Review of Possible External Procedures for Stimulating Stem Cell Activity In Situ

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Summary:

The purpose of this review is to get an in depth overview of the field of using external sources of light or electronics to stimulate or activate biological repair processes *in situ,*
meaning non-invasive procedures employed from the outside of the patient.

The impetus for this inquiry is the hypothesis that as aging occurs, our stem cells become less active and dormant, but can be activated to the youthful state. The whole idea of the Stromal Vascular Fraction (SVF) being the "liquid gold" of stem cell therapy worldwide (not in the US) demonstrates that even in the aged individual, stem cells harvested from adipose tissue via micro-liposuction, and activated by light, perform curative roles when re-injected.

Of course, it is not just stem cells that are playing a role but all the other items in the "soup", including exosomes, cytokines, messanger molecules, endothelial cells and endothelial and other growth factors.

So, the concept that arose in my mind was, if, our stem cells can be activated by the SVF procedure, why can't we activate our stem cells by some external means, or via supplements, or via stem-chemistry using injested or injected compounds…and thus avoid the costly and invasive micro-liposuction collection and manipulation process?

The approaches most studied appear to be Low Level Light Therapy (LLLT), Pulsed Ultrasound, and Pulsed Electromagnetic Fields (PEMF).

Particularly, when considering fat loss, the two approaches seem to be either stimulating the fat cells to release fat, without damage to the cells (as claimed by LLLT); vs using Ultra-sound to destroy the fat cells entirely.

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Part I: Overview of Proven Technologies [covered in this report]

Part II: Specific applications to wound healing, facial rejuvenation, bone regrowth, stem cell activation in vivo, effects on skin, disease conditions [To be Done]

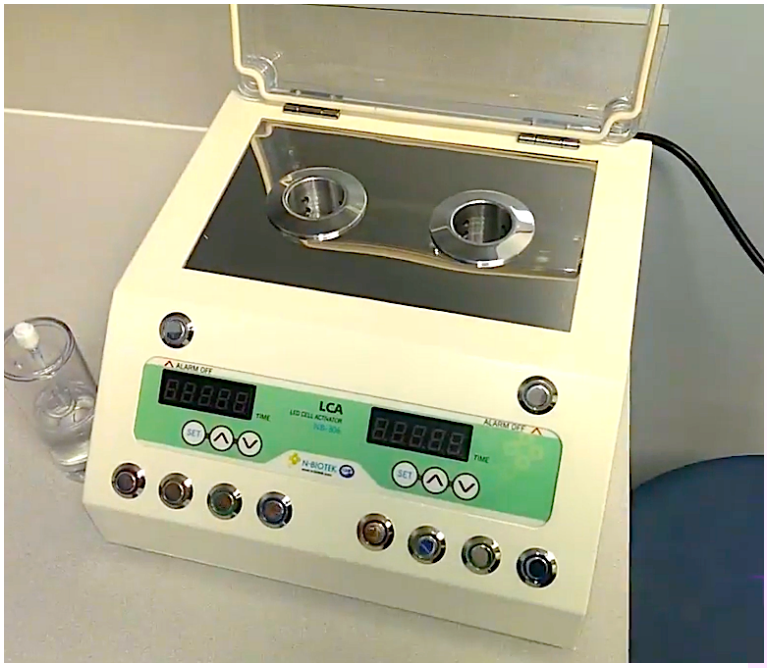
Review of Techniques for Stem Cell Activation In Situ

The idea here is to duplicate as far as possible the effects of SVF therapy (which requires invasive micro-liposuction and photo-activation of harvested stem cells) by the use of external means. The means might be light or laser implementation, or other forms of electronic applications.

We start with the important observation that in the preparation of the Stromal Vascular Fraction from a mini-liposuction, “photoactivation" for 10 seconds is required using an LED light box using all four colors: red, amber,green, blue.

Except for the many papers discussing the benefits of photo-activation of stem cells, using various colors and various intensities and wave lengths, I am not sure where this requirement for use with the SVF fraction originated, only that is clearly used by everyone.

In the Stem Cell Training Course we took, they refer to this light box as Adi-light; however the training video shows a box labeled “N-Biotek Led Cell Activation”. This is a company supporting stem cell research, and the “box” looks like this and is decribed in the PDF.



RED:	To increase cell growth, collagen Synthesis
BLUE:	To help cell’s self-purification, restrain lipolysis
GREEN:	To increase cellular Immunity
YELLOW:	To stimlulate activation

So, we would say that the need for photo-activation for stem cell activation of the SVF is so well accepted as to need no further reference to the literature.

And so this is our starting point.
What *external* procedures can we use that will activate the body's own innate, though dormant, stem cells?

There are many many papers discussing light in an in vitro setting. And for sure, this is like going down the rabbit hole finding this wavelength does this and that wavelength does that. This is to be totally avoided for our purposes. Shining lights on cell cultures may well alter differentiation, proliferation and so forth, but does not get us any closer to having good info for in situ stimulation. A sample paper of the kind one can find relating to in vitro studies is the Wang et al paper relsting to increased proliferation of MSC's with Blue/Green wavelengths, and introducing the term "PhotoBioModulation".

Wang et al, "Red (660 nm) or near-infrared (810 nm) photobiomodulation stimulates, while blue (415 nm), green (540 nm) light inhibits proliferation in human adipose- derived stem cells", https://www.nature.com/articles/s41598-017-07525-w

So, from various in vitro studies, we move on to actual usage of various equipment on patients, and we start out with those procedures used for weight loss, particularly for body sculpting wherein fatty tissue is apparently "melted away" through the external application of light, laser, magnetic and other physical modalities. If fat, i.e. adipose tissue of a patient, can be effectively eliminated through external application, then perhaps the same technology can be used for in situ stem cell activation of dormant stem cells.

In fact, this is what may be actually happening right now. Body sculpting may have the unintended (and unrecognized) side effect of stem cell activation!

A. List of Equipment to be Reviewed

(1) Low Level Laser Therapy www.erchonia.com [635 nm wavelength mentioned]

What is does: "Low level laser therapy (3LT®) has been proven to promote bone repair, wound healing, reduction of inflammation and tendon/ligament repair. Although each application can seem drastically different, the need for energy and stimulation of basic molecules is the same. Low level laser therapy (3LT®) has been shown to stimulate osteoblast, fibroblast, stem cells, skin tissue, nervous tissue, immune cells and a wide assortment of other tissue structures and cells.

Targeting these cells can promote the release of important molecules that support increased blood flow, collagen synthesis, cell recruitment, mineralization, skin cell replication, nerve tissue regeneration, reduction of scar tissue and tissue repair. Each advantage originates from the laser's capability of influencing the energy production of cells (ATP), which is necessary to drive reactions to produce the outcomes mentioned above.

Most non-steroidal anti-inflammatory drugs (NSAIDS) attempt to suppress the mechanism responsible for producing a key immunologic molecule that promotes an inflammatory response and can further damage tissue and impede the rate of healing.
[Reference: https://www.erchonia.com/wp-content/uploads/2020/06/Vet_Brochure_English_US_3192020_Rev_3.pdf]

Research reports collected here:
https://www.erchonia.com/research-articles/

In looking for further input about stemc cells, the following papers seem relevant:

(a)Farivar et al: Biological Effects of Low Level Laser Therapy, J Lasers Med Sci 2014;5(2): 58-62 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4291815/pdf/jlms-5-58.pdf

A good technical introduction to wavelengths, key takeaway is the activation of Platelet derived growth factor C (PDGF-C), A member of the PDGF/vascular endothelial growth factor family and its upregulation can induce mitogenic activity on several mesenchymal cell types….boosts proliferation.

(b) There are many many papers on the use of LLLT on bone regeneration, an example is: Amid et al, Effect of Low Level Laser Therapy on Proliferation and Differentiation of the Cells Contributing in Bone Regeneration, J Lasers Med Sci. 2014 Autumn; 5(4): 163–170. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4281990/

(c) Chang et al: Effects of Photobiomodulation on Stem Cells Important for Regenerative Medicine, Med Laser 2020;9(2):134-141
https://doi.org/10.25289/ML.2020.9.2.134
give this important summary of the field and introduce **Photobiomodulation** as the new term for Low level laser therapy, and cold laser therapy: “Photobiomodulation (PBM), formerly known as low-level laser therapy (LLLT), is a relatively non-invasive technique that has a therapeutic effect on damaged tissue or cells. Recent advances in adapting PBM to stem cell therapy showed that stem cells and progenitor cells respond favorably to light. PBM stimulates different types of stem cells to enhance their migration, proliferation, and differentiation in vitro and in vivo. This review summarizes the effects of PBM on targeted differentiation across multiple stem cell lineages.” The paper falls short, however on actual in vivo examples although a few wound healing cases are noted, most using near Infra-red.

(d) This paper, while only in vitro, showed clearly that PBM of aged mouse stem cells were returned to same as young mice. “The objective of the present study was to fill this gap and improve stem cell therapies—especially the efficacy of autologous stem cell transplantation in older individuals—by rejuvenating stem cells through improvement of their mitochondrial functionality. To test the hypothesis that overall effectiveness of aged stem cells can be restored to the level of young stem cells by PBM, MSCs isolated from bone marrow of both young (3-month-old, 3 m) and old (24-month-old, 24 m) C57BL/6 mice were subjected to different PBM regimens and their proliferation, oxygen consumption rate (OCR), and ATP production were evaluated with respect to those of untreated counterparts, as was expression of senescence/juvenescence markers (i.e., p21, p16, Nrf2, and Sirt1). The findings of the

present study show in particular that consecutive PBM treatments have a lasting rejuvenating effect on aged MSCs. Eroglu et al: Photobiomodulation has rejuvenating efects on aged bone marrow mesenchymal stem cells, Scientifc Reports (2021)11:13067 https://doi.org/10.1038/s41598-021-92584-3 **the autologous transplantation of MSCs is expected to be inefficient in older subjects who most need MSC transplantation.**
(e) AMAZING though poorly designed and poorly written paper: Shows positive effects in vivo in a rat model. In this paper, rats were subjected to whole body LLLT at 890 nm, 80 Hz, 1.5J.cm2, 3 days per week for 60 days and Bone marrow MSC's were harvested and cultured. PBM significantly stimulated the viability and cell proliferation of bone marrow MSC's showing an in vivo effect of the PBM. Mostafavinia et al, Effect of in vivo low-level laser therapy on bone marrow-derived mesenchymal stem cells in ovariectomy-induced osteoporosis of rats, https://pubmed.ncbi.nlm.nih.gov/28846932/

The paper supports the hypothesis that cold laser whole body irradiation can activate possibly dormant stem cells, or at the very least demonstrates that stem cells are affected when whole body laser therapy is applied.

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The Erchonia Emerald Laser for Fat Removal

Introducing EMERALD: A non-invasive low-level laser that emits Ten **532 nm green lasers** onto the skin to treat hypertrophic adipocytes. The treatment emulsifies the adipose tissue and releases excess fatty materials into the interstitial space. From there, the fat is passed through the body during its natural course of detoxification through the lymphatic system; claiming: "light energy at specific wavelengths target fat and work at a cellular level, creating a small transitory pore in fat cells that allows fatty liquids to seep out. The body processes these fatty liquids naturally through its lymphatic system.The fat cells shrink, instead of being killed, a healthier way to safely remove fat from the body."

Emerald Laser

Non-Invasive, In-Office Treatment for Patients up to 40 BMI

Erchonia's green laser was proven and FDA Market Cleared in 3 separate double-blind clinical trials with no known side-effects and produces 23% more energy than our red lasers, making Emerald the safest and most effective way to treat fat.

- Only FDA market cleared laser that treat patients up to 40 BMI.
- The device makes no direct contact with patients skin and is an unattended treatment —minimal staff involvement.
- Non-invasive procedure shrinks fat quickly, safely, and effectively.

Erchonia Zerona Whole Circumference Unit for Fat Loss

The Erchonia Zerona Z6 is a non-invasive cold laser for fat loss. It is the only treatment FDA Market Cleared for overall body circumference reduction and has the greatest results for combined circumference reduction in the industry.

Specifications
Configuration: 6 Line Generated Class 2 Laser Diode Modules
Wavelength: 635nm
Modulation: Constant Wave (CW)
Display: Full Color TFT Touch Screen Control Center
Adjustments:
1) 44” (111.76 cm) Vertical Arm Height Adjustment.
2) Four Independent Adjustable Arms for Desired Laser Concentration
Power Source: 100-240VAC 50-60Hz
Chassis:
1) Metal Frame Powder Coated for Ease of Cleaning
2) 4 Anti-Static Casters (2 Locking)
Housing: Black Carbon Fiber Finish Thermoformed from Non-Allergen Material
Weight: 70lbs. (31.76 kg)
Accessories: 2-Keys, Laser Safety Glasses
Compliant to Quality / ISO 13485 – Medical Device / IEC60825-1 Laser Safety, IEC 60601-1 Safety, IEC 60601-1-2 EMC, CB Mark, CB Cert
FDA Laser Class 2 / FDA Device Class ll EU Device Class IIa, Laser Class II

Lasers for Pain

Foot Fungus Lasers
Lasers for Fat Loss
Radiation Reducer
Therapeutic Stands & Accessories

XLR8® Handheld Cold Laser
Three FDA Clearances in One Handheld

EVL
Erchonia Violet and Red Laser for Veterinarians

EVRL
Erchonia Violet and Red Laser

VLS Vet Laser System

Base Station

FX 635 – Plantar Fasciitis and

Subscribe to our VIP List! Enter your email Subscribe

FX 635 – Chronic Low Back Pain
Laser for Chronic Low Back Pain

Specs for the larger unit:

Specifications
Configuration: (3) Class 2 17.25mW Line Generating Diodes
Wavelength: 635nm
Modulation: Variable
Weight: 65lbs (29.48 kg)
Height: 70in (177.8 cm) (Average – Adjustable)
Two Independent Adjustable Arms For Desired Laser Concentration
4 Locking Anti-Static Casters
Display: Full Color Touch Screen Control
Power Source: 100-240VAC 50-60Hz
Chassis: Powder Coated Aircraft Aluminum
Housing & Covers: Non-Allergenic Material, Spray Finished
Accessories: 2-Keys, Power Cord, Laser Safety Glasses
Compliant to: ISO 13485 Medical Device Quality, IEC 60825-1 Laser Safety, IEC 60601-1-2 EMC, IEC 60601-1 Safety, CE Mark, CB Certified
FDA Laser Class 2 / FDA Device Class ll / EU Device Class IIa, Laser Class II

The Erchonia Summary of the field of Low Level Laser Therapy is terrific. If read with a belief that the observations may all be attributable to stem cell activation, the summary is enlightening, and possibly the best insight into this field of PhotoBiologicalModulation (PMB).

Some excerpts from the article reproduced entirely below:

Effectiveness at a depth of 6 cm!!!!: “In 2001, Neira et al used MRI and scanning electron microscope imaging to assess the depth of biological effects of laser irradiation. The study used an 8mW 635 nm wavelength line laser held above the skin at a distance of 6-8 inches. After 4-6 minutes of exposure, significant (80- 99%) release of fat from fat cells was documented to a depth of 6 cm”.

Even though actual laser penetration far less than 1 mm: “Lasers cannot penetrate the tissue more than a fraction of a millimeter, so there is no other primary responding tissue other than the outer part of the dermis. Still, such irradiation has **secondary systemic effects.**"

Secondary Systemic Effects: “secondary effects (secondary responses) which have been studied and measured in various contexts: increased cell metabolism and collagen synthesisin fibroblasts,increased action potenti al of nerve cells, stimulation of the formation of DNA and RNA in the cell nucleus, local effects on the immune system, increased formation of capillaries by the release of growth factors, increased activity of leukocytes, transformation of fibroblasts to myofibroblasts, and a great number of other measured effects. **Therefore, "deep light penetration is not a necessity per se in biostimulation...The possible reason for this is that cells in the tissues subjected to the light produce substances that then spread and circulate in blood vessels and lymphatic systerns."**

Low Level Laser Therapy

.Effects at the cellular level increase ATP energy and DNA synthesis and benefit acute and chronic musculoskeletal aches and pains, chronic inflammation, acute soft-tissue injuries, as well as other conditions.

I am very pleased to introduce you to Dr. Dan Murphy. He is a seasoned clinicist, having been in practice for 30 years. He is extensively published and considered an expert in cervical spine injuries as well as laser therapy. He has published many articles on the neurophysiology of therapeutic laser. In this article, Dr. Murphy elaborates on a few of the unique physiological effects of laser on cellular structures. I am excited to have Dr. Murphy sharing his extensive knowledge with us and look forward to reading more in the near future..

—William J. Kneebone, DC, CNC, DIHom, FIAMA, DIACT
Department Head

By Dan Murphy DC, DABCO

In 1997, Douglas Wallace wrote an article for Scientific American titled "Mitochondrial DNA In Aging and Disease."[1] In this article, he notes that an intracellular organelle, the mitochondria, is the power plant of cells because it produces ATP energy. "Mitochondria provide about 90% of the energy that cells, and thus tissues, organs, and the body as a whole need to function." Every cell in the body contains hundreds of mitochondria that produce the energy that the body requires.

Each mitochondria contains many copies of DNA, called mitochondrial DNA, or mtDNA. Mitochondrial DNA is separate and distinct from the cell's copy of nuclear DNA. An individual's mtDNA comes from, and is identical to, the mother's mtDNA. Mitochondrial DNA (mtDNA) codes for 13 proteins (enzymes) required for the production ATP energy.

A simplified mechanism of the mitochondrial contribution to the production of ATP energy is illustrated in Figure 1 (after Audesirk[2]). Note that the primary producer of ATP energy is the "electron transport system" of the mitochondria. This is important in the understanding of laser physiology. Wallace further notes: "Anything able to compromise ATP production in mitochondria could harm or even kill cells and so cause tissues to malfunction and symptoms to develop."[1]

The inner membrane of the mitochondria contains 4 protein complexes called the respiratory chain. Electrons from food pass through these protein complexes with the help of Coenzyme Q10, interacting with oxygen and hydrogen to produce water and ATP energy. When discussing low powered laser therapy, it is important to understand that the terminal enzyme of the mitochondrial respiratory chain, the "cytochrome c oxidase" enzyme, also functions as a photoacceptor.[3,4]

"As the respiratory chain participates in energy production, toxic by-products known as oxygen free radicals are given off. These oxygen derivatives carry an unpaired electron and are highly reactive, and can attack all components of cells, including respiratory chain proteins and mitochondrial DNA. Anything that impedes the flow of electrons through the respiratory chain can increase their transfer to oxygen molecules and promote the generation of free radicals."[1] Conversely, anything that improves the flow of electrons through the respiratory chain will increases the production of ATP while reducing the generation of free radicals. This is the key to low-level laser therapy.

Wallace notes: "The mitochondrial theory of aging holds that as we live and produce ATP, our mitochondria generate oxygen free radicals that inexorably attack our mitochondria and mutate our mitochondrial DNA."[1] The accumulation of mitochondrial DNA mutations reduce ATP energy output below optimal levels. "In so doing, the mutations and mitochondrial inhibition could contribute to common signs of normal aging, such as loss of memory, hearing, vision, and stamina."[1]

In support of the writings of Wallace is the 2004 book edited by Rainer Straub and Eugenio Mocchegiani. These authors note: "One of the most accepted theories of aging is the free radical theory of aging. The overproduction of free radicals can induce cell death. Aging, as stated in free radical theory of aging, is characterized by an increased production of free radicals in several tissues or a decreased antioxidant defense leading to chronic oxidative stress."[5] The mitochondria are the major source for the production of free radicals.

As noted, the mitochondrial production of ATP is coupled with the production of Oxygen Free Radicals (Reactive Oxygen Specie,s or ROS). This is undesirable because ROS are major contributors to many diseases, including cancer. Additional support for the deleterious nature of free radical production comes from the authoritative 2006 text by Singh, titled Oxidative Stress, Disease and Cancer. The preface of this text states: "The ability of cells to reduce oxygen to produce energy is fundamental to aerobic life.

"Unfortunately, production of energy by reduction of dioxygen leads to the generation of reactive oxygen species that cause oxidative stress.

"It is now well established that oxidative stress causes extensive damage to cellular components, which can lead to a number of diseases, including cancer."[6]

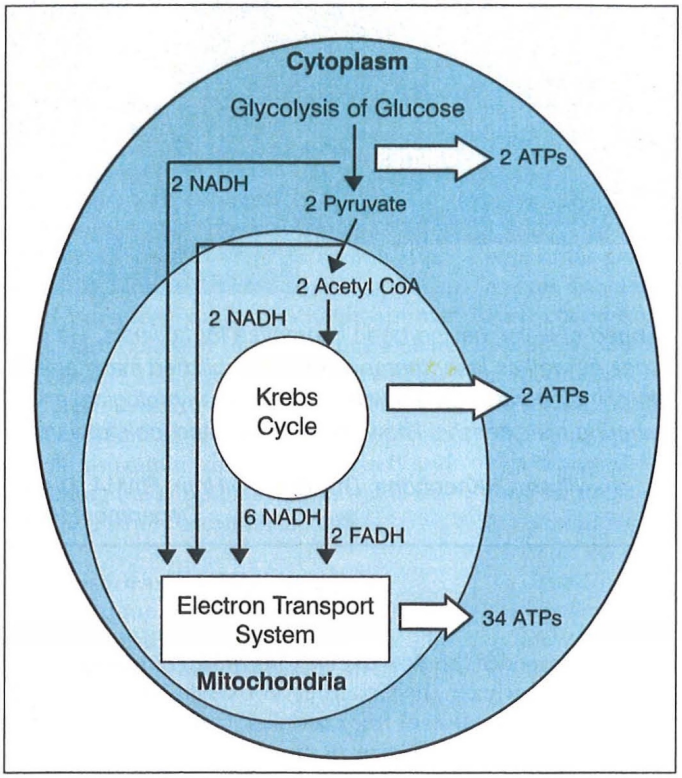

FIGURE 1. *Simplified mechanism of mitochondrial contribution in the production of ATP.*

A recent article by Pieczenik and Neustadt states: "A wide range of seemingly unrelated disorders, such as schizophrenia, bipolar disease, dementia, Alzheimer's disease, epilepsy, migraine headaches, strokes, neuropathic pain, Parkinson's disease, ataxia, transient ischemic attack, cardiomyopathy, coronary artery disease, chronic fatigue syndrome, fibromyalgia, retinitis pigmentosa, diabetes, hepatitis C, and primary biliary cirrhosis, have underlying pathophysiological mechanisms in common, namely reactive oxygen species (ROS) production, the accumulation of mitochondrial DNA (mtDNA) damage, resulting in mitochondrial dysfunction."[4]

Tiina Karu wrote the chapter "Low-Power Laser Therapy" in the book Biomedical Photonics Handbook in 2003.[7] She notes that low-level laser therapy probably works because the laser light is absorbed by the mitochondria photoreceptors, which enhances cellular metabolism. She also notes that the primary reaction of laser light is in the mitochondria, which results in increased ATP energy. "The mechanism of low-power laser therapy at the cellular level is based on the increase of oxidative metabolism of mitochondria, which is caused by electronic excitation of components of the respiratory chain."[7] In her most recent book, Karu notes that the primary component of the mitochondrial respiratory chain being influenced by laser phototherapy is the terminal enzyme of the mitochondrial respiratory chain, the "cytochrome c oxidase" enzyme.[3]

Karu states: "It is known that even small changes in ATP level can significantly alter cellular metabolism."[7] The elevated levels of ATP energy increase the rate of DNA synthesis.

Consequently, the increased levels of ATP energy and DNA synthesis will benefit acute and chronic musculoskeletal aches and pains, inflamed oral tissues, help to heal skin and mucosal ulcerations; treat edema, burns, and dermatitis; relieve pain and treat chronic inflammation as well as autoimmune diseases. Laser therapy is also used in sports medicine and rehabilitation clinics (to reduce swelling and hematoma, relieve pain, improve mobility, and to treat acute soft-tissue injuries). It was shown in the 1980s that laser radiation altered the firing pattern of nerves, which is connected with pain therapy. In 1988, Rochkind et al.[8] noted that the ability of laser irradiation to affect the action potential was dependent upon the wavelength: the effect was strong at 540 nm and 632.8 nm; while laser radiation at 660, 830, 880, 904, and 950 nm had no effect.

The 2002 book by Jan Turner and Lars Hode, titled *Laser Therapy Clinical Practice and Scientific Background*, contains 1,281 references. These authors note:

1) "Today, we can safely say that therapeutic lasers have an important biological effect, and a very positive one at that."

2) "We believe that lasers have a tremendous and as yet untapped potential in the field of healthcare."

3) "Therapeutic lasers have no undesirable side effects in the hands of a reasonably qualified therapist."

4) Lasers are "sterile, painless, and often less expensive than methods already in use," and do not have the side effects as does pharmacotherapy.

5) "Laser therapy of wounds is ideal, since it promotes healing and reduces pain at the same time.

6) Laser light increases the cell's ATP energy."[9]

Therapeutic Implications

A recent representative article regarding low-level laser therapy was published October 2004 in the American Journal of Physical Medicine & Rehabilitation.[10] Researchers injured the knees of 42 rats giving them arthritis. Twenty-one of the rats were given 632 nm low-level laser, applied over the arthritic knee for 15 minutes, three times per week, for 8 weeks; the other 21 rats were not similarly exposed. The results showed a marked repair of arthritic cartilage in the lased rats, but not in the non-lased group. The authors concluded that the 632 nm low-power laser enhances protein production in arthritic joints and repairs the arthritic cartilage.

These authors also noted that lasers are "thought to cause electronic excitation of the photoacceptor molecules, which are thought to be various cytochrome enzymes that are terminal electron carriers in the respiratory chain."[10] This is thought to accelerate electron transfer. "Electron transport in the mitochondrial membrane is one of the main fueling mechanisms underpinning metabolism and proliferation of cells, including generation of adenosine triphosphate (ATP). Low-level laser mediated increase in efficiency of the electron carriers in the respiratory chain would increase generation of adenosine triphosphate, which could manifest itself as increased DNA and protein synthesis and result in cell proliferation, as shown in present study."[10] Thus, their explanation of the physiology of low-level laser therapy is consistent with Karu[7] and Turner[9] above.

Turner notes that "any wavelength will have a biological effect,"[9] while Karu notes that "the 632.8 nm and the 820 nm are the most common wavelengths used in therapeutic light sources."[7]

Turner also notes that "The first company to receive a 510(k) from the Federal Drug Administration (FDA) was Majes-Tec Innovations in the USA and its Erchonia laser."[8] Information pro-

vided by Erchonia[12] notes that the evidence Erchonia used to achieve the FDA 510(k) status involved a group of 50 patients treated for musculoskeletal neck and shoulder pain. The laser used was a 635 nm wavelength line laser using 5 mW of power, applied for 3 minutes over the area of complaint. The laser group showed a 66% improvement in pain and range of motion as compared to the placebo group following a single 3-minute exposure.

In 2001, Neira et al[13] used MRI and scanning electron microscope imaging to assess the depth of biological effects of laser irradiation. The study used an 8mW 635 nm wavelength line laser held above the skin at a distance of 6-8 inches. After 4-6 minutes of exposure, significant (80-99%) release of fat from fat cells was documented to a depth of 6 cm. It is unknown whether the biological effects documented in this study were as a consequence of primary or secondary reactions to the laser irradiation. The reproductions of the electron microscope images in the original article are stunning. Personal investigation revealed that each electron micrograph was produced at a cost of $10,000.

The book by Tuner and Hode also makes the following points: "Treatment with laser therapy is not based on heat development but on photochemical and photobiological effects in cells and tissues."[9]

Lasers "cannot penetrate the tissue more than a fraction of a millimeter, so there is no other primary responding tissue other than the outer part of the dermis." Still, such irradiation has "secondary systemic effects." Therefore, the light "leads in turn to a number of secondary effects (secondary responses) which have been studied and measured in various contexts: increased cell metabolism and collagen synthesis in fibroblasts, increased action potential of nerve cells, stimulation of the formation of DNA and RNA in the cell nucleus, local effects on the immune system, increased formation of capillaries by the release of growth factors, increased activity of leukocytes, transformation of fibroblasts to myofibroblasts, and a great number of other measured effects." Therefore, "deep light penetration is not a necessity per se in biostimulation..." "The possible reason for this is that cells in the tissues subjected to the light produce substances that then spread and circulate in blood vessels and lymphatic systems."[9]

In their literature review, Tuner and Hode also note: "There was also a group of animals on which two wounds were inflicted [bilaterally], only one of which was treated with laser. Even the untreated wound showed better results than the control group. The authors report drew the following conclusion: 'The laser irradiation can thus have released substances in the circulation apparatus so that the tensile strength increased even in the wound on the opposite, untreated side.'"[9]

'Another study notes: "...laser treatment on only the right-hand side of bilaterally inflicted skin wounds increased the healing process on both sides as compared to the control group. This also applied in the case of bilateral burn wounds."[9]

In another study of patients treated unilaterally with chronic neck and shoulder pain, "The pressure pain threshold increased significantly on both the non-treated and the treated side, although the increase was larger on the treated side."

In another animal study evaluating suppressed tuberculin reaction, "The suppression was seen not only on the irradiated side but also on the contralateral, non-irradiated side."[9]

In another study evaluating the effects of laser on the treatment of an anaphylactic reaction in the eyes of rabbits, the healing effect of the laser was obvious, and "Consensual co-reaction could be observed in the contralateral non-irradiated eyes in the experimental group."[9]

Tuner and Hode explain these results on tissues contralateral to the side of laser irradiation stating that: "The non-irradiated 'control' lesion, in fact, benefits from the treated lesion because of the systemic reaction just discussed...conventional laser therapy has both a local effect in the area treated by laser, and a systemic effect through the release of metabolites." The authors also state that "Due to transmission of neural excitation and calcium waves, photobiomodulation is a systemic effect."[9]

Wavelengths and Power Outputs

Therapeutic low powered lasers are commercially available in many different wavelengths and power outputs. In reviewing the new book by Tiina Karu[3] on Laser Phototherapy, it appears clear that there is no one wavelength that is ideal for all appropriately treated clinical syndromes. Few studies compare the outcome of different wavelengths and exposure fluences on measured outcomes. However, a recent study by Carrinho et al[14] compared the tissue repair of injured mouse tendons when treated with either a 685 nm laser or an 830 nm laser, each at fluences of both 3 J/cm^2 and 10J/cm^2. This study used 48 mice that were divided into six experimental groups and are summarized in Table 1.

Laser irradiation started 24 hours after the tenotomy of the Achilles tendon. A total of 12 laser sessions were performed on consecutive days. The rats were killed on day 13, and the injured tendons were surgically removed and analyzed with polarized light microscopy to analyze the organization and molecular order of the collagen fibers. All laser treated groups showed improved healing when compared to injured control group. The best organization and aggregation of the collagen bundles was shown by the animals

Table 1. Study results comparing tissue repair of injured mouse tendons at different wavelengths and dosage.[14]

Treatment conditions	Tissue response compared to control tendons
Group A, tenomized animals, treated with 685 nm laser, at the dosage of 3 J/cm^2	208% improved tissue response over control
Group B, tenomized animals, treated with 685-nm laser, at the dosage of 10 J/cm^2	114% improved tissue response over control
Group C, tenomized animals, treated with 830-nm laser, at dosage of 3 J/cm^2	167% improved tissue response over control
Group D, tenomized animals, treated with 830-nm laser, at dosage of 10 J/cm^2	101% improved tissue response over control
Group E, injured control (placebo treatment)	
Group F, non-injured standard control	

of group A (685 nm, 3 J/cm^2), followed by the animals of group C (830 nm, 3 J/cm^2), and B (685 nm, 10 J/cm^2), and finally, the animals of group D (830 nm, 10 J/cm^2). The authors concluded: "All wavelengths and fluences used in this study were efficient at accelerating the healing process of Achilles tendon post-tenotomy, particularly after the 685-nm laser irradiation, at 3 J/cm^2. It suggests the existence of wavelength tissue specificity and dose dependency."[14]

Interestingly, in this study, the shorter wavelength was associated with the better healing outcome. Counterintuitively, lesser exposure to laser irradiation resulted in an improved healing outcome than higher doses of exposure. These authors note: "The better tissue response was observed after the irradiation with the 685-nm laser, at the dosage of 3 J/cm^2. The animals irradiated with the 830-nm laser, at the dosage of 10 J/cm^2 presented the weaker response to laser irradiation. The best tissue response was obtained after the 685-nm laser irradiation, at the dosage of 3 J/cm^2."

Specifically, the 685-nm laser irradiation at 3 J/cm^2 showed a 16% improved tendon healing over the 830-nm laser at 3 J/cm^2; a 33% improved tendon healing over the 685-nm laser at 10 J/cm^2; and a 54% improved tendon healing over the 830-nm laser at 10 J/cm^2.

Carrinho et al concluded that "Our results suggest that laser irradiation (particularly using the 685-nm laser, at the dosage of 3 J/cm^2) produced an increase of cell proliferation through changes in mitochondrial physiology, subsequently affecting RNA synthesis, which, in turn, alters the expression of various cell regulatory proteins."[14]

Clinical Considerations

Several studies have generated caution concerning higher levels of exposure to low level lasers. Tuner and Hode note that "If a dose above the highest one suitable is administered, weaker or no biological effects will result. With an even greater dose, the bio-suppressive range is entered (inhibiting effect result)."[9]

In 2004, in an article titled "Photobiological Principles of Therapeutic Applications of Laser Radiation," the authors note that the positive action of laser biostimulation is changed "into inhibition of vital activity processes" under large doses of laser radiation, "which is a main hindrance to a successful application of laser therapy and a cause of disappointment."[15]

Possibly the most important article to be aware of regarding the effects of the energy output of lasers was published in the January 2006 issue of the journal Lasers in Surgery and Medicine. This article notes that a lower dose of laser irradiation "...has a stimulatory influence on wounded fibroblasts with an increase in cell proliferation and cell viability without adversely increasing the amount of cellular and molecular damage. Higher doses were characterized by a decrease in cell viability and cell proliferation with a significant amount of damage to the cell membrane and DNA."[16]

These authors further note that by spreading the light out over 3.3 cm, "the light is divergent and is not as harmful as a narrow parallel beam that allows the entire volume of intense laser light to be focused or concentrated on one small area."[16]The laser used in this study used only 3 mW of power.

Summary

Mitochondria present a paradox: not only are they the major producer of ATP energy, but they are also the major producer of free radicals. As the mitochondria produce the ATP energy that our bodies require to function, the mitochondria also produce the free radicals that damage and age our bodies. Lasers increase the mitochondrial production of ATP without increasing the production of free radicals. Anything that increases the production of ATP energy will speed healing and improve symptoms. Since lasers can achieve this with minimal side effects or risks, low-level laser therapy is here to stay. Reviewing the books by Karu,[3] Tuner and Hode,[9] and Baxter,[11] shows the magnitude and diversity of research that has already been completed concerning low-level laser therapy and laser photobiology. Low-level laser therapy has both local and systemic influences, and some laser wavelengths can affect the action potential of neurons. However, there is some evidence that higher amounts of laser energy delivered into the body may not improve clinical outcomes, and there are suggestions that excess exposure to laser irradiation may even be harmful. ■

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He is a contributing author to the books Motor Vehicle Collision Injuries, editions 1 and 2, and Pediatric Chiropractic, and writes a quarterly column in the American Journal of Clinical Chiropractic. He has received numerous awards recognizing his contributions as educator and clinician. Dr. Murphy's reviews of articles regarding alternative health issues can be accessed at www.danmurphydc.com.

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16. Hawkins DH, Tech M, Abrahamse H. Fibroblasts Following Helium-Neon Laser Irradiation, The Role of Laser Fluence in Cell Viability, Proliferation, and Membrane Integrity of Wounded Human Skin. *Lasers in Surgery and Medicine*. 38:74–83 (2006).

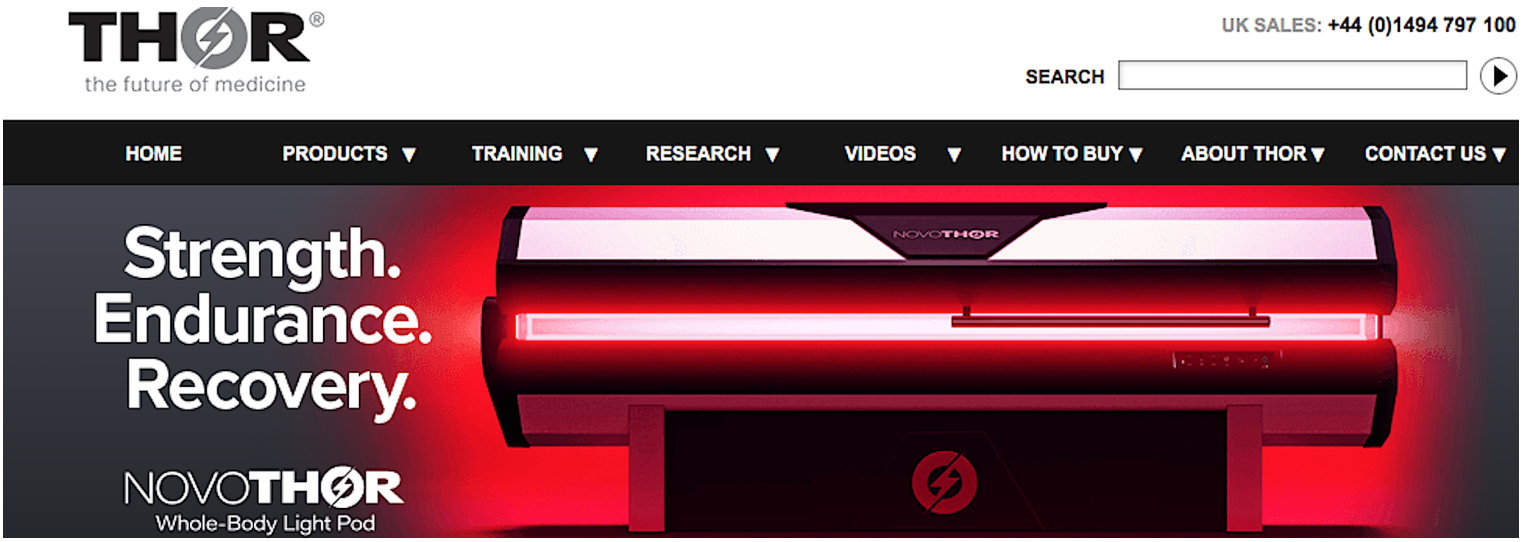
Erchonia Training Videos: https://www.erchonia.com/training-videos/

It is interesting that the hand-held unit, while advertised only for pain, includes on its screen other conditions such as Arthritis, Gut-Brain-Axis, Inflammation, Alzheimers, Autism, Non-healiing wounds, and most likely other conditions:

THOR: Another Cold Laser Product

Thor also makes a cold laser product: https://www.thorlaser.com/LLLT/

They even market a **Whole Body Light Pod**…really terrific for whole body stem cell stimulation!!! Claiming **"Helps Body Heal", and "Increases Blood Flow"**



They present some very good clinical outcomes in a variety of disease/conditions: https://www.thorlaser.com/downloads/research/Autoimmunity-including-RA-&-Thyroiditis.pdf

And present s very nice DOSE REVIEW: https://www.thorlaser.com/downloads/research/Biphasic-Dose-Response-in-Low-Level-Light-Therapy-Harvard.pdf

Lastly, they present some studies where **stem cells were boosted clinically: "A selection of papers demonstrating positive impact of PBM on Stem Cells, with important application in regenerative medicine"**
https://www.thorlaser.com/downloads/research/Stem-cells-for-THOR-website.pdf

Additional Research Reports are here: **https://www.thorlaser.com/thanks_downloads.php**

BEST PRODUCT: The Whole Body Lite Pod: **https://www.novothor.com/index/**

"Red light therapy is a treatment that stimulates natural cellular processes. Similar to how specific wavelengths (at the opposite end of the light spectrum) can be used to treat psoriasis or vitiligo. Red light therapy (also known as photobiomodulation) uses a combination of red and near-infrared light to activate a number of biological processes"

Can be switched between red only and mixed red + near-infrared

Red: 660nm
Near-infrared: 850nm

NovoTHOR handles 2.7kW of power with ease, distributing the power to light engines and drivers that regulate power flow, keeping the precise amount of power to meet strict dosage requirements. A mixture of active and passive cooling keep the LEDs efficient and your customer cool.

An Extremely important paper relating to dose response and the concepts of irradiation vs dose is Huang et al, Biphasic Dose Response in Low Level Light Therapy, Dose Response. 2009; 7(4): 358–383.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2790317/

discusses how **LLLT causes a "cascade of secondary effects that contribute to a range of local tissue and systemic effects"** and presenting these two important Tables:

TABLE 1. Parameters involved in determining the LLLT "medicine"

IRRADIATION PARAMETERS (*The Medicine*)		
Irradiation Parameter	Unit of measurement	Comment
Wavelength	nm	Light is electromagnetic energy which travels in discrete packets that also have a wave-like property. Wavelength is measure in nanometres (nm) and is visible in the 400-700 nm range.
Irradiance	W/cm^2	Often called Intensity, or Power Density and is calculated as Irradiance = Power (W)/Area (cm^2)
Pulse structure	Peak Power (W) Pulse freq (Hz) Pulse Width (s) Duty cycle (%)	If the beam is pulsed then the Power should be the Average Power and calculated as follows: Average Power (W) = Peak Power (W) × pulse width (s) × pulse frequency (Hz)
Coherence	Coherence length depends on spectral bandwidth	Coherent light produces laser speckle, which has been postulated to play a role in the photobiomodulation interaction with cells and subcellular organelles.
Polarisation	Linear polarized or circular polarized	Polarized light may have different effects than otherwise identical non-polarized light (or even 90-degree rotated polarized light). However, it is known that polarized light is rapidly scrambled in highly scattering media such as tissue (probably in the first few hundred μm).

TABLE 2. Parameters involved in determining the LLLT "dose"

IRRADIATION TIME OR ENERGY DELIVERED (*The Dose*)		
Irradiation Parameter	Unit of measurement	Comment
Energy (Joules)	J	Calculated as: Energy (J) = Power (W) x time (s) This mixes medicine and dose into a single expression and ignores Irradiance. Using Joules as an expression of dose is potentially unreliable as it assumes reciprocity (the inverse relationship between power and time).
Energy Density	J/cm^2	Common expression of LLLT "dose" is Energy Density This expression of dose again mixes medicine and dose into a single expression and is potentially unreliable as it assumes a reciprocity relationship between irradiance and time.
Irradiation Time	s	In our view the safest way to record and prescribe LLLT is to define the four parameters of the medicine (see table 1.) and then define the irradiation time as "dose".
Treatment interval	Hours, days or weeks	The effects of different treatment interval is underexplored at this time though there is sufficient evidence to suggest that this is an important parameter.

The main importance of this paper can be summarized as follows:

There is a so-called "optical window" in tissue, where the effective tissue penetration of light is maximized. This optical window runs approximately from 650 nm to 1200 nm. The absorption and scattering of light in tissue are both much higher in the blue region of the spectrum than the red, because the principle tissue chromophores (hemoglobin and melanin) have high absorption bands at shorter wave- lengths, tissue scattering of light is higher at shorter wavelengths, and furthermore water strongly absorbs infrared light at wavelengths greater than 1100-nm. **Therefore the use of LLLT in animals and patients almost exclusively involves red and near-infrared light (600-1100-nm) (Karu and Afanas'eva 1995).**

Ultrasonic Cavitation for Fat Removal

This procedure involves applying pressure on fat cells through ultrasonic vibrations. The pressure is high enough to make the fat cells break down into a liquid form. The body can then get rid of it as waste through your urine.

Ultrasonic cavitation tones the body using radio frequencies and low-frequency ultrasonic waves. These waves form bubbles around fat deposits under the skin. The bubbles then burst, breaking the fat deposits into the interstitial and the lymphatic systems where they are drained. The fat deposits are changed into glycerol and free fatty acids. Glycerol is then reused by the body while free fatty acids travel to the liver and are excreted as waste.

It appears to me that Damage is occurring, the main idea is to damage the fat cells. Will other cells be damaged—i.e. stem cells? Damage can be Good. In the process, will a positive result be obtained in that endothelial cells and endothelial growth factors will be released? I like the idwa of getting in there and stirring things up. This is what we are doing with liposuction and processing of SVF.

However, not all patients would be suitable…non-obese patients and thin patients with limited body fat may not be good candidates.

Other Electronic Equipment for Bone Growth Stimulation

FDA Executive Summary, Prepared for the September 8-9, 2020 Meeting of the Orthopaedic and Rehabilitation Devices Panel:
Reclassification of **Non-Invasive Bone Growth Stimulators**
https://www.fda.gov/media/141850/download

In a 2020 report, the FDA noted this:

Device Description and Current Classification:

Non-invasive bone growth stimulators, currently designated under product code LOF and LPQ, are typically composed of a waveform generator and transducer (e.g., coils, electrodes, and/or ultrasound transducers). Patient-contacting surfaces include the transducers, lead wires, and the device outer casing.

Non-invasive bone growth stimulators utilize an electrical component to produce an output electrical, magnetic, or ultrasonic waveform that is delivered to a treatment site via a non-invasively applied transducer (e.g., electromagnetic coil

or ultrasound transducer) or electrodes. The device also incorporates an internal means to monitor the output waveform and delivery of treatment, and to provide visual and/or audible alarms to alert the user of improper device function.

The induced electrical and/or magnetic fields are generated using one of the following modalities:

- Capacitive Coupling (CC), in which a pair of electrodes are placed on the skin such that a current can be driven across that target site,

- Pulsed Electromagnetic Fields (PEMF), in which a modulated electromagnetic field is generated near the treatment site though an external coil,

- Combined Magnetic Fields (CMF), in which a coil generates a combination of a static and pulsed magnetic field near the treatment site, and

- Low Intensity Pulsed Ultrasound (LIPUS), in which pulsed ultrasonic signals are generated using ultrasonic transducers.

The specific mechanism of action of these devices varies depending on the technology. The underlying theory behind the mechanism of the PEMF, CC, and CMF devices is that the electronic field causes voltage-gated Ca2+ channels in the cell walls of osteocytes to open, changing intercellular and cytosolic Ca2+ levels. This triggers signaling molecules to promote osteoblastic differentiation and formation, and thus upregulating bone formation activity. For LIPUS signals, the ultrasound wave is a mechanical signal that may directly stimulate cellular mechanotransducers resulting in the a signaling cascade resulting the same increase in intracellular Ca2+ levels and upregulation of osteoblastic activity.

Currently, non-invasive bone growth stimulators are grouped under two product codes

- LOF – Stimulator, Bone Growth, Non-Invasive

- LPQ – Stimulator, Ultrasound And Muscle, For Use Other Than Applying Therapeutic Deep Heat

Of the above, PEMF seems to have gotten the most attention, and an excellent review article is here: Cadossi et al, **Pulsed Electromagnetic Field Stimulation of Bone Healing and Joint Preservation: Cellular Mechanisms of Skeletal Response**: J Am Acad Orthop Surg Glob Res Rev. 2020 May; 4(5): e19.00155.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7434032/

As exciting as LLLT therapy seems to be, PEMF has clearly been shown to stimulate bone healing. You are not going to have any "bone stimulation" without the participation of stem cells. So, we can deduce immediately that electromagnetic field pulsing goes deeper into tissue than LLLT and stimulates stem cells in the bone *directly;* as opposed to LLLT, which requires stimulating a "cascade of secondary effects that contribute to a range of local tissue and systemic effects".

From Aaron RK, Boyan B, Ciombor DM, Schwartz Z, Simon BJ: Stimulation of growth factor by electric and electromagnetic fields, Clin Orthop,Number 419, February 2004, we have this, **noting the comparison of Capacitive Coupling to PEMF**:

FromAaron et al Stimulation of Growth Factors

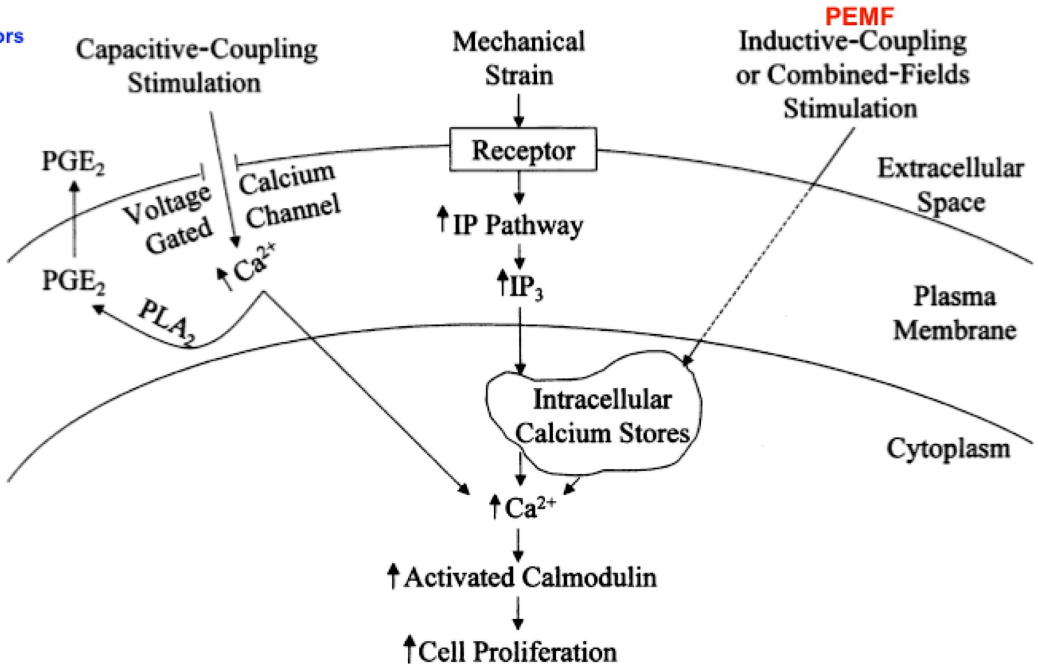


FIGURE 1. Schematic drawings shows the signal transduction pathways followed by electrical fields and mechanical strain. PGE_2 = prostaglandin E_2; PLA_2 = phospholipase A_2; IP = inositol phosphate; IP_3 = inositol triphosphate. Mechanical strain appears to act through receptor mediated IP pathways. Capacitive coupling acts through voltage-gated calcium channels. Inductively coupled fields release intracellular calcium stores. Cellular effects of all stimulation pathways are dependent on activated calmodulin. (Reprinted with permission from Brighton C, Wang W, Seldes R, Zhang G, Pollack S: Signal transduction in electrically stimulated bone cells. *J Bone Joint Surg.* 2001;83A:1514–1523.)

Further Comparison literature research is needed on the specifics!!

For example, Erchonia claims that LLLT stimulates "ostoeblasts, fibroblasts, stem cells, skin tissue, nervous tissue, immune cells" and others. What is the real data for this and also, how does such stimulation compare to the use of PEMF?

Review of Possible External Procedures for Stimulating Stem Cell Activity In Situ Part II

Walter P. Drake
April 22, 2022

Part I: Overview of Proven Technologies [Covered previously]

Part II: Specific applications to wound healing, facial rejuvenation, bone regrowth, stem cell activation in vivo, effects on skin, disease conditions [Covered in this report]

In this Part II, the following modalities were reviewed to determine if there is any clinical evidence of their utility in stimulating stem cells *in vivo:*

Low Intensity Pulsed Ultrasound [LIPUS]

Pulsed Electromagnetic Fields [PEMF]

Low Level Laser Therapy [LLLT]

The purpose of this review was to determine if using these external sources of light or electronics could stimulate or activate biological repair processes *in situ,* and thus address the hypothesis that as aging occurs, our stem cells become less active and dormant, but can be reactivated to the youthful state. The whole idea of the Stromal Vascular Fraction (SVF) being the "liquid gold" of stem cell therapy worldwide demonstrates that even in the aged individual, stem cells harvested from adipose tissue via micro-liposuction, and activated by light, perform curative roles when re-injected.

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LIPUS [Low Intensity Pulsed Ultrasound]

Good Starting Point: Tan et al, “Low-intensity pulsed ultrasound stimulates proliferation of stem/progenitor cells: what we need to know to translate basic science research into clinical applications”. Asian Journal of Andrology (2021) 23, 602–610
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8577250/pdf/AJA-23-602.pdf

The Authors state: “LIPUS has been shown to have many benefts including promotion of tissue healing, angiogenesis, and tissue regeneration; inhibition of in ammation and pain relief; and stimulation of cell proliferation and differentiation”, **but many studies are in vitro.**

However, with respect to bone repair, clinical studies show: LIPUS delivers low-intensity acoustic pressure waves that produce microbiomechanical interactions with the cells to elicit intracellular biological effects. This ultimately results in tissue repair and regeneration, a process named “mechanotransduction.” **Accumulating evidence indicates that LIPUS is effective to stimulate osteoblasts, to promote bone formation, and to activate other stem/progenitor cells.**

Some preliminary clinical reports show LIPUS to be effective in treating chronic prostatitus in human.

Some typical specs for a hand-held unit like SoundCare ($800):

Unique in nature, the SoundCare Plus professional ultrasound device comes complete with two different sound heads - 1 cm^2 and 5 cm^2 - that can be plugged in simultaneously for more targeted therapy. Simple to operate, the user can switch between wands with the single push of a button.

Product Features:

Comes complete with two sound heads
Used for the treatment of chronic and acute muscular pain
User can switch between dual sound heads with the push of a button
Fully functional 1 MHz and 3 MHz frequencies
20 user-defined presets with pulsed and continuous therapy
Ergonomic handle design
Two Year Manufacturer Warranty

Product Specifications:

Acoustic Frequency: 1 MHz ± 10%; 3 MHz ± 10%
Output Power:
0.5 - 10.0 W ± 20%, when duty factor > 80% for 5 cm^2
0.5 - 15.0 W ± 20%, when duty factor < 70% for 5 cm^2
0.1 - 2.0 W ± 20%, when duty factor > 80% for 1 cm^2
0.1 - 3.0 W ± 20%, when duty factor < 70% for 1 cm^2
Pulse Repetition Rate: 100 Hz ± 10%
Duty Factor: 10% - 100%, Stepping 10%
Timer: Adjustable up to 30 minutes
Effective Radiating Area: 5.0 cm^2 ± 20%; 1.0 cm^2 ± 20%
Actual Intensity:
3.0 W/cm^2 ± 20% (1 MHz)
3.0 W/cm^2 ± 20% (3 MHz)
Raw (Max): 5.0
Beam Type: Collimated
Material of Applicator: Aluminum

This paper demonstrated bone healing in patients with “non-unions”, with union occurring by 6 months.

Chaudhry et al, Low intensity pulsed ultrasound (Lipus) as a non-surgical cost effective method of managing atrophic non-union, J Orthop Surg Rehabil. 2019;3(1):1-5.

However, there are many disagreements in thte literature as to if this procedure is effective at all for anything.

Conclusion: Despite many in vitro studies, I could find nothing to support the idea that this procedure might be effective to stimulate stem cells in situ. There is even disagreement as to whether its proposed use in fat reduction is effective, some investigators demonstrating that when the fat returns, it is deposited irregularly and has a worse appearance than before. As noted above, even *in vivo* bone growth stimulation is being hotly debated.

Pulsed Electromagnetic Fields (PEMF)

In which a modulated electromagnetic field is generated near the treatment site though an external coil

To pick up where we left off in Part 1:

Of the above, PEMF seems to have gotten the most attention, and an excellent review article is here: Cadossi et al, Pulsed Electromagnetic Field Stimulation of Bone Healing and Joint Preservation: Cellular Mechanisms of Skeletal Response: J Am Acad Orthop Surg Glob Res Rev. 2020 May; 4(5): e19.00155. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7434032/

As exciting as LLLT therapy seems to be, **PEMF has clearly been shown to stimulate bone healing**. You are not going to have any "bone stimulation" without the participation of stem cells. So, we can deduce immediately that electromagnetic field pulsing goes deeper into tissue than LLLT and stimulates stem cells in the bone directly; as opposed to LLLT, which requires stimulating a "cascade of secondary effects that contribute to a range of local tissue and systemic effects".

Some links to skin (primarily facial) applications:

https://www.skintherapymaster.com/pulse-electro-magnetic-field-pemf

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4479364/

https://www.nualawoulfe.ie/blogs/latest-news/skin-rejuvenation-healing-and-collagen-synthesis

https://www.pemftherapyeducation.com/2017/11/pemf-therapy-gives-healthier-skin/

Here is a PEMF product shaped like a face mask: https://www.prnewswire.com/news-releases/nulife-ventures-introduces-pemf-therapy-device-sedona-face-301190963.html and the User Manual is Here: https://nulifesciences.com/wp-content/uploads/2019/11/SedonaFaceMANUAL.pdf

Conclusion: I am unimpressed with PEMF except for its use in pain management. It is, in the end, an electrical based modality, well suited to nerves and pain issues commonly involving electron transmissions within the human body. As a possible stimulator of stem cell activity, I do not see it, despite many in vitro studies which appear to have no correlation to positive clinical outcomes.

Back to LLLT [Low Level Laser Therapy]

Most lasers have one to four waves; Dr. Casalini's THOR have nine to seventeen synchronized waves, with varying levels of power. ""Our lasers give you multi waves, meaning they're multipurpose," says Dr. Casalini. "So you can work on pain treatment, scar tissue, anti-aging, all in one unit, because each wave gives you a different power."

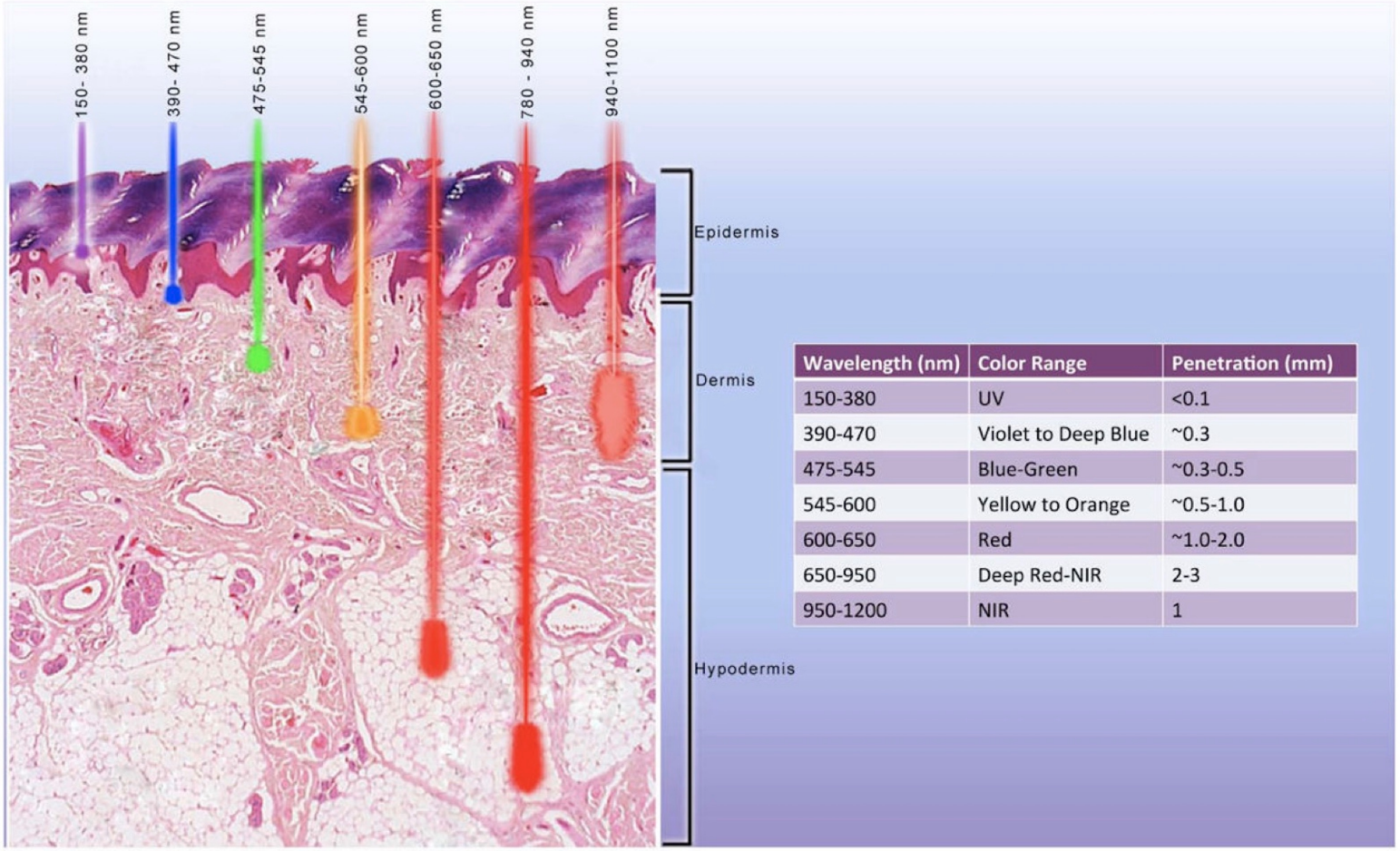
Problem with LLLT: Although LLLT is now used to treat a wide variety of ailments, it remains somewhat controversial as a therapy for 2 principle reasons. First, there are uncertainties about the fundamental

molecular and cellular mechanisms responsible for transducing signals from the photons incident on the cells to the biological effects that take place in the irradiated tissue. Second, there are significant variations in terms of dosimetry parameters: wavelength, irradiance or power density, pulse structure, coherence, polarization, energy, fluence, irradiation time, contact vs non-contact application, and repetition regimen. Lower dosimetric parameters can result in reduced effectiveness of the treatment and higher ones can lead to tissue damage.

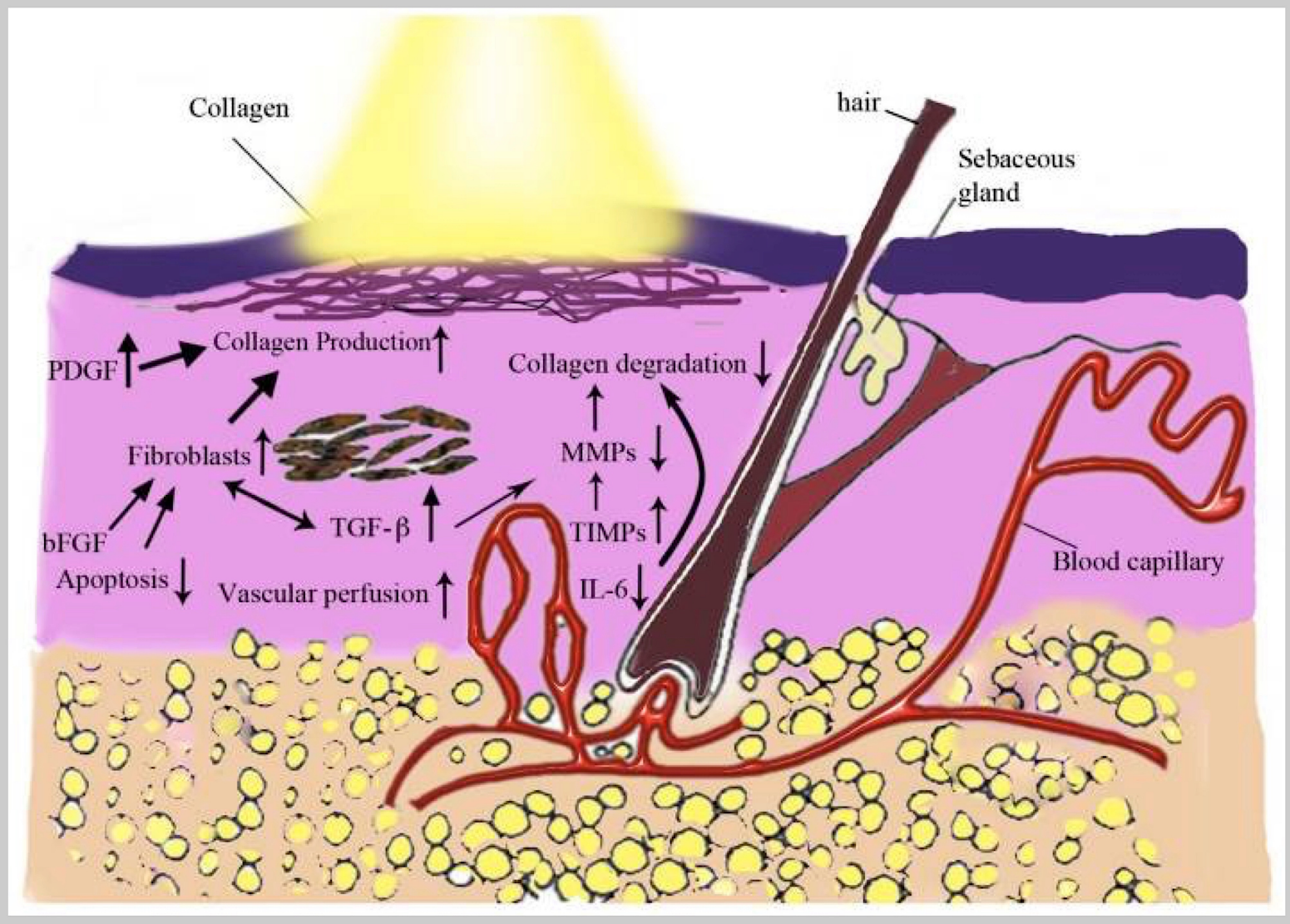
Ref: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4126803/

The best review for the positive effect of LLLT for facial rejuventation is Avci et al, Low-level laser (light) therapy (LLLT) in skin: stimulating, healing, restoring, *Semin Cutan Med Surg*. 2013 March ; 32(1): 41–52
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4126803/

This is a terrific paper that notes this tissue depth vs wavelength:



Wavelength (nm)	Color Range	Penetration (mm)
150-380	UV	<0.1
390-470	Violet to Deep Blue	~0.3
475-545	Blue-Green	~0.3-0.5
545-600	Yellow to Orange	~0.5-1.0
600-650	Red	~1.0-2.0
650-950	Deep Red-NIR	2-3
950-1200	NIR	1



I found this excerpt to be quite helpful and illuminating concerning facial rejuvenation using LLLT, and so wrap up my research with it's quote [numbers in the quote refer to reference numbers in the above cited paper:

LLLT for Skin Rejuvenation

Skin starts showing its first signs of aging in the late 20s to early 30s and it usually presents with wrinkles, dyspigmentation, telangiectasia, and loss of elasticity. Common histologic and molecular-level features are reduction in the amount of collagen, fragmentation of collagen fibers, elastotic degeneration of elastic fibers, upregulation of matrix metalloproteinases (MMPs), especially MMP-1 and MMP-2, dilated and tortuous dermal vessels, and atrophy and disorientation of the epidermis.23,24 Both chronological and environmental influences are responsible for the aging process of skin; however photodamage seems to be one of the most important causes of these changes.

Several modalities have been developed in order to reverse the dermal and epidermal signs of photo- and chronological aging. The main concept of most of these modalities is removing the epidermis and inducing a controlled form of skin wounding in order to promote collagen biosynthesis and dermal matrix remodeling. The most commonly used interventions as of today are retinoic acid (a vitamin A derivative), dermabrasion, chemical peels, and ablative laser resurfacing with carbon dioxide (CO2) or erbium: yttrium-aluminum-garnet (Er:YAG) lasers or a combination of these wavelengths.25–27 However, these procedures require intensive post-treatment care, prolonged downtime and may lead to complications such as long-lasting erythema, pain, infection, bleedings, oozing, burns, hyper- or hypopigmentation and scarring.28,29 These limitations created a need for the development of alternative rejuvenation procedures that were safer, more effective, had fewer side effects and minimum postoperative care and downtime, which in turn led to the emergence of non-ablative rejuvenation technologies.30–32 Non-ablative skin rejuvenation aims to improve photoaged and aging skin without destroying the epidermis.31,32 Irregular pigmentation and telangiectasia can be treated with intense pulsed light sources (IPL), 532 nm potassium-titanyl-phosphate lasers (KTP), and high-dose 585/595 nm pulsed dye lasers (PDL)33. Wrinkle reduction and skin tightening through thermal injury to the dermis (photothermolysis) can be achieved by other IPL sources (ie, low-dose 589/595 nm PDLs, 1064 & 1320 nm neodymium:yttrium-aluminum-garnet lasers, (Nd:YAG) 1450 nm diode lasers, and 1540 nm erbium fiber lasers).33

LED which is a novel light source for non-thermal, non-ablative skin rejuvenation has been shown to be effective for improving wrinkles and skin laxity (Figure 3).34–40 It is not a new phenomenon since the first reports of LLLT effects on increased collagen go back to 1987. Studies by Abergel et al. and Yu et al. reported an increase in production of pro-collagen, collagen, basic fibroblast growth factors (bFGF) and proliferation of fibroblasts after exposure to low-energy laser irradiation in vitro and in vivo animal models (Figure 4).41,42 Furthermore, LLLT was already known to increase microcirculation, vascular perfusion in the skin, alter platelet-derived growth factor (PDGF), transforming growth factor (TGF-β1) and inhibit apoptosis (Figure 4).1,43,44 Lee et al. investigated the histologic and ultrastructural changes following a combination of 830 nm, 55 mW/cm2, 66 J/cm2 and 633 nm, 105 mW/cm2, 126 J/cm2 LED phototherapy and observed alteration in the status of MMPs and their tissue inhibitors (TIMPs).33 Furthermore, mRNA levels of IL-1β, TNF-α, ICAM-1, and connexin 43 (Cx43) were increased following LED phototherapy whereas IL-6 levels were decreased (Figure 4) 33. Finally, an increase in the amount of collagen was demonstrated in the post-treatment specimens 33. Pro-inflammatory cytokines IL-1β and TNF-α are thought to be recruited to heal the intentionally formed photothermally-mediated wounds associated with laser treatments, and this cascade of wound healing consequently contributes to new collagen synthesis.33 LED therapy may induce this wound healing process through non-thermal and atraumatic induction of a subclinical ‘quasi-wound’, even without any actual thermal damage which could cause complications as in some other laser treatments.33 TIMPs inhibit MMP activities, so another possible mechanism for the increased collagen could be through the

induction of TIMPs (Figure 4). When these observations are put together, it is possible that increased production of IL-1β and TNF-α might have induced MMPs in the early response to LED therapy. This may clear the photodamaged collagen fragments to enable biosynthesis of new collagen fibers. Later on, an increase in the amount of TIMPs might protect the newly synthesized collagen from proteolytic degradation by MMPs.33 Furthermore, increased expression of Cx43 may possibly enhance cell-to-cell communication between dermal components, especially the fibroblasts, and enhance the cellular responses to the photobiostimulation effects from LED treatment, in order to produce new collagen in a larger area which even includes the non-irradiated regions.33 In a clinical study performed by Weiss et al., 300 patients received LED therapy (590 nm, 0.10 J/cm2) alone, and 600 patients received LED therapy in combination with a thermal-based photorejuvenation procedure. Among patients who received LED photorejuvenation alone, 90% reported that they observed a softening of skin texture and a reduction in roughness and fine lines ranging from a significant reduction to sometimes subtle changes.36 Moreover, patients receiving a thermal photorejuvenation laser with or without additional LED photomodulation (n = 152) reported a prominent reduction in post-treatment erythema and an overall impression of increased efficacy with the additional LED treatment.36,45 This reduction in post-treatment erythema could be attributed to anti-inflammatory effects of LLLT.40 Using different pulse sequence parameters, a multicenter clinical trial was conducted, with 90 patients receiving 8 LED treatments over 4 weeks.37,46–48 The outcome of this study showed very favorable results, with over 90% of patients improving by at least one Fitzpatrick photoaging category and 65% of patients demonstrating global improvement in facial texture, fine lines, background erythema, and pigmentation. The results peaked at 4 to 6 months following completion of 8 treatments. Markedly increased collagen in the papillary dermis and reduced MMP-1 were common findings. Barolet et al.'s study is also consistent with the previously mentioned studies. They used a 3-D model of tissue-engineered human reconstructed skin to investigate the potential of 660 nm, 50 mW/cm, 4 J/cm2 LED in modulating collagen and MMP-1 and results showed upregulation of collagen and down-regulation MMP-1 in vitro.40 A split-face, single-blinded clinical study was then carried out to assess the results of this light treatment on skin texture and appearance of individuals with aged/photoaged skin.40 Following 12 LED treatments, profilometry quantification demonstrated that while more than 90% of individuals had a reduction in rhytid depth and surface roughness, 87% of the individuals reported that they have experienced a reduction in the Fitzpatrick wrinkling severity score.40

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Finally, I close with the Review by Graeme Ewan Glass, who pulled together all the recent clinical uses of LLLT as of 2021: "Photobiomodulation: The Clinical Applications of Low-Level Light Therapy", Aesthetic Surgery Journal, Volume 41, Issue 6, June 2021, Pages 723–738. https://academic.oup.com/asj/article/41/6/723/6104785?login=true

He starts out with this refreshing note: "In the gray area between the commercial and therapeutic sectors, harnessing the clinical potential in reproducible and scientifically measurable ways remains challenging." Then he goes on to a wonderful summary of the clinical use of LLLT for facial rejuvenation specific skin conditions and wound healing.

CONCLUSION

I was dissappointed at the lack of clinical evidence for all modalities. There has been too much emphasis on in vitro studies and way too much promoting that in vitro observations can achieve comparable clinical results.

I find LLLT to be the most pursuasive modality in general, and the most likely source for some stem cell stimulation in vivo.

Because I am most interested in facial rejuvenation, I looked at all modalities with that in mind, **based on the belief that if facial rejuventation could be achieved, then, in that case one might deduce that stem cell activation was occurring of the same kind that is achieved with the use of SVF injection into the face, or IV.**

Do I believe that any of these modalities comes close to stem cell therapy with Stromal Vascular Fraction from the micro-liposuction of adipose tissue? **No.**

Do I believe that additional clinical studies will show that LLLT or any of the other modalities is equal to the benefits achieved for many diseases and most particularly for facial rejuventation? **No**. What you have now is what it will be. Results will never be any better.

Would I, as an ND, recommend the use of any of the reviewed modalities to any patient? **No**.

There will be a method found to activate dormant stem cells, which hopefully might obviate the need for, or be a substutute for, stem cell therapy using SVF, which is barred in the US.

Possibly this may be found in:

A. Intravenous nutritiional supplementation

B. Transdermal stem-chemistry administering stem cell activating compounds

C. Oral Supplements such as Stem-Kine (lactobacillus cell walls) that boost circulating stem cells.

So, the search continues.