Many of the articles in this issue of the Journal discuss wound healing to varying degrees. One generally accepted definition of wound healing identified by Gottrup is “a reaction of any multicellular organism to tissue damage in order to restore the continuity and function of the tissue or organ.” To be sure, wound healing is a dynamic, continuous, interactive process involving cells and extracellular matrix (material produced by cells and excreted to the extracellular space within tissues), and is dependent on numerous internal as well as external processes.

Wound healing may be divided into three phases: inflammation, proliferation or regeneration, and remodeling. Numerous factors affect the entire wound healing process, including individual tissue cells (such as platelets, polymorphonuclear leukocytes, macrophages, fibroblasts, endothelial cells, pericytes or undifferentiated mesenchymal cells, and epithelial cells); blood circulation and oxygenation; infection; presence of foreign bodies; patient characteristics such as age, and indulgences such as smoking and alcohol.

An in-depth discussion of wound healing is beyond the scope of this article. One area of laser-related study has been commanding increasing attention in the literature in recent years: the effect of low-level laser irradiation on growth factors involved in wound healing. As described by Storgård Jenson, growth factors are a group of polypeptides involved in cellular chemotaxis (directional movement of a cell in response to a chemical concentration gradient), differentiation, proliferation, and synthesis of extracellular matrix. Furthermore, all wound healing events in soft and hard tissues are affected by growth factors, which can be released from the traumatized tissue, brought to the area by macrophages or neutrophils, or harbored in the blood clot.

Growth factors may improve wound healing in numerous ways:
• Recruit specific cells types and stem cells to the wounded area by chemotaxis

THE EFFECT OF LOW-LEVEL LASER THERAPY ON GROWTH FACTORS INVOLVED IN WOUND HEALING

• Induce differentiation of mesenchymal precursor cells to mature secreting cells
• Stimulate mitosis of relevant cells, thus increasing proliferation
• Increase angiogenesis (the formation of new blood vessels)
• Affect secretion and breakdown of extracellular matrix components.

The most significant growth factors involved in the wound healing process, along with some of their primary effects and tissue responses, are summarized below:
• Platelet Derived Growth Factor (PDGF) stimulates cells proliferation and extracellular matrix production of fibroblasts, contributes to the repair of damaged vascular walls, and activates macrophages to debride the wounded area.
• Transforming Growth Factors (TGFs), most notably the TGF-β subtype, strongly promote extracellular production of many cell types including periodontal ligament fibroblasts, and also help regulate the immune and inflammatory processes.
• Epidermal Growth Factor (EGF) encourages cells to continue through the cell cycle, thereby promoting proliferation and wound healing.
• Insulin-like Growth Factor (IGF) combines with other growth factors to stimulate fibroblast proliferation, collagen synthesis, bone formation, and epithelialization.
• Fibroblast Growth Factors (FGFs) support cell survival under stress conditions and stimulate angiogenesis in the early formation of granulation tissue, and regulate tissue vascularization.
• Vascular Endothelial Growth Factor (VEGF) promotes tissue vascularization and angiogenesis in granulation tissue formation during development and repair.
• Bone Morphogenetic Proteins (BMPs) commit undifferentiated pluripotential cells to become bone- or cartilage-forming cells.
**IMPORTANT GROWTH FACTORS INVOLVED IN INTRAORAL WOUND HEALING**

<table>
<thead>
<tr>
<th>Growth Factor</th>
<th>Primary Effect</th>
<th>Tissue Response</th>
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<tbody>
<tr>
<td>Platelet Derived Growth Factor (PDGF)</td>
<td>• Chemotaxis</td>
<td>• Angiogenesis</td>
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<td></td>
<td>• Proliferation</td>
<td>• Macrophage Activation</td>
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<tr>
<td>Tranforming Growth Factors (TGF)</td>
<td>• Chemotaxis</td>
<td>• Collagen Production (Scarring)</td>
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<td></td>
<td>• Production of Extracellular Matrix</td>
<td>• Down-Regulation of Other Cell Types but Fibroblasts</td>
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<tr>
<td></td>
<td>• Proliferation</td>
<td>• Immunoregulation</td>
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<td>Epidermal Growth Factor (EGF),</td>
<td>• Proliferation</td>
<td>• Epithelialization</td>
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<tr>
<td>including TGF-α</td>
<td>• Chemotaxis</td>
<td>• Tooth Eruption</td>
</tr>
<tr>
<td></td>
<td>• Production of Extracellular Matrix</td>
<td></td>
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<tr>
<td>Insulin-like Growth Factor (IGF)</td>
<td>• Proliferation</td>
<td>• Stimulated DNA Synthesis</td>
</tr>
<tr>
<td></td>
<td>• Production of Extracellular Matrix</td>
<td>• Growth Promotion of Committed Cells</td>
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<tr>
<td></td>
<td>• Chemotaxis</td>
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<tr>
<td></td>
<td>• Cell Survival</td>
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<tr>
<td>Fibroblast Growth Factors (FGF)</td>
<td>• Proliferation</td>
<td>• Angiogenesis</td>
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<td></td>
<td>• Migration</td>
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<tr>
<td></td>
<td>• Formation of Extracellular Matrix</td>
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<tr>
<td>Vascular Endothelial Growth Factor</td>
<td>• Proliferation</td>
<td>• Angiogenesis</td>
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<tr>
<td>(VEGF)</td>
<td>• Angiogenesis</td>
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<tr>
<td>Bone Morphogenetic Proteins (BMP)</td>
<td>• Differentiation</td>
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<td></td>
<td>• Proliferation</td>
<td>• Root Cementum Formation</td>
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<td></td>
<td>• Production of Extracellular Matrix</td>
<td>• Dentin Formation</td>
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<td></td>
<td></td>
<td>• Periodontal Ligament Formation</td>
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</tbody>
</table>

*Adapted from Storgård Jensen S*

Shown below are examples of published *in vitro* and *in vivo* animal and human investigations examining the possible effects of low-level laser irradiation on various growth factors involved in wound healing. These and other researchers are building upon the foundation of understanding assembled by Endre Mester and other pioneers of photobiomodulation.

As always, clinicians are advised to review the specific indications for use of their lasers and to review their operator manuals for guidance on operating parameters before attempting laser-assisted wound healing techniques on their patients.

**REFERENCE**

Biostimulatory effects of laser irradiation on cell proliferation and wound healing has been reported. However, little is known about the molecular basis of the mechanism. Interleukin 1β (IL-1β), tumor necrotic factor-alpha (TNF-α), and interferon-γ (IFN-γ) play an important role in inflammation, while platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β) and blood-derived fibroblast growth factor (bFGF) are the most important growth factors of periodontal tissues. The aim of this study was to investigate the effect of low-level He-Ne laser on the gene expression of these mediators in rats’ gingiva and mucosal tissues. Twenty male Wistar rats were randomly assigned into four groups (A24, A48, B24, B48) in which A24 and A48 were cases and B24, B48 were controls. An incision was made on gingiva and mucosa of the labial surface of the rats’ mandibular incisors. Group A24 was irradiated twice with 24 hours interval, while the inflamed tissues of group A48 were irradiated three times with continuous He-Ne laser (632.8 nm) at a dose of 7.5 J/cm² for 300 s. An energy of 5.1 J was given to the 68 mm² irradiation zone. Rats were killed 30 min after the last irradiation of case and control groups, then excisional biopsy was performed. Gene expression of the cytokines was measured using reverse transcriptase-polymerase chain reaction (RT-PCR) technique. Results were analyzed with Kruskal-Wallis and Mann-Whitney U tests. The gene expression of IL-1β and IFN-γ was significantly inhibited in the test groups (P < 0.05), while the gene expression of PDGF and TGF-β were significantly increased (P < 0.05). The case and control groups did not have a significant difference in the gene expression of TNF-α and bFGF (P > 0.05). These findings suggest that low-level He-Ne laser irradiation decreases the amount of inflammation and accelerates the wound healing process by changing the expression of genes responsible for the production of inflammatory cytokines.

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LOW-INTENSITY LASER IRRADIATION STIMULATES BONE NODULE FORMATION VIA INSULIN-LIKE GROWTH FACTOR-I EXPRESSION IN RAT CALVARIAL CELLS

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Background and Objective: We previously reported that low-intensity laser irradiation stimulated bone nodule formation through enhanced cellular proliferation and differentiation. However, the mechanisms of irradiation are unclear. Thus, we attempted to determine the responsibility of insulin-like growth factor (IGF)-I for the action observed. Study Design/Materials and Methods: Osteoblast-like cells were isolated from fetal rat calvariae and cultured with rat recombinant (r) IGF-I, IGF-I-antibody (Ab), and/or the cells were irradiated once (3.75 J/cm²) with a low-intensity Ga-Al-As laser (830 nm). The number and area of bone nodules formed in the culture were analyzed, and IGF-I expression was also examined. Results: Treatment with rIGF-I significantly stimulated the number and area of bone nodules. This stimulatory effect was quite similar to those by laser irradiation, and this stimulation was abrogated dose-dependently by treatment with IGF-I-Ab. Moreover, laser irradiation significantly increased IGF-I protein and gene expression. Conclusion: The stimulatory effect of bone nodule formation by low-intensity laser irradiation will be at least partly mediated by IGF-I expression.

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RESEARCH ABSTRACTS

ACTIVATION OF LATENT TGF-β1 BY LOW-POWER LASER IN VITRO CORRELATES WITH INCREASED TGF-β1 LEVELS IN LASER-ENHANCED ORAL WOUND HEALING

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The term Laser “Photobiomodulation” was coined to encompass the pleiotropic effects of low-power lasers on biological processes. The purpose of this study was to investigate whether transforming growth factor (TGF)-β had a role in mediating the biological effects of low-power far-infrared laser irradiation. We assayed for in vitro activation using various biological forms of cell-secreted, recombinant, and serum latent TGF-β using the p3TP reporter and enzyme-linked immunosorbent assays. We demonstrate here that low-power lasers are capable of activating latent TGF-β1 and -β3 in vitro and, further, that it is capable of “priming” these complexes, making them more amenable to physiological activation present in the healing milieu. Using an in vivo oral tooth extraction-healing model [in 30 human patients], we observed an increased TGF-β1, but not β3, expression by immunohistochemistry immediately following laser irradiation while TGF-β3 expression was increased after 14 days, concomitant with an increased inflammatory infiltrate. All comparisons were performed between laser-irradiated wounds and nonirradiated wounds in each subject essentially using them as their own control (paired T-test p < 0.05). Low-power laser irradiation is capable of activating the latent TGF-β1 complex in vitro and its expression pattern in vivo suggests that TGF-β play a central role in mediating the accelerated healing response.

LOW-LEVEL LASER THERAPY INCREASES TRANSFORMING GROWTH FACTOR-β2 EXPRESSION AND INDUCES APOPTOSIS OF EPITHELIAL CELLS DURING THE TISSUE REPAIR PROCESS

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Background Data: Low-level laser therapy (LLLT) has been reported to modulate the healing of wounds by inducing an increase in mitotic activity, fibroblast number, synthesis of collagen, and neovascularization. Objective: In the present study we evaluated the effect of LLLT on expression of TGF-β2, an immunosuppressive cytokine, at the site of tissue repair, using an experimental rat model to study cutaneous wound healing. In addition, we also investigated the presence of apoptotic cells in epithelial and connective tissue. Materials and Methods: Thirty male Wistar rats were divided into two groups: group 1, which was subjected to surgical skin wounds only (n = 15), and group 2, which was subjected to surgical skin wounds followed by LLLT (n = 15). In group 2, the LLLT was given with these parameters: 15 mW of power, a dose of 3.8 J/cm², for 15 sec for three applications. At 10 d post-surgery and laser application the animals were sacrificed with an overdose of anesthetic and tissue samples from the wounds were submitted to immunohistochemistry and in-situ detection of apoptosis. Results: Most of the inflammatory cells and fibroblasts were TGF-β2-positive, and many apoptotic epithelial cells and fibroblasts were seen in the tissue samples from the LLLT-treated animals. However, a few apoptotic epithelial cells and fibroblasts were also seen in the samples obtained from control animals. Conclusion: Our results indicate that LLLT may be an important inducer of apoptosis during the process of tissue repair. In addition, we demonstrated that LLLT has an immunomodulatory effect on TGF-β2 expression at sites of wound healing.

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Objective: To evaluate the efficacy of low-level laser therapy (LLLT) on collateral circulation and microcirculation if a blood vessel is occluded. Background Data: Investigators have attempted prostaglandin and ultrasound therapy to promote improvements in the vascular bed of deprived tissue after an injury, which may lead to occlusion of the blood vessels. Materials and Methods: Thirty-four adult rabbits were used in this study, two of them considered 0-h reading group, while the rest were divided into two equal groups, with 16 rabbits each: control and those treated with LLLT. Each rabbit underwent two surgical operations; the medial aspect of each thigh was slit, the skin incised and the femoral artery exposed and ligated. The site of the operation in the treated group was irradiated directly following the operation and for 3 d after, one session daily for 10 min/session. The laser system used was a gallium-aluminum-arsenide (Ga-Al-As) diode laser with a wavelength of 904 nm and power of 10 mW. Blood samples collected from the femoral artery above the site of the ligation were sent for examination with high-performance liquid chromatography (HPLC) to determine the levels of adenosine, growth hormone (GH) and fibroblast growth factor (FGF). Tissue specimens collected from the site of the operation, consisting of the artery and its surrounding muscle fibers, were sent for histopathological examination to determine the fiber/capillary (F/C) ratio and capillary diameter. Blood samples and tissue specimens were collected at 4, 8, 12, 16, 24, 48, and 72 h postoperatively from the animals of both groups, control and treated. Results: Rapid increases in the level of adenosine, GH, and FGF occurred. The F/C ratio and capillary diameter peaked at 12-16 h; their levels declined gradually, reaching normal values 72 h after irradiation in the treated group. Numerous collateral blood vessels proliferated the area, with marked increases in the diameters of the original blood vessels. Conclusions: The results indicated that LLLT accelerated collateral circulation and enhanced microcirculation and seemed to be unique in the normalization of the functional features of the injured area, which could lead to occlusion of the regional blood vessels.

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SUPERPULSED LASER IRRADIATION INCREASES OSTEOBLAST ACTIVITY VIA MODULATION OF BONE MORPHOGENETIC FACTORS

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Background and Objective: Laser therapy is a new approach applicable in different medical fields when bone loss occurs, including orthopedics and dentistry. It has also been used to induce soft-tissue healing, for pain relief, bone, and nerve regeneration. With regard to bone synthesis, laser exposure has been shown to increase osteoblast activity and decrease osteoclast number, by inducing alkaline phosphatase (ALP), osteopontin, and bone sialoprotein expression. Studies have investigated the effects of continuous or pulsed laser irradiation, but no data are yet available on the properties of superpulsed laser irradiation. This study thus aimed to investigate the effect of superpulsed laser irradiation on osteogenic activity of human osteoblast-like cells, paying particular attention to investigating the molecular mechanisms underlying the effects of this type of laser radiation. Study Design/Materials and Methods: Human osteoblast-like MG-63 cells were exposed to 3, 7, or 10 exposures to superpulsed laser irradiation (pulse width 200 nanoseconds, minimum peak power 33 W, average out power 200 mW, frequency 30 kHz, total energy 60 J, exposure time 5 minutes), with an administered dose of 6.7 J/cm². The following parameters were evaluated: cell growth and viability (light microscopy, lactate dehydrogenase release), calcium deposits (Alizarin Red S staining), expression of bone morphogenetic factors (real-time PCR). Results: Superpulsed laser irradiation decreases cell growth, induces expression of TGF-β, BMP-4, and BMP-7, type I collagen, ALP, and osteocalcin, and increases the size and the number of calcium deposits. The stimulatory effect is maximum on day 10, that is, after seven applications. Conclusions: Reported results show that superpulsed laser irradiation, like the continuous and pulsed counterparts, possesses osteogenic properties, inducing the expression of molecules known to be important mediators of bone formation and, as a consequence, increasing calcium deposits in human MG-63 cells. Moreover, the data suggest a new potential role for PPARγ as a regulator of osteoblast proliferation.

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IN VIVO EFFECTS ON THE EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR-A165 MESSENGER RIBONUCLEIC ACID OF AN INFRARED DIODE LASER ASSOCIATED OR NOT WITH A VISIBLE RED DIODE LASER

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Objective: This study investigated and correlated the kinetic expression of vascular endothelial growth factor (VEGF)-A165 messenger ribonucleic acid (mRNA) with the associated use or not of an infrared laser and a visible red laser during the wound healing in rats.

Background Data: There is a lack of scientific evidence demonstrating the influence of low-level laser therapy (LLLT) on the expression of VEGF mRNA in vivo.

Materials and Methods: Forty-five Wistar rats were randomly allocated to one of three groups: I (n = 5, nonoperated animals), II (n = 25, operated animals), and III (n = 25, animals operated and subjected to laser irradiation). A surgical wound was performed using a scalpel in the right side of the tongue of operated animals. In group III, two sessions of laser irradiation were performed, one right after the surgical procedure (infrared laser, 780 nm, 70 mW, 35 J/cm²) and the other 48 h later (visible red laser, 660 nm, 40 mW, 5 J/cm²). Five animals each were sacrificed 1, 3, 5, and 7 days postoperatively in groups II and III, and samples of tongue tissue were obtained. The animals of group I were sacrificed on day 7. Total RNA was extracted using guanidine-isothiocyanate-phenol-chloroform method. The results of horizontal electrophoresis after reverse transcription polymerase chain reaction permitted the ratio of VEGF-A165 mRNA and glyceraldehyde 3-phosphate dehydrogenase mRNA expression for groups I, II, and III to be assessed (two-way analysis of variance and Tukey test, p < 0.05).

Results: The expression of VEGF-A165 mRNA in group II (0.770 ± 0.098) was statistically greater than that observed in groups I (0.523 ± 0.164) and III (0.504 ± 0.069) in the first day after surgery (p < 0.05). Significant differences between the groups were not observed in other time periods. Conclusion: LLLT influenced the expression of VEGF-A165 mRNA during wound healing after a surgical procedure on the tongue of Wistar rats.

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