is commercially available now in the USA and world-wide, but a very, very small number has actually made it into the peer-reviewed literature with the vast majority of manufacturers content to ride on the coat-tails of the companies who have done the actual work, both basic science and controlled clinical trials, even though the science of these bandwagon-jumping systems is sketchy at best, and nonexistent or even erroneous at worst. Of particular concern are the 'look-alikes' of far-east origin, particularly China, based on proven systems but with inferior quality LEDs which give neither the rated wavelength, nor sufficient and stable output power. Some of these systems mimic the free-standing planar LED-based units, and others are small hand-held devices with a mesmerizing array of pretty flashing multicolored LEDs, designed for the home-use market. These groups of 'toy' systems are doing more harm than

good to the reputation of LED phototherapy, although one hopes that they are not visiting actual harm on patients with 'no effect' hopefully being the worst that they achieve. The negative impact on 'good' LED phototherapy is, however, very large. Hopefully this entire chapter will go some way to redressing that. Another important point for anyone considering purchasing an LED phototherapy system is that some LED system manufacturers claim that their LEDs are 'NASA technology'. This is totally misleading. Although the new generation of LEDs is based on the 'NASA LED', they are not actual NASA technology, and some of the LEDs thus described are still of the previous generation: *caveat emptor*! The reader must always bear in mind that with LED phototherapy, 'any old LED will ***NOT*** do'.

The treatment categories dealt with in this very important section are based on published literature, not so-called 'white papers', and so the reader can obtain the original articles from online indexing sources such as PubMed, and see in detail what has, and what has not, been scientifically proven and clinically corroborated. The author would like to point out that any suggested treatment protocols are inserted only for example and guidance, and must not be taken as concrete. Manufacturer's recommendations are also only recommendations, and the reader should look to the published literature for more detailed and accurate treatment protocols. It is the hope of the author that the reader will see the true possibilities of LED phototherapy to enhance his or her clinical practice, and will moreover choose an LED system based on the criteria which will appear throughout the section, rather than on hype, pretty flashing colors and pseudo-science. If the actual systems referenced seem to be extremely limited, that is because they are the only ones which have been published in the literature, and the author offers no apologies for this. He can only demonstrate and present to the reader what has been published on systems which have met or exceeded the required criteria.

Non-melanoma Skin Cancers (NMSCs) and Actinic Keratosis

NMSCs, including Bowen's disease and basal cell carcinoma, were the first entity to be treated with LED PDT using specifically-designed 633 nm LED-based system to activate 5-ALA, and the pioneering company was Photo Therapeutics (Fazeley UK and Carlsbad, CA) with their Omnilux® PDT™ system. Having established that an effective activation peak for the relevant porphyrins created from exogenous PDT existed at around 633 nm, a UK company in 1996, working in tandem with the British Cancer Research Council, developed the Paterson Lamp, a filtered xenon-powered lamp which delivered most of its light energy at 633 nm, to be used with exogenous 5-ALA PDT in the treatment of NMSCs.[21] The lamp was the brainchild of Dr. Colin Whitehurst, and it was he who saw the potential for using the new generation of LEDs which became available in 2000 following Whelan's work with the NASA Space Medicine program referenced earlier. The new generation of LEDs emits quasimonochromatic light and do not require filtering, plus they can be mounted in planar arrays to irradiate large areas at the same time, such as the entire face. Dr Whitehurst then helped found Photo Therapeutics, who built the first large-array 633 nm LED therapy source for LED PDT for the treatment on NMSCs. Large-scale clinical trials in the UK and elsewhere in Europe gave excellent results.[22]

The basic protocol which has evolved for 633 nm LED PDT for NMSCs is as follows: please note that differences in LED systems and photosensitizer do not make this an absolute protocol, and the recommendations of the manufacturer of both the LED system being used and the photosensitizer being applied must always be studied and carefully followed. Following thorough cleaning of the treatment area, 5-ALA of the appropriate strength (usually 20%) is applied, and occluded with sterile cling film for the recommended incubation period (up to several hours, depending on the lesion being treated). At the end of incubation, the occlusive dressing is removed and any excess 5-ALA wiped off. Activation of the porphyrins induced in the target tissue is then achieved with 633 nm light, with a dose usually around 96 J/cm^2. This can be extremely painful, and some kind of forced air cooling may be applied during this phase. Following activation, the wound is dressed, and the patient returns after 24 h for dressing removal and the situation is then followed for 4–6 weeks. In a large percentage of lesions, recurrence is not a problem. Persistent lesions are retreated till no recurrence is seen. Figure 14 shows a typical example of 633 nm LED PDT for an NMSC.

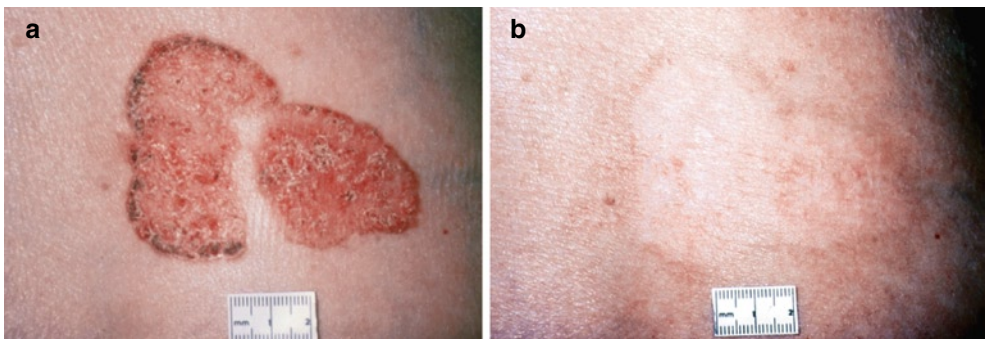

Fig. 14 A basal cell carcinoma before (**a**) and 4 weeks after 633 nm 5-ALA PDT (**b**) (20% 5-ALA, 5 h incubation, 20 min activation at approximately 96 J/cm^2 System used, Omnilux® PDT™, photographs courtesy of Colin Morton MD, Falkirk, Scotland)

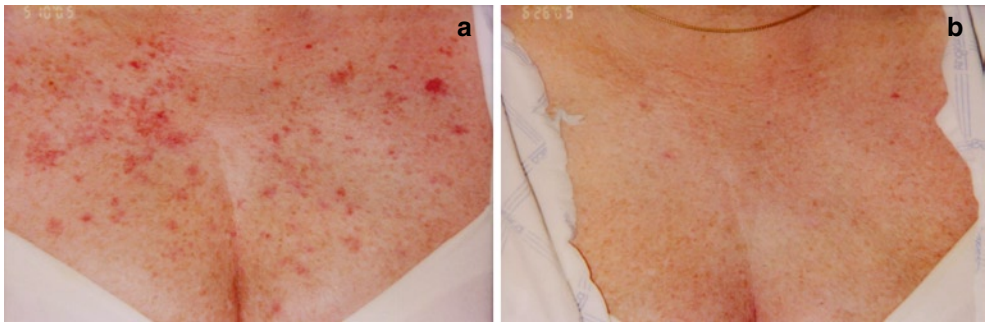

Fig. 15 Actinic keratosis on the décolleté of a 45 year-old female before (**a**) and just over 6 weeks after 633 nm 5-ALA PDT (**b**) (10% 5-ALA, 30 min incubation, 20 min activation at approximately 96 J/cm^2. Same system as in Fig. 14, photographs courtesy of Colin Morton MD, Falkirk, Scotland)

In the case of actinic keratoses (AKs), which are much more superficial than NMSCs, a much lower concentration of 5-ALA is applied with a shorter incubation time. The protocol is otherwise the same and the activation dose is still recommended to be around 96 J/cm^2. Figure 15 shows AK on the upper sternum of a female patient before and after 633 nm LED PDT. One treatment usually suffices for AKs.

Acne Vulgaris

Acne vulgaris still represents a major problem for the practicing dermatologist, despite advances in clinical and medical therapy. Many approaches have been tried with varying degrees of success, but results are inconsistent, even in the same regimen with the same patient. If untreated, or treated improperly, active ace almost always leads to unsightly acne scarring, as disfiguring and psychosomatically troublesome as the active lesions, but more difficult to treat. It therefore made sense to attack and eradicate acne while at the active stage, before scarring was an issue. In addition to the conventional approaches, LED PDT with 633 nm and narrowband blue light LED and non-LED sources at around 410–425 nm attracted attention with good results, but with some downtime and pain associated with the activation stage of the photosensitizer.[23-25] The recurrence rate was still rather high, however. The development of quasimonochromatic LEDs at the peak wavelength of 415 nm offered a new approach, given the extremely high peak in the activation spectra of Pp IX and Cp III, both of which porphyrins are endogenous to active *P. acnes* as already discussed above. With a high enough photon intensity at 415 nm it would therefore theoretically be possible to activate the endogenous porphyrins in *P. acnes* selectively, thereby disabling or eradicating the *P. acnes*.[26,27]

In order to understand why the blue light therapy on its own was achieving good results, but with a still unacceptably high recurrence rate, the etiology of acne must be considered. Acne is often considered as an inflammatory disorder, full stop, with colonization of blocked follicles by *P. acnes* as the main culprit. In fact, acne is multifactorial with major influences other than merely inflammation, such as hormonal and autoimmunological imbalances. Acne is the result of the establishment of a vicious circle set up between *P. acnes* and some t-cells originally homing into the site to help the defence system, but ultimately converted by *P. acnes* to the black side as 'rogue t-cells'. Whereas 415 nm will precisely target the *P. acnes* via the endogenous porphyrins and thereby remove one of the major causes of the inflammation, the rogue t-cells and any hormonal imbalance remain untreated by the 415 nm light, thus leaving the vicious circle unbroken and paving the way for recurrence at some stage in the near future. If light-only therapy for acne were to work well and with robust results, it would therefore be necessary to find another approach whereby the targets not dealt with by the blue light could be attacked with another wavelength. A very interesting paper appeared from Papageorgiou and colleagues in which they achieved excellent and long-lasting results in acne treatment with a combination of filtered blue (415 nm) and red (660 nm) non-LED light applied simultaneously.[28] It was then suggested that sequential rather than simultaneous application of blue and red light might have an even better effect through selective targeting of the different cellular and subcellular wavelength-specific targets, and the quasimonochromatic nature of LED therapy would assist in precise targeting. Two clinical papers were published in 2007 using this sequential approach of 415 nm LED light-only therapy followed by 633 nm red LED treatment, repeated over a 4-week period. One patient group was Caucasian[29] and the other Asian,[30] and both groups had a meaningful number of patients (>25) with a good selection of Burton grades 3–5, representing moderate to severe inflammatory acne. The system used in both studies was the Omnilux® with the blue™ (415 nm) and revive™ (633 nm) heads, and the same protocol was followed in both the USA and Korea study centers. A 2 week washout was imposed for anyone on oral medication. A comedonal scrub was recommended before each treatment session. The blue head was applied first for 20 min, followed at least 48 h later by the red head. This was repeated for 4 weeks. Assessments were performed at pretreatment baseline, at each of the 4 weeks during treatment, and then at 4, 8 and 12 weeks after the final treatment session.

The most interesting point in both studies was that the improvement obtained after the final treatment session, which ranged from 50% to 60% clearance of inflammatory lesions, continued to improve up to 12 weeks after the final treatment with no other therapeutic intervention, reaching from 83% to 90% clearance, and if extrapolated beyond the trial period would have in many patients reached 100%, which from personal communication with the authors of both papers, it in fact did. Figure 16 is a graphic representation of the inflammation reduction curves of the two referenced papers.

No secondary hyperpigmentation was seen in any patients in both studies, which is of particular interest in the Asian skin type. In addition, overall skin condition was subjectively assessed to have improved, and in the case of the Asian population, skin lightening was objectively shown across the population with an instrumental assay. Figure 17 shows examples of the treatment efficacy courtesy of the authors of the papers. At 6 months after the final session, recurrences in both trial centers were extremely few and mild, easily treated with another regimen of the blue/red LED therapy (David Goldberg and Celine SY Lee, personal communication).

As with all approaches not involving excisional surgery, there will always be a small percentage of patients in whom light-only LED phototherapy for acne vulgaris will have disappointing results, but from the above studies the overall efficacy is high enough to warrant applying this approach as the primary treatment of choice. Sequential combination LED phototherapy for acne can be combined with other approaches with even better results and improved maintenance, provided none of these involves any kind of photosensitizing agents, any of which have the potential to create painful and possibly severe side effects. The validity of the addition of a third LED wavelength to the current protocol, namely near infrared at 830 nm with its own unique cellular and tissue targets, is currently being assessed in ongoing clinical studies.

Skin Rejuvenation

Skin rejuvenation and antiageing have become very 'hot' topics. Excessive skin exposure to solar UVA and UVB brings about damaging morphological and metabolic changes in the epidermis and dermal extracellular matrix (ECM), combining with and accelerating the effects of chronological ageing and resulting in the lax, dull and wrinkled appearance of 'old' skin. Oxidative stressors such as singlet oxygen are photochemically generated following absorption of UV radiation in the ECM and damage the matrix integrity with elevated levels of the matrix metalloproteinases (MMPs) 1 and 2, formerly known as collagenase and gelatinase; elastotic damage to the underlying connective tissue occurs, with interstitial spaces appearing in a poorly-organized matrix; the viscosity and quality of the ECM ground substance glycosaminoglycans is reduced; and an inflammatory infiltrate can be identified. As this damage is cause by light, an elegant concept to use the power of light to reverse the damage led to the application of lasers, usually the CO_2 or/and the Er:YAG, in what became known as ablative laser resurfacing. Although still regarded as the 'gold standard' in the rejuvenation of

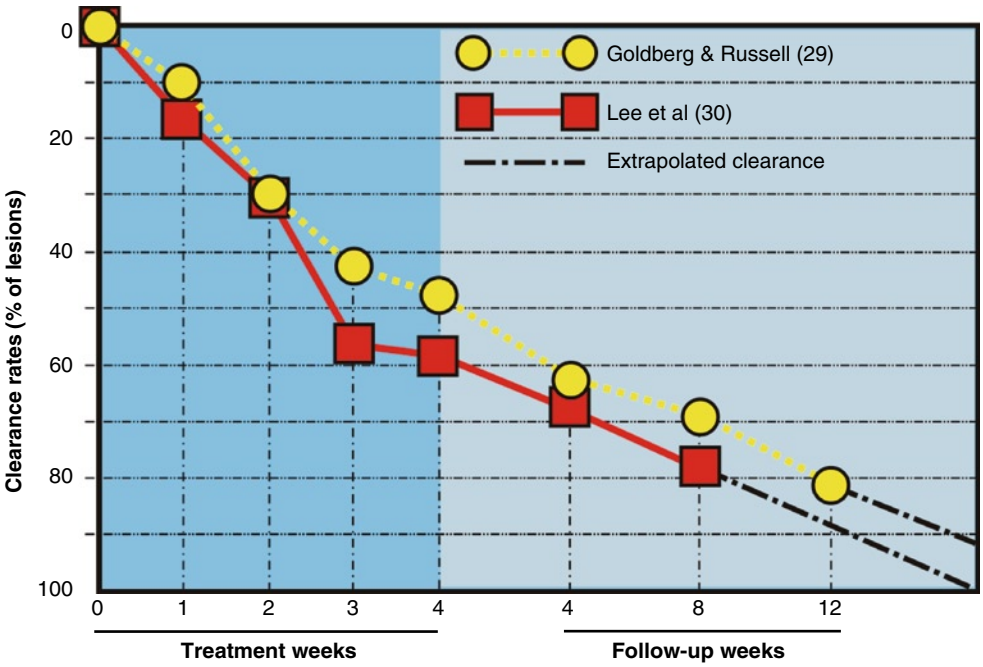

Fig. 16 Inflammatory lesion clearance rates following the blue/red combination LED phototherapy for acne adapted from the studies by Goldberg and Russell[29] and Lee et al.[30] The Goldberg study had a 12-week follow-up after the final treatment, i.e. 16 weeks from baseline, whereas the Lee study had an 8-week follow-up (12 weeks from baseline). However, by extrapolating the clearance rates in both studies, which were clearly linear in nature, the continued improvement is evident. No other therapy was used in either study. The system used was the Omnilux® fitted with the blue™ and revive™ heads

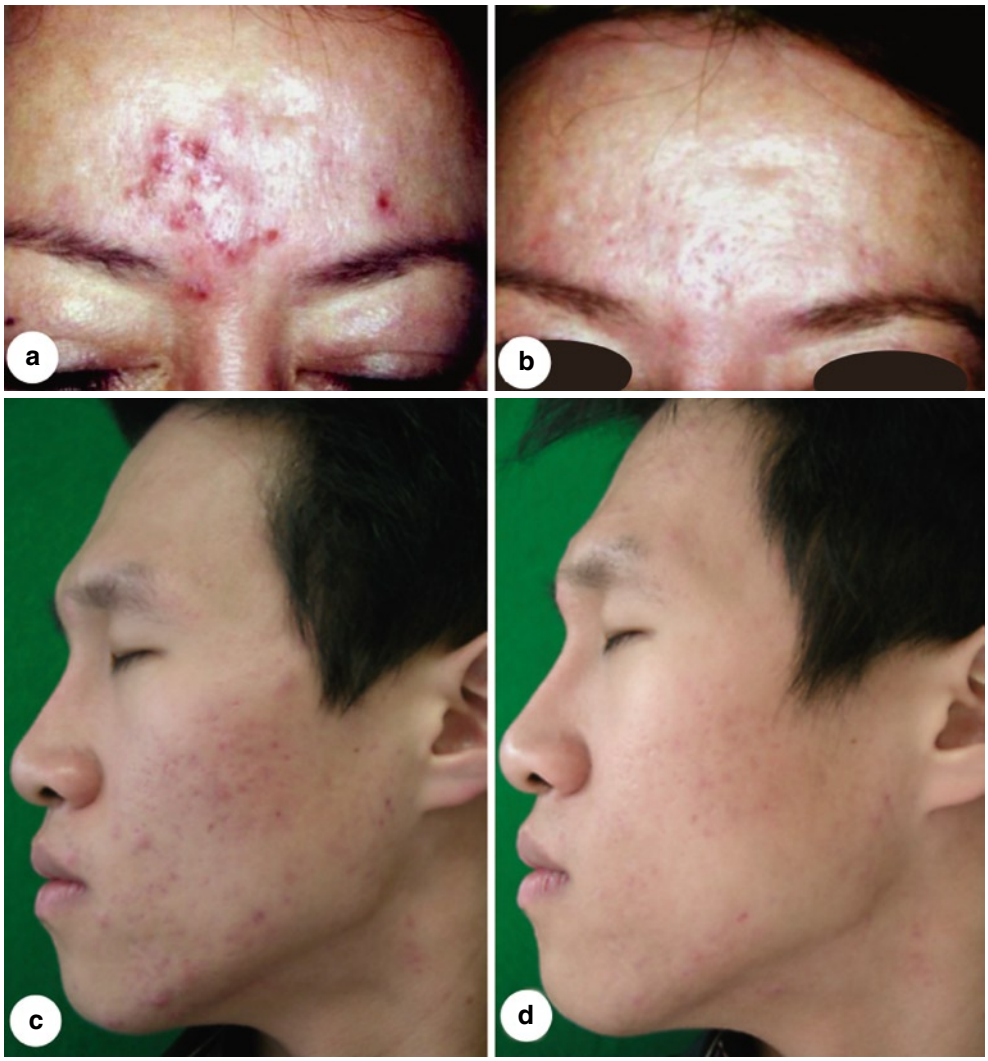

Fig. 17 Representative examples from the Goldberg and Lee studies on light-only combination blue/red LED phototherapy for inflammatory acne. (**a**) Cystic acne at baseline in a 21-year-old female, skin phototype II, from the Goldberg and Russel series. (**b**) Six weeks after the final treatment session (10 weeks from baseline). Excellent clearance and very good cosmesis. Photographs courtesy of Bruce Russell MD. (**c**) Inflammatory acne on the cheek and jaw line of a 19-year-old Korean male patient from the Lee series, skin type IV. (**d**) Eight weeks after the final treatment session (12 weeks from baseline). Good clearance with no secondary hyperpigmentation, a major problem in the Asian skin. The remaining small areas of redness will fade with time (Photographs courtesy of SY Celine Lee MD. Same system used in both trials as in Fig. 16)

severely photoaged skin in general and wrinkles in particular, the possibly severe side effects and a prolonged patient downtime of up to several months associated with this approach drastically reduced its popularity.

To attempt to overcome these problems, so-called nonablative resurfacing was developed using specially adapted laser or intense pulse light sources. The theory was to deliver a controlled zone of deliberate photothermal damage beneath an intact epidermis, so that the wound-healing processes, including collagenesis and remodeling, could occur under the undamaged epidermis, thereby obtaining rejuvenation of the skin without any patient downtime and was popularized as the 'lunch-break rejuvenation'. The theory was good, but in clinical practice patient satisfaction was very low,[31,32] because the good dermal neocollagenesis seen in post-treatment histological analysis was not reflected in a 'younger' epidermis.[33] In an attempt to bridge this gap between ablative and pure nonablative rejuvenation, so-called fractionated or fractional technology was developed whereby many spots of almost grossly invisible epidermal and dermal 'microdamage' were delivered via a scanner or 'stamp-type' head, all surrounded by normal epidermis and dermis to obtain swift reepithelialization and dermal wound healing.[34] Unfortunately, once again the clinical results were not satisfactory to the majority of patients, with good dermal neocollagenesis not being echoed in the epidermis. In both the nonablative laser/IPL and the first generation of fractional technologies, the big problem was that what the patient first sees when looking in a mirror is the *epidermis*, not the dermis. It does not matter to the patient (or her friends) that her dermis is wonderfully better organized if her epidermis remains unchanged, what the author refers to as the SOE syndrome – 'same old epidermis'. Recognizing this, manufacturers of the more recent second generation of fractional systems have returned to the orginal ablative wavelengths, the CO_2 and the Er:YAG, in addition to newer media such as Er-doped fiber, to deliver fractionated microbeams that visibly damage the skin, with a recognizable amount of erythema and some edema post-treatment.

This in some way takes us back towards our gold standard of ablative resurfacing, as once again heat deposition, combined with controlled epidermal damage, becomes a pivotal consideration to achieve the ideal rejuvenation results on a patient-by-patient basis.[35] This approach has been much more successful from the patient satisfaction criterion, although at the cost of a little downtime, because it is involving the epidermis more than the previous nonablative and fractional approaches.

In the meantime, other clinical researchers were wondering if there was a role for LED phototherapy in skin rejuvenation, and the first approach was to use a lower strength of topically-applied 5-ALA activated with 633 nm LED in LED PDT.[36] The results were good, but begged the question as to why more damage, and indeed some pain, should be inflicted to treat what was essentially comparatively mild skin damage. One approach has been to deliver the 5-ALA at very low concentrations (<2%) via liposomes and activate the target tissue using intense pulsed light, achieving complete quenching of the porphyrins and thus avoiding the side effect of residual photosensitivity.[37,38] Because of its totally noninvasive, athermal and atraumatic nature, light-only LED phototherapy for skin rejuvenation has also attracted attention first with a single wavelength system in the visible yellow,[39] but once again a sequential combination technique proved more effective than the single wavelength just as was the case with LED phototherapy for acne.[40,41] The wavelengths used for LED skin rejuvenation have been near IR at 830 nm applied first, followed by 633 nm 72 h later, repeated over 4 weeks. The reasons for these wavelengths and the order in which they are applied are photobiologically based on the precepts of the wound healing cycle, and will be covered in some detail in the next subsection dedicated to wound healing. Both of these wavelengths involve the mother keratinocytes in the basal layer of the epidermis, however, in addition to the target dermal cells, with beneficial effects to both the cellularity and organization of the epidermis, but with no heat and no damage.

Lee and colleagues, in the first and only really detailed controlled study in the peer-reviewed literature, which was published in the very prestigious *Journal of Photochemistry and Photobiology (B)*,[42] compared LED skin rejuvenation in a total of 76 patients randomly assigned to four groups: 830 nm LED therapy on its own, 633 nm LED therapy on its own, the combination therapy with 830 nm and 633 nm and a sham irradiated group. All patients were treated hemifacially, so there was intrapatient as well as intergroup controls. In addition to clinical photography and subjective patient assessment, Dr Lee tested the results with profilometry and instrumental measurement of skin melanin and elasticity. She also carried out histological, immunohistochemical and biochemical assays. She found that wrinkles and skin elasticity were best improved in the 830 nm-treated groups, skin lightening was best in the 633 nm group, so the combination of the two wavelengths was able to achieve the best overall efficacy and high patient satisfaction with the results, with statistical significance seen between all treated groups and the sham-irradiated controls, and a statistically significant improvement between the treated and occluded sides in all of the experimental groups, but not in the sham irradiated group. The clinical photography was backed up by the histological findings for both collagenesis and elastinogenesis, which was proved to take place in all dermal layers down to the deep reticular dermis. No MMP activity was noted, and on the contrary the levels of tissue inhibitors of MMPs (TIMPs) one and two were significantly elevated in all treatment groups, suggesting a photoprotective effect against degradation of the newly-formed extracellular matrix. This was an excellent and thorough study, and the author recommends the reader to get hold of it and read it, all 17 pages of it. It will go a long way to convincing even the most skeptical of the real efficacy of combination LED skin rejuvenation, backed up with real science. An even more recent discussion on the 830 nm/633 nm LED combination has appeared in Viewpoint 3 (Trelles, Mordon and Calderhead) and Comment three (Goldberg) in an article on redressing UV-mediated skin damage in Volume 17 of *Experimental Dermatology*.[43] The wavelengths and systems that have been reported in the five studies cited above are 595 nm (Gentlewaves®, Light Bioscience, VA, USA)[39] and the 830 nm/633 nm combination (Omnilux® plus™ and revive™, respectively: Photo Therapeutics, Fazeley, UK, and Carlsbad, CA, USA).[40-43] Figure 18 shows examples of the efficacy of light-only combination LED skin rejuvenation, including histological findings from the Lee study demonstrating photorejuvenation of both the dermis and epidermis at only 2 weeks after the final treatment session: as remodeling progresses, these histological results will become even better. The important point is the epidermis also shows improved morphology and not just the dermis, thus avoiding the SOE (same old epidermis) syndrome which was the major problem with photothermal nonablative skin rejuvenation. As with LED phototherapy for acne, adjunctive complementary treatment and maintenance techniques will certainly improve the good results consistently shown for light-only LED skin rejuvenation in these studies.

Wound Healing

Wound healing underpins all applications of LED phototherapy involving photon absorption therapy (PAT), and plays a major role in obtaining good cosmetic results in combination LED PDT/PAT for the treatment of acne, and in LED skin rejuvenation, in addition to the treatment of traumatic

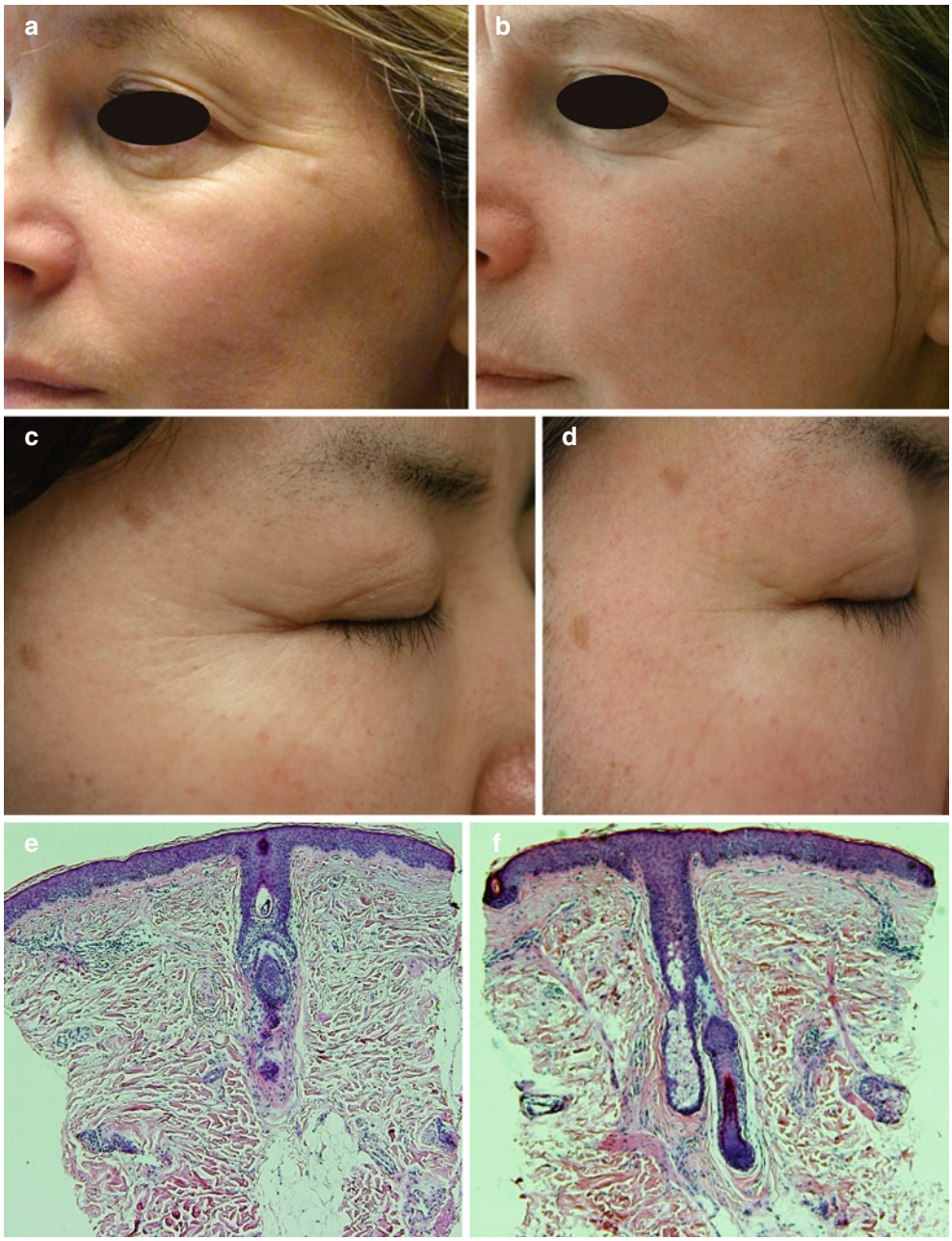

Fig. 18 Representative examples of combination near IR/red light-only LED skin rejuvenation. (**a**) A 29-year-old female, skin type II, at baseline: note the mild rosacea on her cheek. (**b**) The result at 6 weeks after the final treatment session (10 weeks from baseline). Smoothing of the periocular wrinkles can be seen, with overall better skin tone. The rosacea has almost gone. Photographs courtesy of Bruce Russell MD.[40] **c:** Baseline findings in a 26-year-old Korean female, skin type IV. (**d**) Result 12 weeks after the final treatment session. Excellent removal of the fine 'crow's feet' wrinkles and overall improvement and lightening of the skin tone. (**e**) Histological findings at baseline, showing a typical elastotic dermis under a thinned epidermis with a highly disorganized stratum corneum. (**f**) Histology at only 2 weeks after the final treatment session. Note the much better-organized dermal collagen, extending down into the deeper reticular dermis, and the highly visible Grenz layer running under and attached to the basement membrane at the dermoepidermal junction. The epidermis is much thicker with good cellularity and a very well-delineated stratum corneum. (Hematoxylin and eosin, original magnification ×200) (Photographs and photomicrographs courtesy of SY Celine Lee MD.[42] The system used in both studies was the Omnilux® with the plus™ (830 nm) and revive™ (633 nm) heads)

or post-surgical wounds themselves. A brief overview of the wound healing process is therefore warranted. Three distinct phases make up the wound healing process, namely inflammation, proliferation and remodeling, and although they are distinguished by their timing and cellular components, there is always some degree of overlap between them.

The Three Stages of Wound Healing

Inflammation is often regarded as a major problem, but in the wound healing process it is absolutely essential that inflammation occurs before proceeding into the proliferative phase. Inflammation only becomes a problem when it is out of control, such as the end product of the vicious circle instigated by *P. acnes* and rogue t-cells in acne vulgaris.

In the inflammatory phase, from wounding until about day 3–5, mast cells (already present or recruited through chemotaxis), macrophages (already present, recruited or differentiated from monocytes or pericytes) and neutrophils (recruited or differentiated from hematopoietic stem cells) peak in the wound and surrounding tissue. The macrophages ensure that all debris and detritus from the wound are removed through engulfment and internalization, and the leukocytes

are the first line of defence of the autoimmune system against invading pathogens. When they are at work, the macrophages release an important trophic factor, fibroblast growth factor (FGF), and leukocytes are associated with TGFα and β (transformational growth factor). Connective tissue mast cells are granule-filled cells differentiated from CD34-expressing bone marrow precursors which circulate in the ECM till they mature *in situ*, found normally around capillaries and arterioles. Their part in the wound healing process is to release their granules into the ECM. Although the majority of the granules are proinflammatory, which are amongst the first to be released, the later granules contain antiinflammatory chemokines and cytokines, chemotactic factors to recruit more wound-healing cells to the area, and the most powerful antioxidant endogenous to our bodies, superoxide dismutase (SOD). In fact, the mast cell was first described and named by the German physiologist Paul Ehrilch in the latter part of the 1800s. Mistakenly believing that the purpose of the granules was to nourish the ECM, he called the cells '*mastzellen*', (German for 'feeding cells'), giving us our Anglicized version. The combined efforts of the inflammatory stage cells thus leave the ECM in an ideal and favorable condition for the proliferative stage cells.

In the proliferative stage, from around day 4 to day 21, as the inflammatory stage cells decrease in number, fibroblasts and endotheliocytes peak. Fibroblasts, (already in the area or differentiated from pericytes), are an extremely important multifunctional cell. They are not only responsible for synthesizing collagen to replace damaged ECM collagen fibers, but they also produce new elastin to form elastic fibers and additionally manufacture the ground substance, the glycosaminoglycans and glycoproteinous viscous gel-like liquid which lubricates and hydrates the ECM, and which also facilitates intercellular signalling. It is also their task to maintain ECM morphological integrity through constantly monitoring the state of the collagen and elastic fibers, lying along which they can often be seen. In this respect, the quality of both proliferative wound repair and the final wound appearance rests firmly on the back of the fibroblast. Endotheliocytes, (already present in the wound or differentiated from endothelial progenitor cells) clump together to start the neovasculogenesis process, culminating in the repair of damaged blood vessels and production of new blood vessels to oxygenate the newly-forming ECM and provide essential nutrients. From a peak at around day 12–18, the increased number of fibroblasts and endotheliocytes gradually returns by day 20–22 to the pre-wound baseline, leaving the ECM in a regenerated state with newly formed clumps of collagen and elastin fibers, a fresh supply of glycosaminoglycans and well-vascularized.

In the final and much longer stage of the wound healing process, remodeling, which starts around day 19–23, these new fibers and structures gradually mature and are slowly reorganized into better alignment to give a strong, flexible and resilient ECM under an epidermis firmed and tightened by the Grenz layer of collagen fibers running under and attached to the dermoepidermal junction basement membrane. One of the transformational cells of great importance in this phase is the myofibroblast, fibroblasts with smooth muscles at each end of their longitudinal axis. These cells lie along the newly-formed collagen fibres and exert force on them to bring them into good linear alignment. The remodeling process can take up to 6 months, or even longer, to complete, and this is important when thinking of patient education regarding when they can anticipate the final optimal appearance of their treated tissue. Once they have completed their task, the myofibroblasts enter apoptosis and die off, whereas the excess fibroblasts differentiate into quiescent fibrocytes. Figure 19 illustrates in schematic form the time course of the wound healing process, showing the peaks and lows of the cells associated with each of the three phases.

The Influence of Different Wavelengths of Light on the Wound Healing Cells

When we consider LED phototherapy, it is very tempting to go ahead and invent 'new' wavelengths for 'new' photoprocesses. It must never be forgotten that LLLT, laser therapy, has a rich and well-documented history which extends back over the last three decades, so by examining this wealth of published literature it should be possible not to have to reinvent the wheel all over again. Sadly, because the US Food and Drug Administration did not grant 510k approval to a laser therapy system in the process erroneously called 'biostimulation' until 2002, there is not a lot to be found in the US literature until more recently. However, those early US papers which are there, have been quietly forgotten, probably on the principle that if one doesn't understand it, one simply ignores it.

A great deal of literature exists on red light-cell reactions, because the mainstay light source of the early pre-LED investigators was the HeNe laser, delivering 632.8 nm, basically the same as the 633 nm of current array-based LED systems, also in continuous wave (C/W) rather than frequency modulated as discussed already. As mentioned in the Introduction, the effect of red light specifically on subcellular organelles was first published by Fubini and colleagues in the late eighteenth century! The last three decades, however, have added tremendously to the knowledge regarding red light and skin cells. It was reported that 633 nm red light from the HeNe laser induces fibroblast monosheet formation *in vitro* faster and with much better alignment, almost double the speed of the unirradiated controls.[44] Furthermore, a 'wound' created in the monosheet was repaired much faster in the HeNe-irradiated groups. More recent *in vivo* work

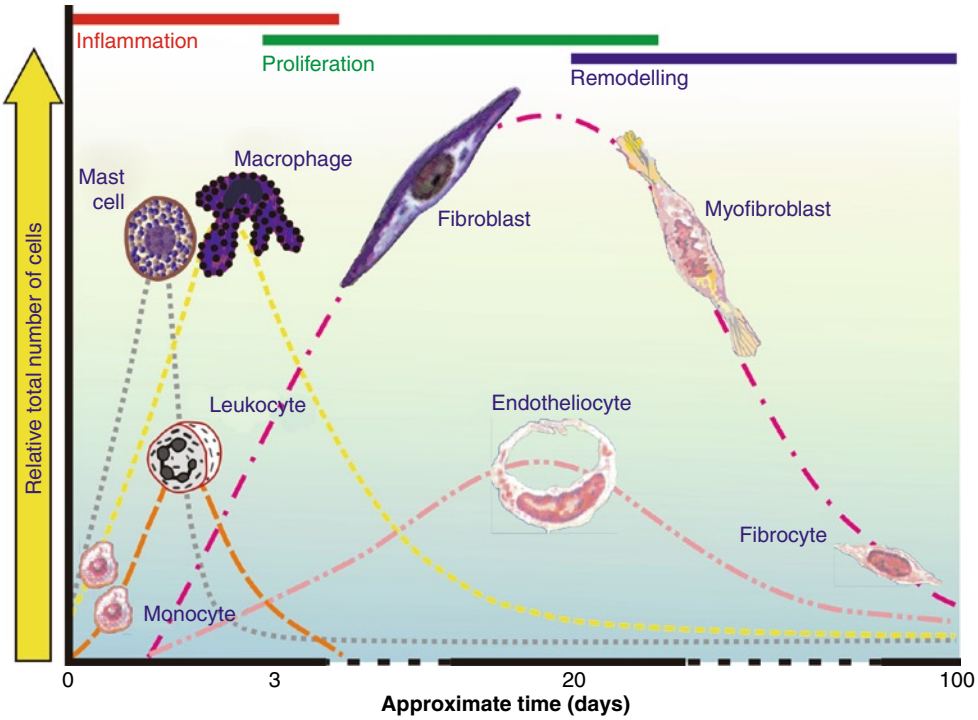

Fig. 19 Schematic illustration of the cell cycles and numbers during the 3 phases of wound healing. During inflammation, which occurs from day zero to day 3–5, the inflammatory cells (leukocytes, mast cells and macrophages) increase in number, peak and then return to baseline levels. During proliferation, the collagen-producing cells, fibroblasts, and neovascularization cells, endotheliocytes, increase in number, and then as remodeling starts, gradually decrease. In the case of fibroblasts, some remain as active fibroblasts, but some transform into myofibroblasts, literally fibroblasts with muscles, whose task is to ensure good linear alignment of the new collagen fibres. It should be noted that the phases overlap, with no clear border between each

with 633 nm LED energy in human subjects demonstrated dramatic fibroplasic changes in specimens from irradiated subjects compared with unirradiated controls.[45] Tiina Karu, probably the most well-known living photobiologist, has produced an enormous amount of work in her lifetime on the effects of low incident levels of light on cells and their organelles. She confirmed the much earlier work by Fubini and further identified the specific target for 633 nm light as the cytochrome-c oxidase resident at the end of the mitochondrial respiratory chain.[46] She also showed that coherent light was not essential to achieve effects *in vivo*, provided the photon intensity at the target was high enough. Mast cells have been stimulated *in vitro* and *in vivo* to degranulate when irradiated with 633 nm light, and much faster than when unirradiated: stimulation with 830 nm speeds up degranulation even more.[47,48] The author has very recently shown that, 48 h after a single irradiation with 830 nm LED energy, mast cells in the forearms of healthy human subjects had almost 50–70% degranulated compared with specimens from unirradiated controls, where no degranulation was seen at all (Unpublished data). Near IR energy at 830 nm stimulates macrophages to perform their chemotactic, phagocytic and internalizing functions better and faster, while releasing almost 30-fold the amount of fibroblast growth factor (FGF) compared with unirradiated controls,[49] and the same is true for neutrophils.[50,51] The epidermal basal layer keratinocyte is too often forgotten in LED phototherapy, but research has shown that both 633 and 830 nm noncoherent light both *in vitro* and *in vivo* can activate the keratinocytes to release a large amount of cytokines which drop down into the dermis to assist with the dermal wound healing processes, so much so that keratinocytes have been nicknamed 'cytocytes'.[52] Additionally, the photoactivated keratinocytes can improve the cellularity and organization of the epidermal strata, with a better organized stratum corneum.[42]

If the wound healing cells, including epidermal keratinocytes, are examined for increased wavelength-specific action potential based on the last 30 years of both LLLT and non-laser light source literature, the results could be presented as in Table 4. The wavelengths which have the most verified and published results at a cellular and subcellular level are 633 and 830 nm in the near IR, but very importantly they do not have the same efficacy in the same cell types. Near IR at 830 has excellent results in activating the activity levels of the inflammatory stage cells, mast cells, macrophages and neutrophils, in addition to epidermal keratinocytes. On the other hand, red at 633 nm is best for photoactivating fibroblasts *in vivo*, due to its superior penetrating powers compared with 595 nm yellow, and epidermal keratinocytes. This is why the skin rejuvenation protocol was set to start always with 830 nm and then, 48–72 h later, 633 nm, because of the specific cellular targets and their temporal appearance in the wound healing process, because with LED therapy at these wavelengths, although there is no wound, exactly the same response is achieved as seen after any examples of the nonablative photothermal damage approach.

The data displayed in this table can also help to explain why the 830 nm/633 nm combination is effective for skin

Table 4 Phototherapeutic wavelength-specific actions in raising the action potentials of dermal and epidermal cells specifically associated with wound healing and skin rejuvenation . All results are for low incident power densities (15 mW/cm^2 to 1.0 W/cm^2) and a range of doses (2–60 J/cm^2) delivered in continuous wave with the exception of the yellow waveband which is a frequency modulated (so-called 'pulsed') beam and 904 nm which is from a true pulsed diode laser. Many of these studies in the 633 and 830 nm rows have also been replicated *in vivo* with LED energy

Nominal wavelength (nm)	Wound healing phase/cell types and action level					
	Inflammation			Proliferation	Remodeling	All
	Mast	Neutro	Macro	Fibro	Fibro-Myo	Keratino
590–595	?	?	?	+++[a]	?	?
633	++[a,b]	+[a]	++[a]	+++[a,b]	±	+++[a,b]
670	?	?	++[a]	++[a]	?	?
790	++[a]	?	?	++[a]	?	?
830	+++[a,b]	+++[a]	+++[a]	+[a]	+++[b]	+++[a,b]
904	–	?	±[a]	–	–	?
1,064	?	?	?	+[b]	?	?
10,600	?	?	?	++[b]		

[a]*in vitro* studies;

[b]*in vivo* studies; *Mast* mast cells, *Neutro* neutrophils, *Macro* macrophages, *Fibro* fibroblasts, *Fibro-Myo* fibroblast to myofibroblast transformation, *Keratino* keratinocytes. Degree of action potential: +++, very high; **++**, high; **+** some; **±** little or none; **–** , retardation; ?, unknown

rejuvenation, even though it is not wound healing *per se*. The 830 nm energy first degranulates the mast cells, dumping a load of proinflammatory substances into normal tissue, such as heparin, trypsin, histamine and bradykinin. This gives the tissue the impression that it has been 'wounded', even though there is actually no wound because of the athermal and atraumatic action of LED phototherapy. Macrophages are also photoactivated, helping to give a clean ECM 'seeded' with FGF, with some TGF released from neutrophils recruited into the area by the degranulating mast cells. Because the inflammatory stage has been established especially by this mast cell-mediated 'quasi-wounding', the tissue has no option but to proceed into the next stages of the wound repair process, starting with proliferation in which 633 nm has its best effect on fibroblasts. When this 830 nm/633 nm sequence of irradiation is repeated over 4 weeks, separated each week by 2–3 days, the dermal cells (and epidermal keratinocytes) are upregulated in a step-wise manner and maintained in the inflammatory/proliferative stages. After the final treatment session the remodeling is allowed to start, and this explains why the best results are not seen at this immediately post-treatment stage, but later on at 4, 8 and 12 weeks or more after the final treatment, as was the case in the acne and skin rejuvenation studies already mentioned.[29,30,42]

The same sequential wavelength principle applies to frank wound healing, whether it is accidental or iatrogenic trauma. Burns, for example, are an ideal injury for LED phototherapy, because of the noncontact and hands-free application, and the large area of the treatment heads. In a recent study, the 830 nm/633 nm combination produced excellent results in large area burns, as illustrated in Fig. 20.[53] As mentioned already above, ablative laser resurfacing lost popularity due to the potential of serious side effects, especially edema and prolonged erythema, leading to prolonged patient downtime.

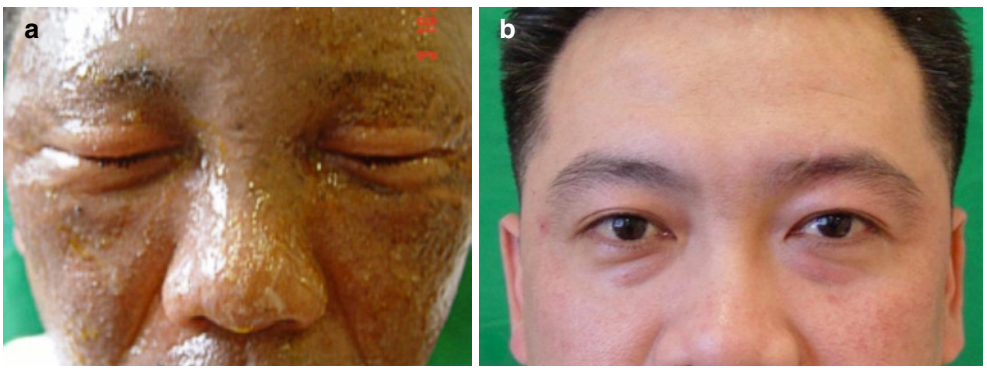

Fig. 20 A 39-year-old male patient with severe full facial electric spark burn injury before (**a**) and 3 months after the final treatment with combination 830 nm/633 nm LED phototherapy. One full 4 week session was performed with the wavelengths being sequentially applied as usual, a resting period of 4 weeks, and then another 4-week regimen (Photographs courtesy of Prof Jin-wang Kim MD PhD, Burns Center, Haelym University School of Medicine, Seoul, Korea. System used: Omnilux® with the plus™ (830 nm) and revive™ (633 nm) heads)

The wound left following laser ablative resurfacing is simply a full facial burn. In a recent publication Trelles and coworkers used the 830 nm/633 nm combination LED therapy following laser ablation of the face with a combined Er:YAG/CO_2 laser system.[54] There were two groups of patients, 30 in each group. The experimental group received the LED therapy following laser ablative treatment, and the control group received sham treatment from the standby setting of the 633 nm head only. The average healing times (full reepithelization and resolution of erythema) for the control and experimental groups were 13 weeks and 6 weeks respectively. The extent of post-procedure pain, bruising and erythema was significantly less for the LED-treated group (60.1%, 72.3% and 59.7%, respectively), whereas improvement in the skin condition was much more clearly seen in the LED-treated group, with a satisfaction index (SI) of 89% compared with 51% for the control group. The SI was calculated by adding only the number of 'excellent' and 'very good' scores from a standardized 5-element scoring system, and expressing the result as a percentage of the total population. Healing following upper blepharoplasty and periocular laser resurfacing in a hemifacial study was reported to be cut by one-half to one-third following LED therapy at 633 nm, and the improvement was subjectively rated as 2–4-fold better compared with the unirradiated side.[55] LED treatment (830 nm/633 nm) following Er:YAG laser ablation of deep and extensive plantar warts roughly halved the healing time, cut the postoperative pain by at least one-tenth and gave less than 6% recurrence rate in 121 cases.[56] Long-term nonhealing ulcers which healed following low incident levels of red light (HeNe laser, 633 nm) was the first subject to appear in the literature from the Godfather of phototherapy, the late Prof. Endre Mester of Semmelweis University, Budapest, Hungary, and started all the controversy surrounding LLLT in the early 70s.[57] Very interestingly, Mester reported that ulcers on the limb contralateral to the one treated also eventually healed, although more slowly than the irradiated wounds. This was the first report on the systemic theory in phototherapy, whereby photoproducts created in the irradiated tissue were carried systemically through the body to have an effect wherever they were required. More recent studies with 830 nm showed even quicker healing of recalcitrant crural ulcers.[58] Near IR 830 nm does not only work in soft tissue wounds, but also in bone where it accelerates the union of fractures, even in the case of delayed union healing, replacing the usual poorly-organized callus with better-quality bone so that the remodeling stage is much shorter.[59,60]

Some of the mechanisms behind the efficacy of LED phototherapy-accelerated wound healing at the appropriate wavelength have already been at least partly elucidated, such as the wavelength-specific activation of the dermal and epidermal cells associated with the three phases of wound healing. Karu has suggested that the latency effect of phototherapy in cells actually continues in subsequent generations of the irradiated cells in a chapter of her latest book (*Ten Lectures on Basic Science of Laser Phototherapy*, 2007, Prima Books AB, Grängesberg, Sweden), which is an important consideration in skin rejuvenation.[61] Another important mechanism involves improvement of blood flow following irradiation with 830 nm, and this has been shown to positively impact on flap survival in the rat model.[62] Improved blood flow not only brings in oxygen and nutrients, but establishes a higher oxygen tension in the treated area which can establish gradients between the wound at the surrounding tissue, used as 'superhighways' by the reparative cells.[63] In the case of bony tissues, 830 nm has been shown to increase the metabolism of osteoblasts,[64] and to upregulate some of the genetic pathways leading to better differentiation of new, active osteoblasts from mesenchymal cells.[65]

In conclusion, the sequential application of 830 and 633 nm LED energy, and even of each of these wavelengths used on its own, has been shown to enhance all aspects of wound healing, always provided the incident irradiance (power density, photon intensity) is sufficiently high and an appropriate dose is given. In addition to the excellent and growing reputation of LED phototherapy as a stand-alone light-only therapy, this means that LED therapy has proved to be an ideal adjunctive therapy to any of the conventional approaches seen in dermatological practice and this is perhaps the most exciting aspect of LED phototherapy in the future. No matter how the dermatologist alters the epidermal or dermal morphology of his or her patient, be it through microdermabrasion, ablative and nonablative skin rejuvenation, fractional technology or conventional surgery, the addition of an appropriate LED phototherapy regimen will help to improve already good results but at a very reasonable cost, thus improving the satisfaction rates of both the clinician and the patient.

Other Clinical Indications

The indications already discussed have been well-researched, and are being reported in the literature. Some other applications exist which are very much at the experimental stage, but which should be mentioned to prepare the reader for what's coming in the not-too-distant future and for which LED phototherapy is proving very interesting. At this stage the author cannot go into details, because of the early stage of the clinical and related basic science experiments, but the reader should watch for articles on LED therapy and eczema, psoriasis, stretch marks, vitiligo and even cellulite reduction, although in the last-mentioned LED therapy is being used adjunctive to other approaches. Particularly in psoriasis, several multinational pilot studies have produced very interesting results, and this may well be the first of the list to appear

in print in a peer-reviewed article. Yet another exciting field is the potential use of LED phototherapy in combination with platelet-rich plasma (PRP) for wound healing and for skin rejuvenation. PRP is well-established as a valid method in wound healing to speed up the process and give good cosmesis or in recalcitrant healing situations. Knowing how cell-specific certain LED wavelengths are, the obvious step is to combine the two approaches to achieve even better results, even faster. Some preliminary studies are currently underway in Tokyo, and the early results indicate that this will be a field to watch closely.

Safety with LEDs

- LED phototherapy is intrinsically safe
- Eye protection sometimes required against potential optical hazards
- LED phototherapy is essentially side effect free
- Few contraindications exist, but sensible precautions should be taken
  - Patient history must be checked for any photosensitivity-related diseases or conditions.
  - Drugs, ointments and even cosmetics being used by the patient must be checked for photosensitizing elements.

Surgical lasers and even intense pulsed light systems are by their very nature designed to create thermal damage and are thus subject to stringent safety codes to prevent accidental irradiation of tissue, other than the planned target tissues. Because LEDs are incapable of creating photothermal damage in tissues, the same stringent codes regarding accidental irradiation of tissue do not apply. However, as all of the LED systems discussed above operate in the visible and near infrared waveband, there is a potential for optical damage, as the eye is capable of gathering this waveband and focusing the light onto the retina at the back of the eye, particularly the macula and fovea, the area responsible for visual acuity. This will be looked at in a little more detail below.

Most LED phototherapy systems are run from conventional mains electricity, and so present potential hazards in common with any other such mains-driven equipment as, for example, DVD players and television sets. Common sense dictates the safe handling of this group of equipment, leading to the following guidelines:

DO NOT connect or disconnect the mains plug with wet or damp hands
DO NOT pull the plug from the mains socket using the power cable
DO NOT place any containers with liquid in them on top of the unit (e.g., coffee mugs) to prevent damage from accidental spillage. If such spillage should occur immediately turn off the system and have it serviced before using it again.
DO NOT attempt to perform and unauthorized servicing of the system which involves opening up the case and/or defeating any interlocks.
DO connect the mains cable to the system before plugging the mains plug into the socket
DO check that the power to the wall socket is off before inserting the mains cable plug
DO switch off the wall socket before removing the mains plug

Apart from these rather obvious points, common sense should prevent any electrical-related damage to therapist or patient.

Optical Hazards with LED Phototherapy

As already mentioned above, any LED system operating in the visible to near-infrared waveband emits light which the human eye can gather, and focus onto the back of the retina as illustrated in Fig. 21. If the incident power density is great enough,

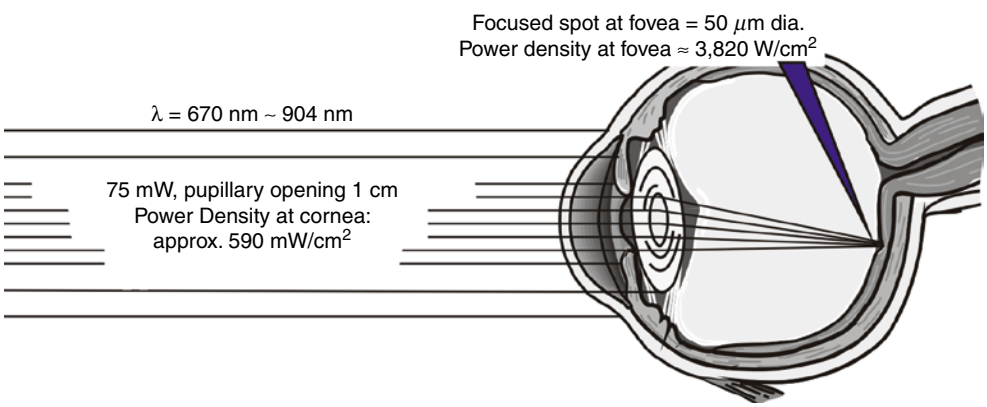

Fig. 21 Schematic illustration of how a low incident power of 75 mW is capable of being focused by the unaccommodated eye into a very small spot, with damaging power densities, right in the center of the fovea. The importance of appropriate protective eyewear is quite clear

permanent damage to the fovea could occur leading to uncorrectable loss of visual acuity. For example, an incident power density of as low as 75 mW focused to a 50 μm spot produces a power density of over 3,800 W/cm^2, perfectly capable of severely damaging target biological tissue. However, a set of values has been established for the maximum permissible exposure, or MPE, to light at a range of wavelengths. If an LED phototherapy system has been independently tested to deliver light below the MPE for its nominal wavelength, then even prolonged direct viewing of the beam is theoretically safe. In clinical practice, however, visible light LED arrays are extremely bright, even when below the MPE for their wavelength, so some form of eye protection is usually a good idea if only for patient comfort. Small, opaque eye cups held in place with an elasticated cord are popular, which will still allow the light to reach the periocular region in the case of LED phototherapy for skin rejuvenation. However, if the system delivers light which is over the MPE, then protective eyewear becomes mandatory for the patient, and also for any ancillary staff spending any length of time in the treatment room to help protect their eyes against diffuse reflection from the target tissue. For shorter visible wavelengths such as the blue waveband, the inherent photon energy of the light is approximately one-third as high again as visible red light even though the incident power density is the same, as discussed above, and so has greater potential for optical damage. Appropriate eyewear is necessary in this case.

The 'blink reflex' is nature's way of helping us protect our own eyes against an over-bright visible light source, but near-IR light cannot be 'seen' by the human eye and so the blink reflex is not triggered by energy in this invisible waveband. Near-IR is still gathered and focused by the unaccommodated eye just as visible light is, however, so suitable protective eyewear is thus mandatory for LED systems delivering energy in the near-IR waveband.

If the goggles or glasses are not opaque, then they have to be specifically sourced with an appropriate optical density for the wavelength of the system. Eyewear designed for red light will not protect adequately against IR or visible blue light, for example. The eyes of the patient, and indeed anyone with the patient in the treatment room during LED therapy, must be assiduously protected even though LEDs are often discounted as inherently 'safe', compared with a surgical laser or IPL. It is better to err on the side of caution!

Finally, national and federal regulatory agencies, such as the US Food and Drug Administration (FDA), issue approvals of systems for specific applications for which they have been proved '**safe and effective**'. Although some manufacturers have received such approvals, they are few and far between. Some less than truthful manufactures will claim FDA approval, when in fact all they hold is a letter from FDA recognizing that their LED system is a **nonsignificant risk device**, or NSRD. This is NOT the same as a system's having gone through the due regulatory process to obtain what is known as a **510(k) approval**, based on which, and only on which, can that device be legally sold in the USA for clinical use. 510(k) approvals for existing LED systems can be searched for on the FDA website (www.fda.gov/cdrh/510khome.html).

Side Effects

Once again, the inherently 'safe' output of LED systems helps to keep unwanted side effects to a minimum, but with any kind of phototherapy there is always the outside chance of triggering such a side effect. These are almost 100% photosensitivity-related, so a careful history of the patient must always be taken to identify the existence of pre-existing photosensitivity issues. For example, if a patient reports that he or she regularly comes out in an itchy rash when exposed to terrestrial sunlight, LED phototherapy should not be given. Some skin types, such as the Asian skin, are incredibly sensitive to other wavelengths despite being very resilient to UV skin damage. Particularly in the Asian skin, secondary hyperpigmentation can occur without any apparent physical insult, and a carefully-taken history will show if the patient is predisposed to this very upsetting side effect. A very small proportion of patients treated with LED therapy have reported post-treatment headaches of varying magnitudes, all of which have resolved spontaneously. No reason has been elucidated for this, and treatment with mild analgesics has been found to speed up the resolution of the headache. Almost all of those so afflicted have been undergoing LED phototherapy for facial skin rejuvenation, but interestingly only a very few have actually stopped turning up for their treatments. The main point is to take a very careful and thorough patient history to identify the potential of any LED therapy-related problems, but they are very much extremely few and far between. For longer sessions of LED phototherapy, for example in facial skin rejuvenation, the main side effect is that the patients tend to fall asleep during the treatment and 'wake up' feeling great!

Contraindications

Leading on from the previous subsection, any kind of endogenous or exogenous photosensitivity is a contraindication to LED phototherapy. Patients with any form of porphyria, for example, should never be treated with LEDs. Those whose history includes solar-mediated eruptions are likewise not good subjects. The careful dermatologist should also ascertain what the patients are putting on their skins prior to an LED therapy session. Ointments or creams containing known photosensitizers such as coumarins or porphyrins must be discontinued at least 2 weeks before any LED treatment.

Even some perfumes contain recognized photosensitizers. The application or ingestion of photosensitizing drugs including systemic retinoids and the recent use of topical retinoic acid should also be carefully considered, and some acne treatments such as Roaccutane® (isotretinoin) are all contraindications.

Other potential, possibly more 'emotional' contraindications include patients who are pregnant or lactating, although these are not as absolute as the in the previous paragraph and if the LED therapy is not being delivered over the womb as in the case of facial skin rejuvenation for example, then it can be given at the discretion of the treating clinician, and with the informed consent of the patient. In fact, possible benefits may well accrue to both the mother and fetus from the systemic nature of blood-borne beneficial photoproducts.

The most emotional contraindication is for patients with some form of cancer. It is true that cancer cells irradiated in a favorable *in vitro* environment will replicate at a much faster rate than control cells. However the *in vitro* environment is totally different to the living body, where the cancer cell is seen as an out-and-out enemy by the autoimmune system, and is in a very *unfavorable* environment surrounded by potential killer cells. As mentioned above, the very first application of red LED PDT phototherapy was in the *treatment* of skin cancers, and application of low incident levels of light have been shown to cause regression or even complete removal of aggressive tumors in animal models,[66] and induce significantly prolonged survival times in terminally-ill cancer patients.[67] Furthermore, low incident levels of light energy have been shown to boost the autoimmune system. Once again, however, discretion should be used in the case of a patient with a cancerous condition who is seeking LED phototherapy for something else at a site distant from the cancer, such as skin rejuvenation. As a final note, in the more than four decades since the laser and other light sources have been used in medicine and surgery, not one case of iatrogenic, phototherapy-linked cancer has been reported in the literature.

In short, LED phototherapy systems from reputable manufacturers are inherently safe, provided they are used according to the manufacturer's recommendations regarding eye protection, and approved treatment protocols. LED phototherapy systems are basically side-effect free, apart from the beneficial side effects, and will give continue to deliver side-effect free therapy provided the list of contraindications discussed above, and always provided by responsible manufacturers, is carefully applied, in addition to the taking of a careful patient history. However, as already said, not any old LED system will do, and the careful dermatologist must ensure that the system has appropriate quasimonochromatic wavelengths, appropriate photon intensities over a sufficiently large area, has a proven track record in the published literature and has marketing approval from regulatory bodies such as the US FDA.

LED Phototherapy: Quo Vadis?

- 'New' LED wavelengths not likely, but possible
- Further technical advances will increase scope of LED phototherapy
- Potential for LED therapy in the home and OTC market
- LED phototherapy is safe, effective, easily applied, side effect-free and well-tolerated by all patients.
- Effective as a stand-alone light therapy, LED phototherapy will also prove even more invaluable in adjunctive therapy to complement all existing modalities in dermatological practice.

There is no doubt that LED phototherapy has come a very long way in a very short time, with the 'NASA LED' being introduced only a decade ago. The big question now is, where can it go? I believe the answer depends on three main areas: wavelength; other technical developments; and applications.

New Wavelengths

It is possible that a 'new' (and useful) wavelength will be 'discovered', but unlikely. The current main wavelengths are:

- Soret band short wavelength visible blue light, with the optimum peak at 415 nm, the peak of the action spectrum of the porphyrins endogenous to *P. acnes.*
- Visible yellow at 590–595 nm, the highest peak of the action spectrum of cytochrome c oxidase in the mitochondrial redox chain to instigate ATP production, although in practical clinical application this waveband is extremely limited by its poor penetration into living tissue due to absorption in the biological pigments melanin and hemoglobin, which are its preferential chromophores. However, this waveband also has potential in the treatment of inflammatory rosacea and other very superficial entities.
- Visible red light from 620–680 nm, with a 'best case' at 633 nm because of the minor absorption peak in the action spectrum of the porphyrins associated with exogenous 5-ALA PDT, the major peak in the absorption spectrum of cytochrome c oxidase, and its deep penetration into living skin. 633 nm is the optimum wavelength to activate fibroblasts *in vivo* even in the deeper dermis. This wavelength also has a tremendous published database, because it is the same as the HeNe laser.
- Near infrared at 830 nm, because it has the deepest penetration of any wavelength due to its being at the bottom of the water absorption curve, and has a well-proven

track record as the most useful laser therapy wavelength in near infrared diode laser therapy for musculoskeletal and neurogenic pain, enhancement of local blood and lymphatic flow, and wound healing. This wavelength has a good body of literature showing prefertial effects in degranulation of mast cells, and photoactivation of neutrophils and macrophage cells with associated release of trophic factors.

There are now many LED devices currently available that have wavelengths the same or very near to those given above, but we have to remember that in many cases an action spectrum peak has a very narrow shoulders, so a difference of even 5 nm from the peak can dramatically lower the action potential. There are other wavelengths which have been proved very useful in the laser therapy literature, in particular 780 nm for neurological applications and 904 nm for dental and other hard tissues, including bone. In the case of neurological indications, such as repairing transected or crushed nerves, the punctal nature of laser energy with its extremely high photon intensity delivered within the very small treatment area is essential to the treatment technique: it is impossible to concentrate LED energy from an array onto such a small spot, so LED systems at this wavelength would not have the same effect as a laser diode-based handpiece. In the case of 904 nm in hard tissue, the laser systems used were based on a GaAs (gallium arsenide) laser diode. GaAs laser diodes are capable of generating outputs in the range of 15–45 W, but cannot be operated in continuous wave because of the tremendous heat they generate and the need to provide a dedicated cooling system. The GaAs laser diode is therefore operated in a true pulsed mode, with pulse widths in nanoseconds and interpulse intervals of milliseconds, so the average power seen by the tissue is in milliwatts, usually three orders of magnitude less than the peak power: a 15 W peak power would this give an average power at the tissue of 15 mW. However, these ultrashort pulses create effects in tissue other than photoabsorption, such as photoosmotic and photoacoustic effects, and in hard tissues such as bone, it was believed that a physical resonance was set up which acted in coordination with the photoabsorption effects to give excellent bone healing, even in the case of slow union fractures. No LED phototherapy system is capable of delivering a true pulsed beam, and simply modulating a continuous wave beam of tens or even hundreds of milliwatts per square centimeter will not produce the same effect as the very tightly delivered true pulsed diode laser beam, so this wavelength is also not an appropriate one for an LED system, given that one of the main advantages of LED arrays is the ability to irradiate a large area of tissue rather than a very small treatment target. In all of the wavelengths bulleted above, and in their clinical application, the targets are not precise points, and the ability to irradiate a large tissue mass is an advantage. Despite the present and future claims, I do not believe that we will see a 'new' wavelength for light-only LED phototherapy.

New Technical Developments

LED technology is constantly evolving, as seen in the current transition from the individually board-mounted dome-type LED to the latest generation of 'on-board' chips which are actually part of the circuit board. There has been speculation about developing whole-body LED arrays, particularly in the sports medicine and sports clinic environment, so that athletes could have a complete LED 'photobath' after a strenuous workout to help relax overused muscles and dissipate lactic acidosis, thereby enabling total retention of tonus in a deliberate overtraining program to reach absolute peak power and stamina. The application of such whole-body arrays in the spa and beauty market would also be very interesting. The problem with such large arrays is heat. The dome-type LEDs do not in themselves generate a lot of heat because they are solid state, but when mounted in arrays they require on-board driver circuitry, and this does generate a fair amount of heat which must be dissipated otherwise overheating of the LEDs will result in movement away from their rated wavelength, in addition to shortening of their useful life. Efficient cooling of the circuit boards is thus essential and this is not an easy matter even with the current size of arrays, to the extent that the method of cooling has actually been patented in some systems. In order to cool the number of large arrays which would be necessary for a whole-body LED generator would require a dedicated and powerful cooling system, and this raises the dual problem of developing an appropriate method of extracting heat, whether it was based on air- or water-based cooling, and where the heat extracted from the LED arrays would go. In a small treatment room, the heat build-up could be very noticeable, as was the case in the first generation of large laser systems. The development of on-board chips have gone some way to solving this problem, and the emergence of electrostatic cooling systems which could be built directly into or onto the LED boards is very interesting. This is a field which will be very interesting to watch.

Another area for development may well be in the optics of the LEDs themselves. Currently LEDs deliver a divergent beam, typically in the region of 60° steradian. It is the deliberate overlapping of these divergent beams which causes the phenomenon of photon interference, which, coupled with scattering of light in target tissue, allows LED arrays to deliver very useful photon intensities over large areas of tissue. It would, however, be possible to build corrective optics into the acrylic which encapsulates and protects the actual diode chip. Collimation could be forced on the light emitted

from the chip with a series of condensing optics, as is currently the case with laser diode-based pointers, so that an almost parallel beam would emerge: this would obviously instantly increase the incident photon intensity of the beam. Alternatively, a lens could be incorporated in the capsule, to deliver a beam with a fixed focal length, i.e., coming to a focused point at a predetermined distance from the LED. This would increase the incident photon intensity even more. An array of convergent and individually focused LEDs would therefore offer a real alternative to a laser diode, probably still at less cost than a laser diode-based system, once the problem of the cost of the optics has been resolved. Again, this is an area which deserves watching carefully.

The final area is the home market, which is tied into the previous subsections as far as appropriately selected LEDs are concerned. There are already a large number of very pretty colored, happily twinkling LED-based systems being touted as suitable for home use, however the vast majority of them are mere toys, especially the ones with multicolored LEDs, and the poor user might as well stand in front of their christmas tree lights as use these systems. This does not mean that responsible manufacturers have not been researching correct combinations of appropriate wavelengths and intensities in ergonomically-designed hand-held self-contained units which will be safe and effective for home use: some indeed have. It is anticipated that these units will be available in a number of ways: for prescription by a dermatologist or other specialist as a maintenance program for their in-office LED treatment regimen; as an over-the-counter product from chemists or pharmacists with product-related training; or from reputable self-health mail-order companies. The author is aware of one company who has two such self-contained hand-held products, one blue/red for treatment of acne, and one infrared/red for skin rejuvenation, which are in the final stages of FDA approval process. Despite their size, they have the same high quality LEDs delivering the same intensity in mW/cm^2 as the medical versions of the systems based on LED arrays. When used for the recommended time they will thus deliver exactly the same dose as their much larger cousins. Naturally they cover a very much smaller area than the full-sized planar arrays, but because of their lightweight nature, it is anticipated that the user will be able to watch TV or listen to music while irradiating the target area one bit at a time, and they will be absolutely ideal for a maintenance program following office or clinic treatment with the full-sized systems. Yet another area to be watched with great interest.

Applications: Combination Is Key

The applications for light-only LED phototherapy continue to grow in a pan-speciality manner, so that a large range of clinicians are finding useful applications for LED phototherapy of appropriate wavelength and incident photon intensity. However, as with lasers, a saturation point will be reached. This can be postponed by combining the effects of different LED wavelengths, such as the blue/red combination for acne, and the near-infrared/red combination for wound healing and skin rejuvenation. I firmly believe that we should go beyond that, as in fact is already happening, and use LED phototherapy in combination with the existing more conventional approaches to achieve even better results. A perfect example where this is happening is full face laser ablative resurfacing. Initially hailed as a superb approach to rejuvenating severely photoaged skin, in recent years it has declined dramatically in popularity because of potential side effects such as scarring, unpleasant-looking sequelae and a very long downtime before the patient can once again return to work or to society. However, everyone agrees it is still the 'gold standard', particularly for deep wrinkles and severe photodamage. Some reports have now appeared on the use of near infrared/red combination LED phototherapy together with ablative laser resurfacing. The controlled study already discussed above by Trelles et al. is an excellent example which compared two groups of full-face ablative resurfacing patients[54]: one group was also treated with combination LED phototherapy, and the control group was not, but otherwise the resurfacing and wound care regimens were exactly the same. The healing time in the LED-treated group was cut by more than one-half, postoperative pain was cut by more than 70% and the erythema cleared in less than 7 weeks compared with 4–6 months.

LED phototherapy can and does offer even better results in any case where the dermatologist has in some way altered the epidermal and dermal architecture of his or her patient, whether it is as mild as an epidermal powder peel, through chemical peels to nonablative resurfacing with lasers or IPL systems, and full-face ablative resurfacing. The adjunctive application of LED phototherapy will, I believe, drive its acceptance even more strongly than its use as a stand-alone modality, and the major advantage of LED-based systems is their very competitive pricing in addition to their portability and versatility. Combination therapy is the key.

Conclusions (and Questions You Should Ask)

- LED phototherapy is here to stay!
- 'Any old LED' will NOT fit the bill!
- When considering buying an LED system, ask the right questions!
- Combination treatment is the key!

LED phototherapy is certainly here to stay, but unfortunately the medicoscientific waters are being muddied by a number of manufacturers who have jumped on the LED bandwagon, making extravagant claims and barefacedly using the data amassed by those companies who have been responsible enough to go through regulatory approvals, such as FDA 510k clearances, as if the data were their own. Statements such as '…. uses NASA technology ….' are common, but totally misleading. The current generation of LEDs actually exceeds the 1990s NASA technology as far as output power and quasimonochromaticity are concerned, and in fact have absolutely nothing to do with NASA! Even worse than these manufacturers are the companies which import 'lookalikes' from countries such as China and Korea. They may be cheap, but they are certainly not cheerful, and the heart of an LED system is the quality and pedigree of the LEDs used in its arrays: you get what you pay for. To make sure you get what you actually want, i.e., an LED phototherapy system that will actually do something that you want it to do, and make you and your patients happy, please see the following, which will not only summarize the main points of the chapter but will also reinforce my favorite maxim which is; 'Any old LED will NOT do'.

Caveat Emptor (Let the Buyer Beware!)

When considering purchasing an LED-based phototherapy system for his or her practice, the wise dermatologist should always ask the manufacturer or salesperson the following questions (and take a written note of verified or verifiable answers!).

What Regulatory Approvals Does the System Have?

This means appropriate FDA 510k approvals in the USA (no LED system has yet got full premarketing approval, PMA), Health Canada in Canada, TGA in Australia, Ministry of Health, Labour and Welfare (*Kohseishou*) in Japan, appropriate CE marking for medical devices in Europe, and so on. It does not mean having 'NASA technology LED's' or 'Approved by the FDA', the latter of which usually simply means a letter from FDA recognizing that the system is a nonsignificant risk device (NSR) or minimal risk device (MSR). This is not an approval to market, but is simply a guide based on which the institutional review board (IRB) of a research center can classify the system when it does take part in a properly structured study.

What Is (Are) the Wavelength(s)?

As has been said many times, wavelength is the most important single factor when attempting to achieve a photoreaction: no absorption, no reaction. Some targets require a fairly broad waveband of 30 nm or so, but most of the targets in LED phototherapy are much more specific. Ask what the nominal wavelength of the system is, and what is the deviation either side. For example, the Omnilux® revive™ mentioned elsewhere in this chapter has a nominal wavelength of 633 nm, and the spread is ±3 nm. That means that the vast majority of the light is at the nominal wavelength of 633 nm, and will therefore optimally target wavelength-specific chromophores at that wavelength such as cytochrome c oxidase, and the porphyrins Cp III and Pp IX. Visible red at 670 nm, for example, will still have some effect on cytochrome c oxidase, but that wavelength just misses the boat as far as porphyrin activation is concerned.

While on the subject of wavelength, some manufacturers offer all the colors of the rainbow in the one system, in one particular system mounted in a semicircular bar which scans over the face with the claim that 'blue is for serenity, green is for inner peace, yellow is for well-being, and red is for relaxation'. In fact, this manufacturer is not offering phototherapy, but 'chromotherapy' also known as 'colorology' which is an alternative medical approach based on 'chakras' and their associated colors to achieve balance in an unbalanced system.[68] As with reflexology, the origin of the approach is Russian, as is a great deal of the literature, but chromotherapy has a large following. The methods and English language studies used to prove that it works, however, have been severely criticized.[69] In addition, as Karu and colleagues have well-demonstrated, the intermingling of wavelengths way well include some which cancel each other out thus having no effect, or indeed downregulate cellular activity compared to the wavelengths applied individually.[10] The fact that the light is scanned over the face should sound another warning bell, since this dramatically lowers the dose, even if the photon intensity were high enough (which it is not). The answer? Keep to well-proven wavelengths, applied singly. This does not mean to say they cannot be applied in combination, but sequentially …. indeed they should, but a suitable period (48–72 h) must be allowed between applications to allow the first wavelength to do what it is supposed to do at a cellular and tissue level before the second wavelength involves its specific targets.

What Is the Intensity?

You are looking for answer here in mW/cm^2 (milliwatts per square centimeter) of the entire array, not the 'lumens' of an

individual LED or indeed the whole head. If in doubt about this parameter and its paramount importance, next to wavelength, please re-review subsection 2.3 above on photon density, another way of saying 'intensity'. A good range, depending on wavelength, would be anywhere from 40 mW/cm^2 up to 150 mW/cm^2 , although the higher the intensity, the more problems will exists in keeping the head cool enough to avoid discomfort to the patient and a drift away from the LED nominal wavelength. If this range seems low compared with a diode laser therapy system, for example, always bear in mind that the better LED systems cover a large area of tissue, for example some offer an active array area of 220 cm^2, unlike the laser therapy system which usually irradiates a spot of only a few mm in diameter per 'shot'.

If you get an answer in joules, ignore it … better still, laugh loudly. If you get an answer in joules per square centimeter (J/cm^2), that's better, but it is actually the answer to the next question! The incident intensity or power density is extremely important, because a higher power density enables a shorter irradiation time, and it has been reported for a continuous wave system that shorter irradiation times with a higher intensity got significantly better results in first passage human gingival fibroblast proliferation *in vitro* compared with longer irradiation times at a lower intensity, even though the dose (in J/cm^2) was the same.[70] Of course, the Arndt-Schultz curve must always be remembered (subsection 2.3 above), and the upper limit of photoactivation must never be exceeded or a photothermal reaction will occur.

What Is the Recommended Dose?

This is where the J/cm^2 unit should be the correct answer, but NOT the dreaded joule. If you see a joule running around an LED system, kill it. As discussed above, the joule is simply a unit of energy and has no significance whatever on the clinical effect in a prescribed area of target tissue. Correlate the dose with the recommended irradiation time. As a matter of interest, if you cannot find out the intensity from the manufacturer, by dividing the dose (J/cm^2) by the irradiation time (in seconds), you will end up with the intensity in W/cm^2.

For this category, here is no 'correct' dose, although it should certainly be higher than 40 J/cm^2 depending on the wavelength. If the intensity or power density is correct, then it is almost impossible to overdose. Overdosing is not recommended, however, simply because it wastes time and will not often produce dramatically better results than the recommended dose, which the responsible manufacturer will have arrived at by conducting dose-ranging response-related studies. If the recommended dose is, for example, 120 J/cm^2 over 20 min, increasing the irradiation time by 10–30 min will not get a 50% better effect, but on the other hand, cutting the time down by half to 10 min may well give a result well below 50% of that achieved at the recommended time. If the system supports heads with different wavelength, the manufacturer may well have standardized the treatment time to the same for each of the heads, but the dose will almost always be different for each wavelength, simply because of a combination of LED characteristics, wavelength/tissue interactions and the individual photon energy associated with each wavelength.

How Is the LED Energy Delivered?

The answer here will be 'in continuous wave (or CW)', which is good; or 'frequency modulated (also known as photomodulated)' which is not so good; or 'pulsed', which is actually the incorrect way of saying the second answer and is totally wrong! Light at a given wavelength already contains its own frequency, as discussed in subsection 2.5 above, and light represents 'information' to cellular targets. Imposing a secondary frequency on that primary frequency cannot only disrupt the flow of information, it also cuts down on the dose since there is no light incident on the target cells when the source is switched off. It is true that cells have a 'dark reaction' time as shown by Karu,[71] but it occurs well after irradiation, and not in the short off-duty interval in a frequency modulated beam cycle (cf Fig. 10b). Figure 22 is by the same independent research group, Almeida-Lopes and colleagues at the University of Sao Paulo, Brazil, as the data on power density in reference 70, and shows the growth pattern of first

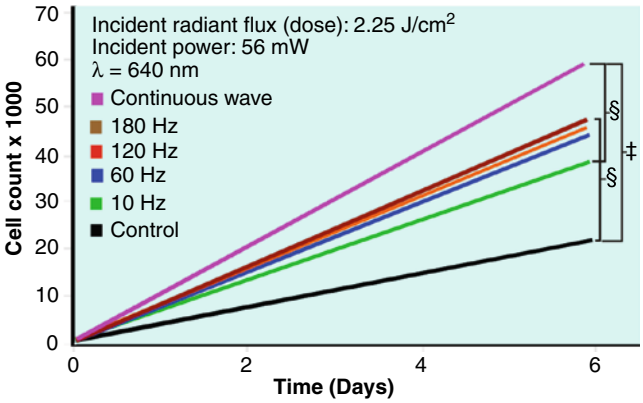

Fig. 22 An *in vitro* experiment to assess the effects of various frequencies in frequency modulated beams compared with a continuous wave beam and an unirradiated control on the cell proliferation of first passage pooled human gingival fibroblasts. The dose was kept constant at 2.25 J/cm^2. All of the frequencies were statistically significantly better at increasing proliferation (§, $p < 0.01$ for all). The CW beam, however, was significantly more efficient than both the control and the frequency modulated beams (‡, $p < 0.001$ and §, $p < 0.01$, respectively). The results represent the averaged data from 10 repeated experiments (Used with the permission of Pra. Luciana Almeida-Lopes, personal communication, data as yet unpublished)

passage human gingival fibroblasts exposed *in vitro* to 640 nm at several frequencies and continuous wave (CW), with constant incident power density and dose as ascertained in earlier studies. There was a significant difference seen between the group of frequencies and the unirradiated controls ($p > 0.01$ for all), with the higher frequencies inducing better cell proliferation, but the continuous wave beam induced greatest and most significant proliferation compared with both the unirradiated controls ($p < 0.001$) and the frequency modulated beams ($p < 0.01$). The experiment was repeated 10 times and the results averaged. (Data and graph reproduced with the permission of Pra. Luciana Almeida-Lopes, as yet unpublished data). Cells, especially fibroblasts, seem to prefer CW to frequency modulated energy.

What Has Been Published on the System/Technology?

What you are looking for here are papers by reputable authors published in the indexed and peer-reviewed literature, or at least in well-established and peer-reviewed journals (15 or more volumes) which have not yet been indexed by MedLine and/or PubMed but which do none-the-less have scientific credence. An example of the latter is *Laser Therapy* (Editor-in-Chief, Toshio Ohshiro, published by JMLL, Tokyo, Japan, now entering its 17th edition). An alternative source is appropriate chapters in books from reputable publishers. What you are NOT looking for are so-called 'white papers' which any manufacturer can produce to look like a genuine publication, or articles from the commercially-oriented medical press unless they are also in turn backed up by 'real' papers. All of the referenced works in this chapter fall under this latter category. Also, make very sure that the articles offered by the manufacturer/salesperson are on their specific system and wavelength(s). Very often articles on approved systems will be cited as 'proof' that their (unapproved) system works, even though sometimes the intensity, dose or even wavelength is not the same as in the published articles.

Finally …

Despite the moaning of the sceptics, LED phototherapy has definitely arrived, has been proven to work in many areas, and is finding a steadily increasing number of applications both within and outside dermatology. It is comparatively inexpensive, robust, easy to administer, safe, effective, pain free (in fact it can be used to treat pain), side-effect free and minimally contraindicated. It offers the possibility of a stand-alone noninvasive phototherapy method, but when used together with any of the methods currently used by the dermatologist to alter his or her patient's skin, already good results can be expected to become even better. LED phototherapy will not turn a poor dermatologist into a good one, but it will help the good dermatologist to become even better, with happier patients. And finally, please remember, above all, not any old LED will do the job!

References

1. Giese AC. *Living with Our Sun's Ultraviolet Rays*. New York: Springer; 1976.
2. Fubini S. Influenza della luce sulla respirazione del tessuto nervoso. *Annali Universali di Medicina e Chirurgia*. 1897; Serie 1, 250: Fascicolo 7.
3. Finsen NR. Om de kemiske Straales skadelige Virkning paa den dyriske Organisme. In: *Behandlung af Kopper Med Udestaengning af de kemiske Straaler*. Copenhagen: Hospitalstidende; 1893.
4. Zheludev N. The life and times of the LED – a 100-year history. *Nat Photonics*. 2007;1:189-192.
5. Whelan HT, Houle JM, Whelan NT, et al. The NASA light-emitting diode medical program- progress in space flight and terrestrial applications. *Space Technol Appl Int Forum*. 2000;504:37-43.
6. Whelan HT, Smits RL, Buchmann EV, et al. Effect of NASA light-emitting diode (LED) irradiation on wound healing. *J Clin Laser Med Surg*. 2001;19:305-314.
7. Smith KC. *The Science of Photobiology*. New York: Plenum Press; 1977.
8. Ohshiro T, Calderhead RG. *Low Level Laser Therapy: A Practical Introduction*. Chichester: Wiley; 1988.
9. Asagai Y, Ueno R, Miura Y, Ohshiro T. Application of low reactive-level laser therapy (LLLT) in patients with cerebral palsy of the adult tension athetosis type. *Laser Ther*. 1995;7:113-118.
10. Karu T. Primary and secondary mechanisms of action of visible to near-IR radiation on cells. *J Photochem Photobiol B*. 1999;49:1-17.
11. Smith KC. The photobiological basis of low level laser radiation therapy. *Laser Ther*. 1991;3:19-24.
12. Kudo C, Inomata K, Okajima K, Motegi M,Ohshiro T. Low level laser therapy pain attenuation mechanisms, 1: histochemical and biochemical effects of 830 nm gallium aluminium arsenide laser radiation on rat saphenous nerve Na-K-atpase activity. *Laser Ther*. 1988;Pilot Issue: 3-6.
13. Dougherty TJ. Photodynamic therapy (PDT) of malignant tumors. *Crit Rev Oncol Hematol*. 1984;2:83-116.
14. Garcia-Zuazaga J, Cooper KD, Baron ED. Photodynamic therapy in dermatology: current concepts in the treatment of skin cancer. *Expert Rev Anticancer Ther*. 2005;5:791-800.
15. Gold MH, Goldman MP. 5-aminolevulinic acid photodynamic therapy: where we have been and where we are going. *Dermatol Surg*. 2004;30:1077-1083.
16. Charakida A, Seaton ED, Charakida M, Mouser P, et al. Phototherapy in the treatment of acne vulgaris: what is its role? *Am J Clin Dermatol*. 2004;5:211-216.
17. Kjeldstad B, Johnsson A. An action spectrum for blue and near ultraviolet inactivation of Propionibacterium acnes; with emphasis on a possible porphyrin photosensitization. *Photochem Photobiol*. 1986;43:67-70.
18. Sigurdsson V, Knulst AC, van Weelden H. Phototherapy of acne vulgaris with visible light. *Dermatology*. 1997;194:256-260.
19. Arakane K, Ryu A, Hayashi C, Masunaga T, et al. Singlet oxygen (1 delta g) generation from coproporphyrin in *Propionibacterium acnes* on irradiation. *Biochem Biophys Res Commun*. 1996;223:578-582.
20. Lavi R, Shainberg A, Friedmann H, Shneyvays V, et al. Low energy visible light induces reactive oxygen species generation and stimulates

an increase of intracellular calcium concentration in cardiac cells. *J Biol Chem*. 2003;278:40917-40922. Epub July 7, 2003.
21. Morton CA, Whitehurst C, Moseley H, et al. Development of an alternative light source to lasers for photodynamic therapy. Clinical evaluation in the treatment of pre-malignant non-melanoma skin cancer. *Lasers Med Sci*. 1995;10:165-171.
22. Babilas P, Kohl E, Maisch T, Backer B, et al. *In vitro* and *in vivo* comparison of two different light sources for topical photodynamic therapy. *Br J Dermatol*. 2006;154:712-718.
23. Pollock B, Turner D, Stringer M, Bojar RA, et al. Topical aminolaevulinic acid-photodynamic therapy for the treatment of acne vulgaris: a study of clinical efficacy and mechanism of action. *Br J Dermatol*. 2004;151:616-622.
24. Hong SB, Lee MH. Topical aminolevulinic acid-photodynamic therapy for the treatment of acne vulgaris. *Photodermatol Photoimmunol Photomed*. 2005;21:322-325.
25. Omi T, Bjerring P, Sato S, Kawada S, et al. 420 nm intense continuous light therapy for acne. *J Cosmet Laser Ther*. 2004;6:156-162.
26. Webber J, Luo Y, Crilly R, Fromm D, Kessel D. An apoptotic response to photodynamic therapy with endogenous protoporphyrin *in vivo*. *J Photochem Photobiol B*. 1996;35:209-211.
27. Nitzan Y, Kauffman M. Endogenous porphyrin production in bacteria by δ-aminolevulinic acid and subsequent bacterial photoeradication. *Lasers Med Sci*. 1999;14:269-277.
28. Papageorgiou P, Katsambas A, Chu A. Phototherapy with blue (415 nm) and red (660 nm) light in the treatment of acne vulgaris. *Br J Dermatol*. 2000;142:973-978.
29. Goldberg DG, Russell B. Combination blue (415 nm) and red (633 nm) LED phototherapy in the treatment of mild to severe acne vulgaris. *J Cosmet Laser Ther*. 2004;8:71-75.
30. Lee SY, You CE, Park MY. Blue and red light combination LED phototherapy for acne vulgaris in patients with skin phototype IV. *Lasers Surg Med*. 2007;39:180-188.
31. Trelles MA, Allones I, Luna R. Facial rejuvenation with a nonablative 1320 nm Nd:YAG laser: a preliminary clinical and histologic evaluation. *Dermatol Surg*. 2001;27:111-116.
32. Nikolaou VA, Stratigos AJ, Dover JS. Nonablative skin rejuvenation. *J Cosmet Dermatol*. 2005;4:301-307.
33. Orringer JS, Voorhees JJ, Hamilton T, Hammerberg C, et al. Dermal matrix remodeling after nonablative laser therapy. *J Am Acad Dermatol*. 2005;53:775-782.
34. Rahman Z, Alam M, Dover JS. Fractional laser treatment for pigmentation and texture improvement. *Skin Ther Lett*. 2006;11:7-11.
35. Wanner M, Tanzi EL, Alster TS. Fractional photothermolysis of facial and nonfacial cutaneous photodamage with a 1,550-nm erbium-doped fiber laser. *Dermatol Surg*. 2007;33:23-28.
36. Lowe NJ, Lowe P. Pilot study to determine the efficacy of ALA-PDT photo-rejuvenation for the treatment of facial ageing. *J Cosmet Laser Ther*. 2005;7:159-162.
37. Alster TS, Surin-Lord SS. Photodynamic therapy: practical cosmetic applications. *J Drugs Dermatol*. 2006;5:764-768.
38. Christiansen K, Peter Bjerring P, Troilius A. 5-ALA for photodynamic photorejuvenation - optimization of treatment regime based on normal-skin fluorescence measurements. *Lasers Surg Med*. 2007;39:302-310.
39. Weiss RA, Weiss MA, Geronemus RG, McDaniel DH. A novel non-thermal non-ablative full panel LED photomodulation device for reversal of photoaging: digital microscopic and clinical results in various skin types. *J Drugs Dermatol*. 2004;3:605-610.
40. Russell BA, Kellett N, Reilly LR. A study to determine the efficacy of combination LED light therapy (633 nm and 830 nm) in facial skin rejuvenation. *J Cosmet Laser Ther*. 2005;7:196-200.
41. Goldberg DJ, Amin S, Russell BA, Phelps R, et al. Combined 633-nm and 830-nm led treatment of photoaging skin. *J Drugs Dermatol*. 2006;5:748-753.
42. Lee SY, Park KH, Choi JW, Kwon JK, et al. 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*. 2007;88:51-67. Available online as Epub ahead of print.
43. Trelles M, Mordon S, Calderhead RG, Goldberg D. (Viewpoint 3 and comment 3) How best to halt and/or revert UV-induced skin ageing: strategies, facts and fiction. *Exp Dermatol*. 2008;17:228-240.
44. Rigau J, Trelles MA, Calderhead RG, Mayayo E. Changes in fibroblast proliferation and metabolism following *in vitro* helium-neon laser irradiation. *Laser Ther*. 1991;3:25-34.
45. Takezaki S, Omi T, Sato S, Kawana S. Ultrastructural observations of human skin following irradiation with visible red light-emitting diodes (LEDs): a preliminary *in vivo* report. *Laser Ther*. 2005; 14:153-160.
46. Karu T. Identification of the photoreceptors. In: *Ten Lectures on Basic Science of Laser Phototherapy*. Grangesberg: Prima Books AB; 2007.
47. Trelles MA, Rigau J, Velez M. LLLT *in vivo* effects on mast cells. In: Simunovic Z, ed. *Lasers in Medicine and Dentistry (Part 1)*. Switzerland: LaserMedico; 2002:169-186.
48. Trelles MA. Phototherapy in anti-aging and its photobiologic basics: a new approach to skin rejuvenation. *J Cosmet Dermatol*. 2006;5:87-91.
49. Young S, Bolton P, Dyson M, Harvey W, Diamantopoulos C. Macrophage responsiveness to light therapy. *Lasers Surg Med*. 1989;9:497-505.
50. 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 Ther*. 1990;2:123-134.
51. Dima VF, Suzuki K, Liu Q, Koie T, et al. Laser and neutrophil serum opsonic activity. *Roum Arch Microbiol Immunol*. 1996;55(4): 277-283.
52. 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;3(1):96-101. Epub September 1, 2003.
53. Kim JW, 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, Neuhann-Lorenz C, eds. *Innovations in Plastic and Aesthetic Surgery*. Berlin: Springer; 2007:36-42.
54. Trelles MA, Allones I, Mayo E. Combined visible light and infrared light-emitting diode (LED) therapy enhances wound healing after laser ablative resurfacing of photodamaged facial skin. *Med Laser Appl*. 2006;21:165-175.
55. Trelles MA, 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.
56. Trelles MA, Allones I, Mayo E. Er:YAG laser ablation of plantar verrucae with red LED therapy-assisted healing. *Photomed Laser Surg*. 2006;24:494-498.
57. Mester E, Szende B, Spiry T, Scher A. Stimulation of wound healing by laser rays. *Acta Chir Acad Sci Hung*. 1972;13:315-324.
58. 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.
59. Glinkowski W. Delayed union healing with diode laser therapy (LLLT); case report and review of literature. *Laser Ther*. 1990;2:107-110.
60. Pretel H, Lizarelli RF, Ramalho LT. Effect of low-level laser therapy on bone repair: histological study in rats. *Lasers Surg Med*. 2007;39(10):788-796.
61. Karu T. Irradiation effects are detectable in the cells of subsequent generations. In: *Ten Lectures on Basic Science of Laser Phototherapy*. Grangesberg: Prima Books AB; 2007.

62. 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.
63. Niinikoski J. Current concepts of the role of oxygen in wound healing. *Ann Chir Gynaecol*. 2001;90(Suppl 215):9-11.
64. Kim YD, Kim SS, Hwang DS, Kim SG, et al. 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*. 2007;39:441-450.
65. Nagasawa A, Kato K, Negishi A. Bone regeneration effect of low level lasers. *Laser Ther*. 1991;3:59-62.
66. Dima VF, Ionescu MD. Ultrastructural changes induced in walker carcinosarcoma by treatment with dihematoporphyrin ester and light in animals with diabetes mellitus. *Roum Arch Microbiol Immunol*. 2000;59:119-130.
67. Skobelkin OK, Michailov VA, Zakharov SD. Preoperative activation of the immune system by low reactive level laser therapy (LLLT) in oncologic patients: a preliminary report. *Laser Ther*. 1991;3:169-176.
68. Simpson S. *Chakras for Starters*. 2nd ed. Nevada City: Crystal Clarity Publishers; 2002.
69. Carey SC. *A Beginner's Guide to Scientific Method*. 2nd ed. Belmont: Wadsworth Publishing Inc; 2004.
70. Almeida-Lopes L, Rigau J, Zângaro RA, Guidugli-Neto J, Jaeger MM. Comparison of the low level laser therapy effects on cultured human gingival fibroblasts proliferation using different irradiance and same fluence. *Lasers Surg Med*. 2001;29:179-184.
71. Karu T. *Ten Lectures on Basic Science of Laser Phototherapy*. Grangesberg: Prima Books AB; 2007.