

laser

international magazine of laser dentistry

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LLLT activated latent TGF- β 1

| laser

Graduation of the 10th year of the master programme “Lasers in Dentistry”

| meetings

UAE International Dental Conference & Arab Dental Exhibition — WFLD took part at AEEDC 2010





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Dr Georg Bach

Dear Reader,

_Globalization progresses steadily and has also found its way into laser dentistry. This issue of laser—the international magazine of laser dentistry is going to prove this thesis.

We, whose goal it is to treat patients with monochromatic light, realize that the individual problems we are facing in our daily routines are, in fact, quite diverse.

We have been dealing with an array of problems: hesitant acceptability of laser dentistry within the universities and professional dental societies, availability of laser wavelengths and equipment and problems with reimbursements.

If we choose to stick together, we will realize the outstanding and notable level today's laser dentistry has reached, a level many didn't think was achievable only a few decades ago.

Invigorated by this awareness, each and every one of us should play their part and contribute to a truly global laser network for the benefit of the community.

laser is an excellent platform for scientific exchange. I hope you will enjoy this issue and welcome your feedback!

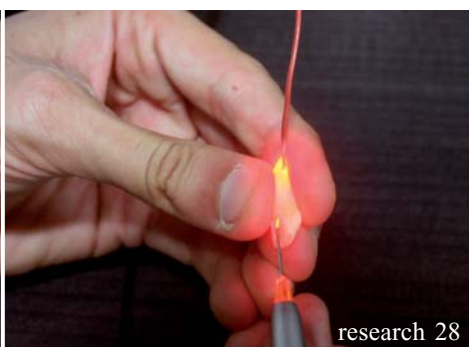
Yours sincerely,

A stylized, handwritten signature in black ink, consisting of a large, fluid 'h' shape.

Dr Georg Bach
Guest Editor



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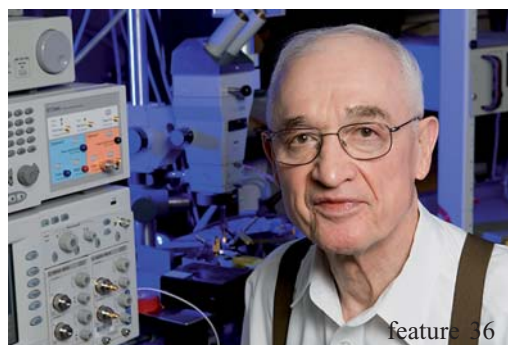
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LLLT activated latent TGF- β 1

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A potential molecular pathway mediating the nexus between inflammation and wound healing in oral tissues.

Low-level laser therapy in dentistry

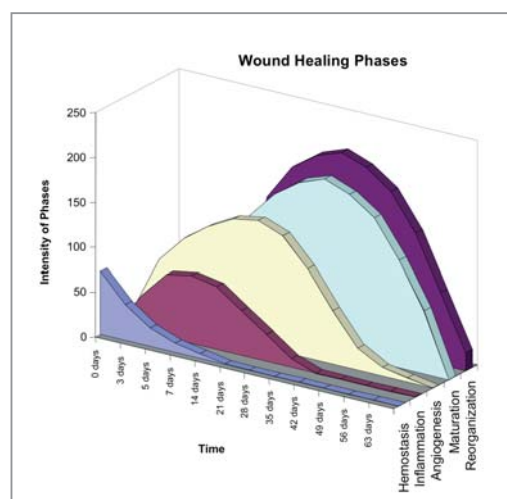
For over 30 years lasers have been a part of dentistry and oral surgery predominantly as surgical tools. Surgical lasers currently used in dental practice include CO₂ lasers, Nd:YAG lasers, Er:YAG lasers, and diode lasers.¹ CO₂ lasers have been used to remove superficial tissue layers while leaving underlying tissues undamaged and are especially valued for their coagulation effects. Er:YAG lasers have been used for ablation of soft and hard tissues and to sterilize root canals and periodontal pockets while Nd:YAG lasers have been used for debridement of calculus and the reduction of endodontic microbes. The diodes have been used for variety of low level applications from analgesia to stimulating healing.

Low-level laser therapy (LLLT) is considered a non-invasive and painless process that uses photonic energy to provide biological therapeutic ad-

vantages, including analgesic capabilities.² While these types of lasers are still used surgically, clinicians have been increasingly using LLLT in the past ten years. Rather than cut or ablate, low-level lasers take advantage of certain photobiological processes, the mechanistic molecular basis of which are yet to be fully characterized. These lasers function in the milliwatt range instead of the higher wattage (0.5 to over 1 W) used by the surgical lasers. The clinical applications of low-power laser for patient care in dentistry have been used to reduce inflammation, relieve pain and discomfort including hypersensitive dentine and promote wound healing.³ There are some clinical studies but few rigorously controlled trials to demonstrate the efficacy of LLLT definitively as well as a paucity of basic science research to probe its mechanistic underpinnings in its various dental applications. This short review does not attempt to comprehensively overview the state of field but highlights some of the recent human clinical studies that have attempted to directly explore the efficacy of LLLT on inflammation and healing in oral tissues.

Inflammation

Inflammation is a complex reaction to injurious agents such as microbes and damaged, usually necrotic, cells that consist of vascular response, migration, and activation of leukocytes, and systemic reaction.⁴ Inflammation is usually a protective pathophysiological response of the body to help prevent noxious damage and return to a homeostatic physiological state. But in scenarios of persistent stimuli or uncontrolled inflammatory reactions, this mechanism can turn pathological and harm the host instead.



Wound Healing and Regeneration

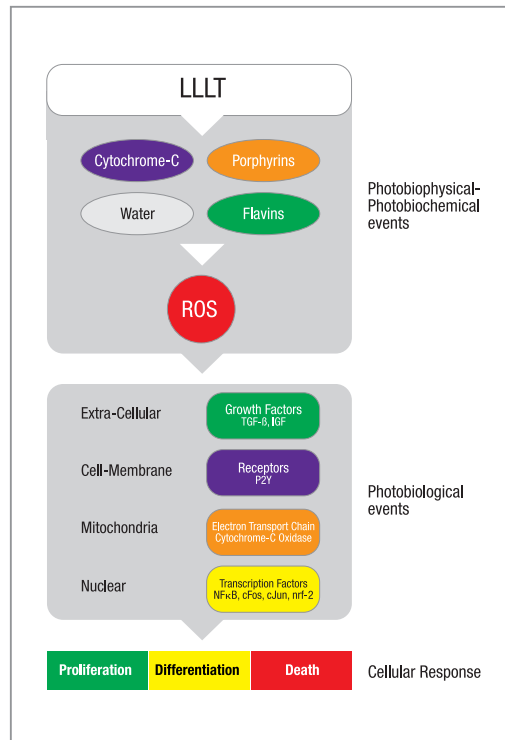
Wound healing, on the other hand, is the resolution of inflammation that succeeds the inflammatory reaction. The ultimate goal of healing is to remove all traces of the inflammatory reaction, along with the noxious stimuli, and return tissues to their original structural and functional homeostatic state. The ideal outcome of wound healing is a complete restoration of the damaged tissue and is termed regeneration. There are two possible modes of regeneration although these two processes are not sharply delineated and may co-exist in the certain scenarios. The mode of regeneration involves proliferation of material preceding development of the new part termed 'Epimorphosis' while the other involves transformation directly into a new organism, or part of an organism without proliferation at the cut surfaces termed 'Morphallaxis'.⁵

The nexus of inflammation and healing with timing of LLLT

While inflammation is critically important and precedes healing, a persistent inflammatory reaction will interfere with effective healing. The ability to modulate the inflammatory response by changing the initial milieu of factors can potentially direct the eventual healing process. The use of LLLT attempts to do just this by delivering photonic energy in this early inflammatory, post-injury scenario that could activate or inactivate specific molecular pathways, accelerating the resolution and the subsequent healing process. The early or repeated use of LLLT during the persistence of the inflammatory phase is therefore a central aspect in defining its clinical efficacy. The use of LLLT in a chronic inflammatory scenario will probably be inefficacious due to the recurrent, persistent noxious stimuli and the poor healing milieu. We believe LLLT does not create a novel *in vivo* scenario but aids in the re-establishment of homeostatic mechanisms often accelerating its natural trajectory.

LLLT in Gingivitis and Periodontitis

Gingivitis generally is not associated with significant pain and thus the LLLT studies have focused on its anti-inflammatory effects.¹ In one study, 10 female subjects refrained from all oral hygiene for 28 days in efforts to induce gingivitis. On the 21st and 24th days, the marginal gingival, buccal to the one of the lateral mandibular incisors, was irradiated for 4 minutes by LLLT. Results showed no statistical difference between the laser and control sites in regards to the level of plaque formation or gingival bleeding.⁶ In a more recent



study, patients were subjected to ten LLLT sessions with 670 nm laser to treat gingival inflammation. Clinical parameters such as the gingival index, plaque index and probing index at 1, 3 and 6 months after laser or conventional oral hygiene therapy were assessed. While both methods are successful at reducing gingivitis, the authors concluded that LLLT leads to better therapeutic results.⁷

Periodontitis, due to pathogenic bacterial species, often presents with bleeding and swelling of the gums, halitosis, gingival recession, and if untreated can lead to tooth loss. Qadri et al showed that treatment with LLLT along with routine oral hygiene measures reduced gingival inflammation.⁸ In a split mouth, double-blind study, patients with moderate chronic periodontitis were treated with a 635 nm InGaAlP diode laser at 4.5 J/cm² and a 820 nm GaAlAs diode laser at 8.75 J/cm² following basic periodontal treatments of scaling, root planing, and oral hygiene instructions. Following treatment, plaque and gingival indices as well as pocket depth were all reduced for the laser-treated side indicating a reduction in inflammation. Additionally, analyses of gingival crevicular fluid showed decrease Matrix Metalloproteinase-8 (MMP-8) in the laser treated side that has been linked directly to the severity of inflammation. Another study by the same group observed that the longer coherence length of an HeNe laser had a more pronounced biological effect than an InGaAlP diode laser on gingival inflammation.⁹

In a study performed to evaluate LLLT as an initial treatment for periodontitis, 30 subjects ranging from ages 20 to 60 who had periodontal pockets of at least 5 mm deep in each quadrant underwent treatment in which half of their mouth was treated with traditional scalpel and root planning (SRP) procedures and the other half was treated with for SRP and a Nd:YAP laser. The Nd:YAP laser was used at 10 W with a 200 nm fiber, time and total fluences were not reported. Evaluations were done at day 0 and day 90 based on the quantity of plaque, gingival inflammation, bleeding on probing (BOP), pocket probing depth (PPD) and clinical attachment level (CAL). The analysis showed that although both methods were equally effective in treating periodontitis, there was no difference in post-operative pain as reported by the patients.¹⁰ Similarly, another study used a He-Ne laser at 0.2 mW, 10 min for 8 days in the first 3 months to treat advanced chronic periodontitis (probing pocket depth over 5 mm) in 16 patients and evaluated supragingival plaque (PL), BOP, PPD and probing attachment level (PAL) were recorded at baseline and at 3, 6, 9, and 12 months. Their results also showed no additional clinical benefit with the He-Ne laser compared to conventional periodontal therapy.¹¹

Other studies, however, using diode laser treatment as a therapeutic method for periodontitis proved to be more promising. In a study done in Greece, 30 patients diagnosed with aggressive periodontitis in all four quadrants were initially evaluated for plaque index, BOP, PPD, and CAL at 2 weeks, 12 weeks and 6 months after treatment. Each quadrant was randomly assigned to either SRP alone, SRP with laser, laser alone or control. In this study, a 980 nm diode laser in continuous mode at 2 W was used. Plaque samples obtained six months after treatment showed a statistically significant reduction in total bacterial load, PPD and CAL in the SRP plus laser group compared to either treatment alone, however there was no difference in plaque index and BOP.¹² In a similar LLLT study using a diode laser (630–670 nm) in combination with SRP in 60 patients randomly sorted into three treatment groups where the first group received only SRP treatment for four days, the second group received SRP treatment for four days followed by five days of laser treatment while the third group received four days of SRP treatment followed by ten days of laser treatment. The clinical parameters measured included the plaque index, gingival index and BOP demonstrated a statistically significant improvement with both LLLT groups.¹³

While there appears to be some discrepancies in clinical outcomes of these studies, there appears to be a large variation in type and manner of lasers used

to perform these LLLT studies. Another important aspect is the varying clinical scenario and the nature of underlying patho-physiological processes in each of these diseased states that might need a more tailored therapeutic LLLT regimen for its clinical efficacy.

_LLLT and oral wound healing

A study by Amorim JC et al used LLLT on gingivectomy wounds in twenty patients with periodontal disease using the split mouth design. They used a 685 nm, 50 mW laser at and 4 J/cm².¹⁴ The authors observed a significant improvement in clinical parameters evaluated in the laser group at 21 and 28 days post surgery compared to the control sites. They postulated that the improvement likely derived from higher collagen production leading to a better remodeling of connective tissue and a reduction of the probing depth, the latter in turn aiding oral hygiene and synergistically contributing to limiting inflammation.

In a similar split mouth study design by Ozcelik et al. also showed that LLLT could enhanced epithelization and improved wound healing after gingivectomy and gingivoplasty procedures.¹⁵ Using a Mira-2-tone solution to visualize areas of epithelialization, the investigators treated patients with a 588 nm diode laser at 120 mW and 4 J/cm² for seven days post surgery. They observed a significant decrease in the non-epithelialized surfaces following LLLT suggesting that besides stimulating collagen production, LLLT might facilitate fibroblast and keratinocyte motility, angiogenesis and growth factor release contributing to decreased inflammation and improved wound healing.

Two recent studies have looked at the physiological mechanism implicating Mast cell degranulation following LLLT. Sawasaki et al. and Silveira et al. used histological evaluation of hypertrophic gingival tissues (epulis fissuratum) irradiated with 670 nm AsGaAl laser at 8 J/cm². Both groups observed significantly increased degranulation indexes of mast cells in the irradiated samples than in the non-irradiated controls. This increase of degranulated mast cells and the resultant release of histamine would lead to increased inflammation. While this would seem counterintuitive to the anti-inflammatory effects of LLLT, it is suggested that hastening the inflammatory response by the degranulation of mast cells and, hence, heralding inflammatory resolution could in turn expedite the succeeding wound healing process. The intricate interplay following Mast cell degranulation by LLLT on monocyte-macrophage influx and fibroblast proliferation and collagen synthesis remains to be investigated.^{16, 17}

Our clinical study recruited 30 patients scheduled to undergo multiple extractions for complete dentures. Following institutional ethical approval and obtaining informed consent, two sites in each patient were used in our study, each patient acting as their own control. Following tooth extraction, one site was irradiated with a 10 mW, 904 nm GaAs laser in contact for 5 min for a total dose of 3 J/cm². A small soft tissue biopsy was obtained from the two sites and wound healing parameters like inflammatory infiltrate, vascularity, matrix synthesis-organization and TGF- β 1 expression were assessed using routine histopathology and immunostaining. We observed a better organized healing response in laser irradiated oral tissues and it is significant to note that the laser accelerated healing did not preclude any normal wound healing phase, demonstrating all the usual phases but seem to occur at a more rapid pace (Fig. 1). This accelerated laser healing correlated with an increased expression of TGF- β 1 immediately post laser irradiation. A major regulatory step in defining the physiological role of TGF- β *in vivo* is its activation from a naturally-secreted latent complex. Various physico-chemical modalities like heat, extreme pH, proteases and reactive oxygen species (ROS) that all induce a change in the conformation of the latent complex causing dissociation and, hence, activation of the TGF- β 1 dimer. Therefore, the ability to activate the latent TGF- β (LTGF- β 1) complex would provide a precise and natural manner of exploiting its role in various biological processes. The histological analysis from our clinical study suggested that a potential source of LTGF- β 1 could be the abundant degranulating platelets from the serum present in the early wound environment that are among known potent source of *in vivo* LTGF- β 1. We then used a cell-free system with serum and assessment by an isoform-specific ELISA and a reporter based (p3TP) assay system to demonstrate the ability of LLLT to activate the latent TGF- β complexes *in vitro* at varying fluences from 10 sec (0.1 J/cm²) to 600 secs (6 J/cm²). We conclude that activation of latent TGF- β 1 by LLLT could contribute to the photobiomodulatory effects and promote oral wound healing.¹⁸

Potential mechanisms of LLLT on inflammation and healing

Despite the increased clinical popularity of LLLT due to its non-invasive, physiological mode of action, lack of information on the precise molecular mechanisms and well-controlled clinical trials have prevented LLLT from being more widely accepted as a routine treatment option. LLLT broadly utilizes wavelengths in the red and near-infrared

spectrum to change intra-cellular photoreceptors such as endogenous growth factor complexes, porphyrins, flavins, surface transmembrane receptors and cytochrome c oxidase in the respiratory chain. To broadly categorize these intermediates, we outline a putative hierarchical level of interaction from the literature in the context of the LLLT and cell-tissue compartments (Figure 2).

Our work with a latent growth factor complex Transforming Growth Factor- β (TGF- β), a multifaceted cytokine, and LLLT has unraveled one such molecular pathway providing an attractive molecular mechanism for photobiomodulation.¹⁸ TGF- β plays key roles in biological processes like development, wound healing and malignancies and has a myriad range of effects based on its spatio-temporal expression on a wide range of cells from epithelial keratinocytes to fibroblasts, endothelial, neural and inflammatory cells. The intricate role of TGF- β on inflammatory cell subsets displays a fascinating dichotomy between its immune-suppressor versus immune surveillance functions and is an ongoing area of intense lab investigation. Interestingly, although primarily identified as a pro-matrix, fibrosis promoting wound cytokine, TGF- β transgenic mice have shown a startling variety of healing phenotypes further indicating its diverse roles on epithelial migration and survival, chemotaxis of monocytes-macrophages and mechanical homeostasis of the matrix milieu.¹⁹ The activation of such a multifaceted growth factor by LLLT with its broad effects on various component of inflammatory-healing process could 'short-circuit' or 'kick-start' the complex cascade of biological events effecting the eventual healing and regenerative outcomes. Clinically, one of the most attractive features of exploiting this mechanism is the activation of endogenous levels of TGF- β and thus, potentially only gently nudging the natural physiological process along, without a major perturbation of the biological system as seen with addition of exogenous factors. We speculate that there might be more such latent molecular complexes amenable to low power laser modulation in the inflammatory and early wounding scenarios. Our present research has established that the photophysical and photochemical events can correlate with large magnitudes of laser fluences. In contrast, the photobiological events are tightly limited within a narrower range of laser fluences through an unknown biological regulatory mechanism. This mechanism along with potential chromophores, wavelength and fluence parameters affecting the latent TGF- β activation process by LLLT for oral wound healing and other biological applications are our present focus of research.