

# Platelet Derived Growth Factor Secretion and Bone Healing After Er:YAG Laser Bone Irradiation

Gavriel Kesler, DMD<sup>1</sup>

Dana Kesler Shvero, DMD<sup>2</sup>

Yariv Siman Tov, DVM<sup>3</sup>

George Romanos, DDS, DMD, PhD<sup>4\*</sup>

Er:YAG laser irradiation has been reported to enhance wound healing. However, no studies have evaluated the synthesis of growth factors after laser irradiation. The present study investigated the effects of laser irradiation on the amount of secretion of platelet derived growth factor (PDGF) in the wound, clarifying the effects of the Er:YAG laser on the bone healing. Osteotomies were prepared in the tibiae of 28 rats using an Er:YAG laser (test group). Maximum power of 8 watts, energy per pulse of 700 mJ, and frequency up to 50 Hz were used. The laser was used with external water irrigation, a spot size of 2 mm, energy per pulse of 500 to 1000 mJ/pulse, and energy density of 32 J/cm<sup>2</sup>. Twenty eight additional rats served as a control group and their osteotomies were prepared with a drill 1.3 mm in diameter at 1000 rpm, with simultaneous saline irrigation. Two rats from the tested group and 2 from the control group were sacrificed on each day following surgery (1–14 days), and the tissue specimens were prepared for histologic evaluation. Immunohistochemical staining with anti-PDGF was performed after histologic examination. The difference between the PDGF staining intensities of the 2 treatment groups was analyzed using a multivariate logistic regression test. A significant rise in PDGF staining occurred in both groups 2–3 days following surgery. However, while high PDGF counts remained for the 2-week experimental period in the laser group, PDGF levels in the control group returned to baseline levels 8 days post surgery. The 2 groups (laser and control) were found to be different throughout the experiment, and the rat type was found to be a significant predictor ( $P = .000011$ ). The present study demonstrated that Er:YAG laser irradiation seems to stimulate the secretion of PDGF in osteotomy sites in a rat model. It is possible that the high levels of PDGF are part of the mechanism that Er:YAG irradiation enhances and improves the healing of osteotomy sites.

**Key Words:** bone healing, Er:YAG laser, PDGF

<sup>1</sup>Dental Laser Clinic, Department of Oral Rehabilitation, The Maurice and Gabriela Goldschleger School of Dental Medicine, Tel Aviv University, Tel Aviv, Israel.

<sup>2</sup>Hadassah Dental School of Medicine, Jerusalem, Israel.

<sup>3</sup>Assaf Harofeh Medical Center, Rishon LeZion, Israel.

<sup>4</sup>Eastman Dental Center, Division of Periodontology, Unit of Laser Dentistry, University of Rochester, Rochester, NY.

\*Corresponding author, e mail: Georgios.Romanos@urmc.rochester.edu

DOI: 10.1563/AAID JOI D 09 00120.1

## INTRODUCTION

It has been proven that the Er:YAG laser (2940 nm) ablates bone effectively and efficiently with minimal thermal damage because the emission of the wavelength is mainly absorbed by water.<sup>1,2</sup> Absorption in water is the primary absorption for this wavelength due

to relatively broad water band around 3000 nm.<sup>3,4</sup> This is due to water normally being present among the crystals of enamel, dentin, cementum, and bone. In carious tissue there is an even greater quantity of water replacing the lost minerals. The key to understanding hard tissue ablation by this wavelength is that it is primarily due to the wavelength's absorption in water and the superheating of the water below the surface. In this order, enamel, dentin, bone, cementum, and carious tissues have descending mineral density and ascending water composition.<sup>5,6</sup> In addition, there is a small absorption at around 2800 nm by the hydroxyl group of the (carbonated) hydroxyapatite mineral in the tissues,<sup>7-9</sup> but this is far outweighed by the effect on the water.

Unfortunately, many studies on the effect of the laser on hard tissues have perpetuated the erroneous thinking that dental mineral strongly absorbs these wavelengths. Not only is this incorrect, but it misleads us in understanding the mechanism of how laser application at this wavelength causes ablation to occur, and causes misunderstanding in the use of the device. If laser light is effectively absorbed by direct ablation of the mineral, the crystals themselves may be heated above their melting point, and some disruption of the crystal structure occurs with subsequent resolidification in a different form. There is also conductive heat transfer to interstitial free water.<sup>10-12</sup> The development of Er:YAG and Er,Cr:YSGG wavelengths have enabled bone ablation to be carried out using relatively high-energy fluence with minimal adjacent damage. The use of erbium lasers in dentoalveolar surgery represents a less traumatic experience for the patient, when compared with the intense vibration of the slow-speed surgical bur. As with tooth tissue ablation, tissue cutting is a thermally induced explosive process.<sup>13,14</sup> It is essential to maintain coaxial water spray to prevent heat damage, which

would delay healing. Bone composition is very similar to dentin from the perspective of laser/tissue interactions. The mineral is similar and the protein content is similar, as is the water content; thus, in maxillary alveolar bone, the speed of laser cutting is comparable to that of a bur, and in the mandible it is slightly slower, reflecting the greater mineral density of cortical bone. It is important that excessive power parameters be avoided to reduce the "stall-out" effect of debris and to minimize blood-spatter. Laser power values of 350–500 mJ/10–20 pps (average power range 3.5–7.0 watts) with maximal water spray appear to produce good ablation rates.

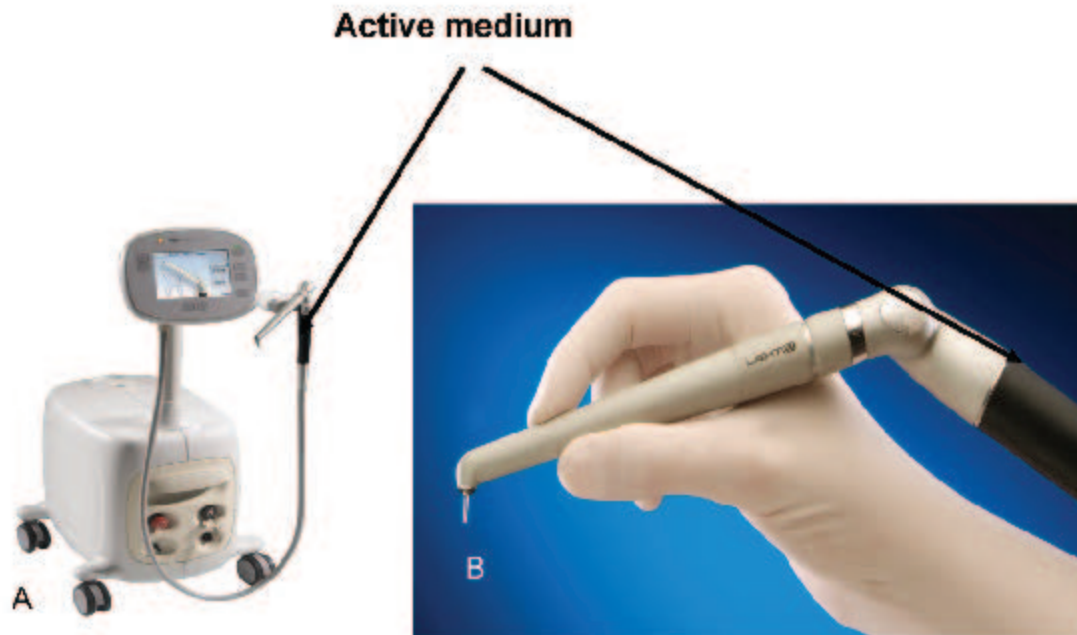
Studies on the healing of laser-ablated bone support the contention that the reduction in effects, such as physical trauma, tissue heating, and bacterial contamination may lead to uncomplicated healing processes when compared with conventional use of a surgical bur.<sup>15-17</sup> The microanalysis of the surface of bone that has been ablated using lasers shows little evidence of thermal damage, and any char layer appears to be restricted to a minimal zone 20–30  $\mu\text{m}$  in depth.

Increased bleeding is noted after Er:YAG laser treatment due to the low thermal effect, and the decortication effect of this laser promotes new bone formation especially during the first few days.<sup>20,21</sup>

Er:YAG laser bone ablation compared to bur drilling creates an irregular microsurface without a smear layer, increasing fibrin attachment and greater aggregation of red blood cells due to the mechanical trapping effect of the laser roughened bone surface. This is advantageous for blood clot attachment, helping to promote new bone formation.<sup>16,22</sup>

Er:YAG laser irradiation has a low-intensity laser-like effect by activating the surrounding cells, and has a biostimulatory effect on the processes of wound healing,<sup>23</sup> fibroblast proliferation,<sup>22</sup> collagen synthesis,<sup>26</sup> and bone regeneration.<sup>25</sup> Pourzaran-





**FIGURE 1.** The Er:YAG laser—The LiteTouch, manufactured by Syneron, Israel. (A) Laser overview: the active medium is located in the handpiece. (B) Handpiece overview.

dian et al<sup>26</sup> reported that Er:YAG laser irradiation stimulated the proliferation of cultured human fibroblasts through the production of platelet derived growth factor (PDGF). Kesler et al<sup>25</sup> demonstrated in a pilot rabbit study that implant site preparation with the Er:YAG laser results in good healing and a significantly higher percentage of bone to implant contact as compared with conventional osteotomies. Lubart et al<sup>27</sup> reported that the Er:YAG laser enhances wound healing by releasing reactive oxygen species.

To date there are no detailed histologic and histochemical assessments of the mechanisms of bone healing following Er:YAG laser irradiation. The aim of the present study was to test the hypothesis that the improved healing of bone defects following Er:YAG laser irradiation is at least partially due to local accumulation of PDGF in the osteotomy sites.

#### MATERIALS AND METHODS

This study was approved by the Local Institutional Research Ethics Committee of Assaf Harofeh Medical Center, Israel.

Fifty-six male Sprague-Dawley rats weighing 350–400 g (4 months old) were divided into 2 groups of 28 each. All rats were weighed to the nearest gram. Food and water were provided ad libitum throughout the experiment. The rats were anesthetized using volatile gas (enflurane) and shaved, scrubbed, and draped to provide a surgical field. A 1.5-cm incision was made on the medial proximal surface of the tibia above the tibial protuberance. Tissue was reflected to expose the flat portion of the tibia below the joint.

#### Laser group

The Er:YAG laser used was the LiteTouch, manufactured by Syneron (Yokneam-illit, Israel). The machine had a direct delivery system, the active medium built into the handpiece base, a maximum power of 8 watts, the energy per pulse of 700 mJ, and a frequency utilized up to 50 Hz. In the experiment we used a regular handpiece and external water irrigation, a spot size of 2 mm, energy per pulse of 500–1000 mJ/pulse, and an energy density of 32 J/cm<sup>2</sup> (Figure 1).

Twenty-eight rats served as the laser group. Experiments were typically performed

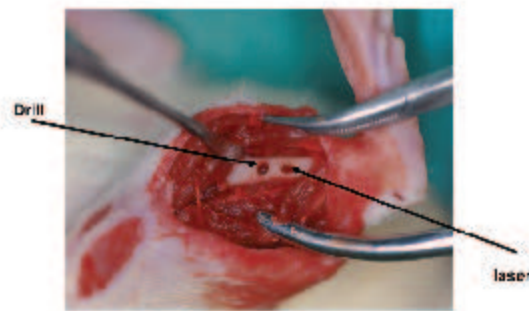


FIGURE 2. Bone sites prepared with laser and drill.

using 10–15 pulses to drill through the tibia. The bone volume removed per pulse was  $1.4 \text{ mm}^3$ . Assuming that the energy is above the ablation threshold, the crater depth for the 2-mm spot size was calculated for the regular handpieces at 0.66 mm per pulse. Primary closure was achieved for each animal by approximating the muscle layers with sutures and closing the skin with surgical staples (Figure 2).

#### **Control group**

Twenty-eight rats served as a control group. Conventional osteotomies were prepared with a 1.3-mm diameter surgical implant drill (1000 rpm) proximal to the tibial protuberance. Copious warm saline irrigation was used to create an oblique-transverse osteotomy, traveling through the medullary canal up to opposite cortical shaft.

#### **Follow-up**

Animals were followed postoperatively for 14 days. Two rats from each group (2 test and 2 controls) were sacrificed daily from day 1 to day 14, and were prepared for histologic evaluation.

#### **Specimen preparation for histologic evaluation**

Tibiae with the surrounding tissues were removed, cleaned of soft tissue, and fixed in 10% buffered formalin. After fixation, the bones were placed into a decalcification solution (DECAL-RAPID, BioGenex, San Ramon, Calif) for 3 days. Slices of bone seg-

ments (one from each rat) of the treated area were sectioned serially in a vertical plane from mesial to distal at 3- to 5-mm width. The tissue samples were embedded in paraffin blocks; 5- $\mu\text{m}$  sections from each block were stained with hematoxylin and eosin. Two sections from each rat were analyzed.

#### **Immunohistochemistry**

The antibodies used in the present study are currently available for in vitro diagnostic use. The immunogen used is a synthetic peptide of PDGF-B conjugated with hemocyanin.

The clone used was PDGF 88. The kit used is a recommended detection system Link-Label staining system (BioGenex) for rat species. This monoclonal antibody was designed for the specific localization of PDGF-B in formalin—fixed paraffin—embedded tissue sections. The slides were counterstained with Mayer's hematoxylin. In general, the demonstration of antigens by immunohistochemistry was a 2-step process involving first, the binding of a primary antibody to the antigen of interest, and second, the detection of bound antibody by a chromogen.

After paraffin removal and hydration, the sections were immersed in 10 mM citrate buffer, placed in a pressure cooker chamber, and microwaved for 10 minutes for optimal antigen retrieval. Endogenous peroxidase activity was blocked by the incubation of slides in 3% hydrogen peroxide in methanol for 10 minutes, and nonspecific epitopes were eliminated by incubation with a blocking solution (UltraVision; Lab Vision Corporation, Fremont, Calif) for 10 minutes. The slides were incubated with the primary antibodies raised against human platelet derived growth factor receptor (PDGFR)- $\alpha$  (1:200 dilution; Lab Vision) for 60 minutes. After the slides were rinsed, they were incubated with biotin as a secondary antibody, followed by enzyme-labelled streptavidin for 10 minutes (UltraVision detection system, anti-polyvalent horseradish peroxidase/diaminobenzidine; Lab Vision).



**FIGURES 3 AND 4.** **FIGURE 3.** Immunohistochemical staining for platelet derived growth factor (PDGF) 2 days after surgery ( $\times 400$ ). (a) Laser group negative ( $\times 400$ ). (b) Control group negative staining. **FIGURE 4.** Immunohistochemical staining for PDGF 4 days after surgery ( $\times 400$ ). (a) Laser group strong positive staining. (b) Control group positive staining.

The PDGF positive staining was distributed in cytoplasm. Two sections from each of the 4 rats that were sacrificed each day were blindly examined and scored by 3 independent experience examiners to give microscopic evaluation of color intensity. Interpretation of the staining results was evaluated in each section and scored for the intensity of staining as follows: intensity (+0) = no staining; intensity (+1) = slight intensity staining; intensity (+2) = moderate intensity staining; and intensity (+3) = high intensity staining.

The results of the 3 examiners for each sample were averaged to give 1 result for each rat.

#### **Statistical evaluation**

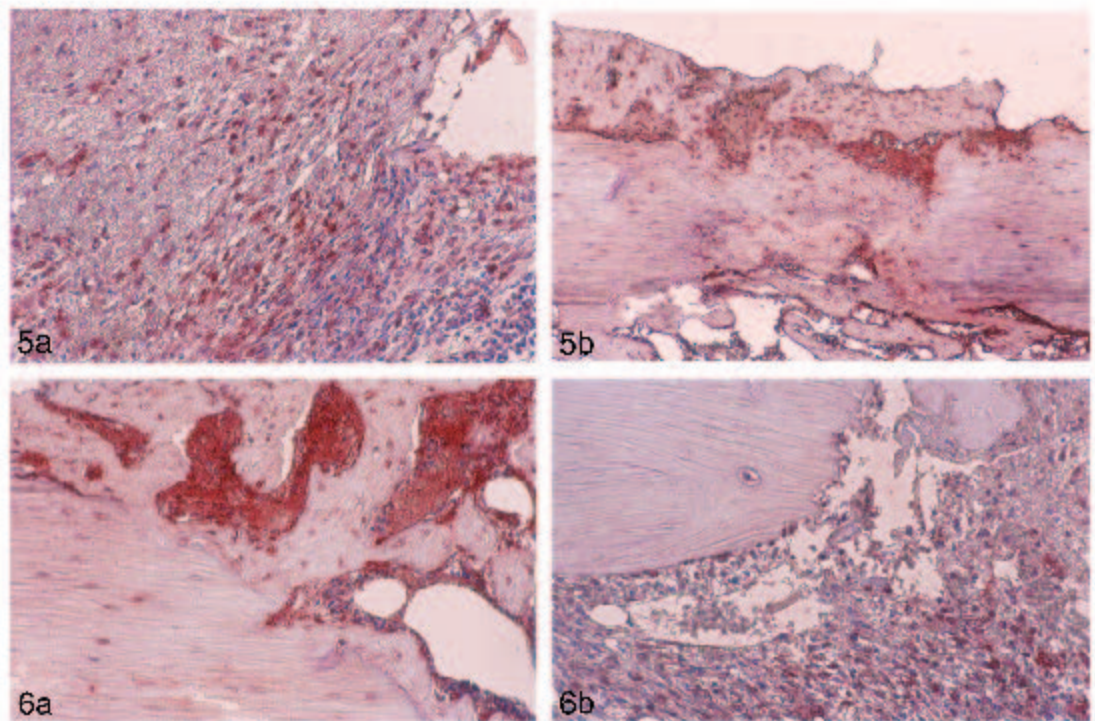
The difference between the effects (the PDGF staining intensities) of the 2 groups

(test and control) over the study period was analyzed using a multivariate logistic regression test. The variability of the PDGF staining intensities (0 through 3 as an ordinal dependent variable) was examined against the type of the rat (laser vs control) and the day of sacrifice.

#### **RESULTS**

The stimulatory effect of laser irradiation on PDGF secretion was evaluated and examined as described, to determine whether the faster healing described previously was possibly mediated by PDGF.

During the first 2 days following surgery, the PDGF staining scores were very low for both type of treatments. In the laser group, PDGF levels started to rise from the third



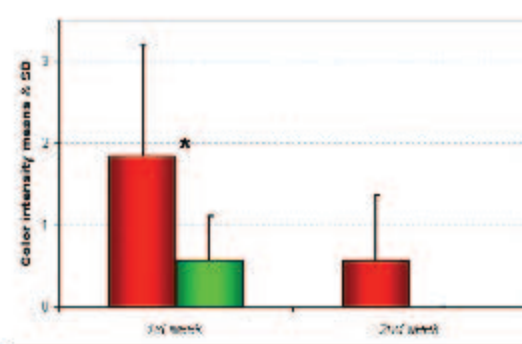
**FIGURES 5 AND 6. FIGURE 5.** Immunohistochemical staining for platelet derived growth factor (PDGF) 8 days after surgery ( $\times 400$ ). (a) Laser group positive staining. (b) Control group negative staining. **FIGURE 6.** Immunohistochemical staining for PDGF 14 days after surgery ( $\times 400$ ). (a) Laser group positive staining. (b) Control group (0) negative staining.

day, reached a peak on day 4, and remained high for the rest of the study period (day 14). The strongest staining was seen between days 4 and 6 (Figures 3 through 7).

In the control group (drill osteotomies), PDGF levels started to rise 4 days following

surgery (1 day later compared to the laser group). Between days 4 and 7, the staining score remained high, but on day 8 (following surgery) PDGF levels fell to baseline levels, while they remained high in the laser group (Figures 3 through 7).

The variability of the PDGF staining intensities (0–3 degrees), as an ordinal dependent variable, was examined against the type of the rat (laser vs control group) and the day of sacrifice (Table). The rat type (the type of treatment) was found to be a significant predictor ( $P = .000011$ ).



**FIGURE 7.** Immunohistochemical staining for platelet derived growth factor (PDGF) results for the laser and control groups, 2 weeks after bone surgery. The PDGF was significantly higher in the laser group for at least 2 weeks ( $P = .000011$ ). Analysis of variance with repeated measures:  $*P = .008$ . Treatment effect:  $P = .002$ . Time effect:  $P = .029$ .

TABLE			
Results of multivariate logistic regression, treating the platelet derived growth factor staining as an ordinal dependent variable			
	Degree of Freedom	Wald	<i>P</i>
Intercept	3	39.11773	.000000
Day	1	0.94176	.331827
Rat type	1	19.28514	.000011



## DISCUSSION

The Er:YAG laser delivers a wavelength that is highly absorbed by water and hydroxyapatite and is one of the most effective lasers for bone ablation with minimal thermal damage to the surrounding intact tissues.<sup>3,30</sup>

In the present study we demonstrated that the healing pattern of bone after Er:YAG laser irradiation was improved and is even faster when compared with mechanical bur treatment. Kesler et al<sup>25</sup> reported that there is not delayed healing in comparison to the report of Nelson et al<sup>29</sup> using a rabbit tibia. Other studies have addressed issues related to bone healing after Er:YAG laser irradiation. Buchelt et al<sup>20</sup> and Lewandowski et al<sup>21</sup> reported no difference in the amount of newly formed bone after treatment by Er:YAG laser as compared to the drill.

There are discrepancies in the findings of previous studies, which may be partly due to the use (or none) of water irrigation during ablation, as well as differences in output energy repetition rate, animal model, and treatment in tissues with differing vascularity. In the present study, the use of water spray during Er:YAG laser treatment prevented major thermal damage. Er:YAG laser irradiation without air/water coolant may cause slight charring of the irradiated surface and result in the formation of toxic products that may delay the healing process.<sup>19</sup> Studies performed by Belal et al<sup>30</sup> demonstrated that Er:YAG laser irradiation may improve the effectiveness of periodontal therapy.

In general, the process of bone healing follows 3 distinct but overlapping stages. In brief, it begins with initial injury and the early inflammation phase, followed by a granulation/proliferative phase, and is completed by the late remodeling phase.<sup>31,32</sup>

Platelets are characteristically activated at sites of injury, creating a physical barrier to limit blood loss and accelerating the generation of thrombin to intensify the coagulation process.<sup>33</sup> Platelets can also release local

mediators with phlogistic potential, interacting with leukocytes and endothelial cells to modulate the inflammatory reaction.<sup>34</sup> In addition to hemostasis and inflammation, platelets are involved in wound healing and repair of mineralized tissues.<sup>35</sup> The biologic activities of platelets occur as an immediate response to a fracture, while the degranulation of platelets takes place during hematoma formation. Vascular disruption also occurs in microfractures without any clinical signs of injury.<sup>36</sup> Platelet-released growth factors are chemoattractant for mesenchymal cells of external soft tissue and bone marrow.<sup>37</sup> Growth factors that are highly abundant in platelets can stimulate the proliferation and differentiation of periosteal cells and attract granulocytes and macrophages to the fracture hematoma.<sup>38</sup> Platelet-derived growth factor is involved in almost all wound healing processes. The immediate presence of a potent growth factor in the blood clot at the injured site (eg, bone fracture) would be expected to promote the bone repair.

PDGF is a glycoprotein with a molecular mass of approximately 30 kD.<sup>39,40</sup> Although it is the primary growth factor in platelets, it is also synthesized and secreted by other cells, such as macrophages and endothelial cells.<sup>41</sup> It exists mostly as a heterodimer of 3 chains, termed PDGF AA, PDGF BB, and PDGF AB, of about equal size and molecular mass (approximately 14 to 17 kD). Homodimers of A-A and B-B chains are also present in human platelets and have the same effects on bone regeneration.<sup>39,40</sup> All forms bind with high affinity to 2 cell surface protein tyrosine kinase receptors, PDGFR- $\alpha$  and PDGFR- $\beta$ . The biological effects of PDGF are mediated by these 2 receptors. PDGF has been studied for its clinical utility in wound healing and revascularization. It is one of the major factors activated in wound healing and plays an important role as an endogenous promoter in epithelial tumor formation. PDGF can lead to excessive production of



extracellular matrix components including various collagens, proteoglycans, and laminin. It has been found to stimulate the proliferation of the veins in smooth muscle cells, but only in cultures treated with hyaluronidase. PDGF is one of the most potent activators of stromal cells. PDGF modulates the synthesis of extracellular matrix components. The change in the basic fibroblast growth factor/PDGF ratio may alter the composition of the extracellular matrix of the vein wall during aging, and thus its susceptibility to various diseases. Proliferation and migration are important responses to mesangial cell injury. PDGF is a prime candidate to mediate these responses in glomerular disease, and PDGF and PDGFR are usually elevated in the mesangium during glomerular injury. The immediate presence of a potent growth factor in the blood clot at the injured site (eg, bone fracture) would be expected to promote faster repair and thus a better chance of survival. As such, PDGF in particular seems to have numerous positive effects on wound healing. By virtue of the presence of platelets in the blood clot, it is the first growth factor in the wound and leads towards revascularization, collagen synthesis, and bone regeneration.

Platelet-derived growth factors are dispersed throughout the wound as platelets degranulate. There are approximately 0.06 ng of PDGF per 1 million platelets.<sup>42</sup> This calculates to about 1200 molecules of PDGF per individual platelet.<sup>43</sup> Such numbers underscore the potency of PDGF and emphasize the profound potential for enhanced wound healing and bone regeneration. Its effects are mediated when the PDGF binds to cell membrane receptors. This binding activates an internal cytoplasmic signal protein with a high-energy phosphate bond (kinase activity).<sup>42</sup>

The life span of a platelet in a wound and the direct influence of its growth factors are fewer than 5 days. The extension of healing and bone regeneration activity is accom-

plished by 2 mechanisms. The first is the increase and activation of marrow stem cells into osteoblasts, which then secrete transformation growth factor beta (TGF- $\beta$ ) and insulin like growth factor (IGF) into the osteoid matrix. The second and more dominant mechanism seems to be the chemotaxis and activation of macrophages, which replace the platelets as the primary source of growth factors after the third day.<sup>18</sup> The macrophages are attracted to the bone by actions of PDGF. As PDGF fades in influence, macrophage-derived growth and angiogenic factors take over (days 5 to 7).<sup>43</sup> Macrophage-derived growth factors and angiogenic factors may actually be identical to PDGF, only synthesized by macrophages. The marrow stem cells will secrete TGF- $\beta$  and IGF to continue self-stimulation of bone formation as an autocrine response.<sup>43</sup>

Despite these results, it remains unclear whether PDGF is the only factor that mediates the interaction of platelets with blood cells. The biologic profile of PDGF, including its ability to recruit osteoprogenitor cells, makes it particularly well-suited to address the skeletal defects that are seen with comorbid conditions, such as osteoporosis, diabetes, and the effects of smoking. The clinical success and safety that have been demonstrated with use of recombinant human PDGF (rhPDGF) in the repair of periodontal defects have led to US Food and Drug Administration (FDA) approval of rhPDGF for this indication.<sup>39</sup>

The use of the Er:YAG laser with parameters  $<60$  mJ/pulse and 10 Hz<sup>32</sup> together with rhPDGF has been proven to be a potent stimulator and strong mitogen for human periodontal ligament cells.

In our study, the effect of PDGF in laser-assisted osteotomies was up to 2 weeks in comparison to 4–7 days in conventional bone surgery. Studies showed that laser-initiated PDGF formation may result in higher bone density in Er:YAG laser osteotomies than in



conventional bone preparation. The roughened bone surface created by Er:YAG laser irradiation, which provides a mechanical trap for cell attachment, is a fundamental requirement for bone healing and may help to produce the results noted in the present study. This finding implies that healing occurs more rapidly in the Er:YAG laser-irradiated bone tissue. The accelerated bone healing after Er:YAG irradiation could be explained by the attachment of more extracellular matrix components to the laser irradiated surface, early domination of inflammatory cells, and greater proliferation of capillaries.

Hollinger et al<sup>30</sup> demonstrated that there is a direct effect of rhPDGF on tooth supporting osseous defects. The high amount of PDGF secretion is one indication that there may be more mechanisms involved in this process. If this theory is correct, additional studies will be necessary to determine the ideal laser parameters. It is very important to clarify the promising role of the Er:YAG laser on bone healing via secretion of PDGF, which may promote bone regeneration. Further investigations may be required to validate this hypothesis.

#### CONCLUSIONS

Based on the present results, Er:YAG laser bone surgery may initiate several mechanisms that may explain these results: bone regeneration due to low level laser therapy-like effects and bone formation. Bone surgery with Er:YAG laser, when compared with the mechanical drill, may enhance the bone regeneration by increasing the amount of growth factors present in the rabbit stem cells.

The Er:YAG laser may magnify the natural bone regeneration pathway that is known to continue to a mature graft.

#### ABBREVIATIONS

IGF: insulin like growth factor

PDGF: platelet derived growth factor

PDGFR: platelet derived growth factor receptor

rhPDGF: recombinant human PDGF

TGF- $\beta$ : transformation growth factor beta

#### ACKNOWLEDGMENTS

The authors would like to thank Professor R. Koren, Department of Pathology, Sackler Faculty of Medicine, Tel Aviv University, for her support and technical assistance in this study as well as Dr Michael Yunker for his help in the preparation of the manuscript.

#### REFERENCES

1. Keller U, Hibst R, Mohr W. Experimental animal studies on laser osteotomy using the erbium:YAG laser system [in German]. *Dtsch Z Mund Kiefer Gesichtschir*. 1991;15:197-199.
2. Hibst R. Mechanical effects of erbium:YAG laser bone ablation. *Lasers Surg Med*. 1992;12:125-130.
3. Nuss RC, Fabian RL, Sarkar R, Pullafito CA. Infrared laser bone ablation. *Lasers Surg Med*. 1988;8:381-391.
4. Seka W, Fried D, Featherstone JD, Borzillary SF. Light deposition in dental hard tissue and simulated thermal response. *J Dent Res*. 1995;74:1086-1092.
5. Niu W, Eto JN, Kimura Y, Takeda FH, Matsumoto K. A study on microleakage after resin filling of Class V cavities prepared by Er:YAG laser. *J Clin Laser Med Surg*. 1998;16:227-231.
6. Meister J, Franzen R, Fomer K, et al. Influence of the water content in dental enamel and dentin on ablation with erbium YAG and erbium YSGG lasers. *J Biomed Opt*. 2006;11:34030.
7. Wigdor H, Abt E, Ashrafi S, Walsh JT Jr. The effect of lasers on dental hard tissues. *J Am Dent Assoc*. 1993;124:65-70.
8. Nelson DG, Featherstone JD. Preparation, analysis, and characterization of carbonated apatites. *Calcif Tissue Int*. 1982;34(suppl 2):S69-S81.
9. Featherstone JD, Nelson DG. Laser effects on dental hard tissues. *Adv Dent Res*. 1987;1:21-26.
10. Moshonov J, Stabholz A, Leopold Y, Rosenberg I, Stabholz A. Lasers in dentistry. Part B. Interaction with biological tissues and the effect on the soft tissues of the oral cavity, the hard tissues of the tooth and the dental pulp [in Hebrew]. *Refuat Hapeh Vehashinayim*. 2001;18:21-28, 107-108.
11. Keller U, Hibst R. Ablative effect of an Er:YAG laser on enamel and dentin [in German]. *Dtsch Zahnarztl Z*. 1989;44:600-602.
12. Curti M, Rocca JP, Bertrand MF, Nammour S. Morpho structural aspects of Er:YAG prepared class V cavities. *J Clin Laser Med Surg*. 2004;22:119-123.
13. Apel C, Meister J, Ioana RS, Franzen R, Hering P, Gutknecht N. The ablation threshold of Er:YAG and Er:YSGG laser radiation in dental enamel. *Lasers Med Sci*. 2002;17:246-252.



14. Peavy GM, Reinisch L, Payne JT, Venuogopalan V. Comparison of cortical bone ablations by using infrared laser wavelengths 2.9 to 9.2 micron. *Lasers Surg Med.* 1999;25:421-434.
15. Wang X, Ishizaki NT, Suzuki N, Kimura Y, Matsumoto K. Morphological changes of bovine mandibular bone irradiated by Er,Cr:YSGG laser: an in vitro study. *J Clin Laser Med Surg.* 2002;20:245-250.
16. Pourzarandian A, Watanabe H, Aoki A, et al. Histological and TEM examination of early stages of bone healing after Er:YAG laser irradiation. *Photomed Laser Surg.* 2004;22:342-350.
17. O'Donnell RJ, Deutsch TF, Flotte RJ, et al. Effect of Er:YAG laser holes on osteoinduction in demineralized rat calvarial allografts. *J Orthop Res.* 1996;14:108-113.
18. Fried NM, Fried D. Comparison of Er:YAG and 9.6 microm TEA CO<sub>2</sub> lasers for ablation of skull tissue. *Lasers Surg Med.* 2001;28:335-343.
19. Sasaki KM, Aoki A, Ichinose S, Ishikawa I. Ultrastructural analysis of bone tissue irradiated by Er:YAG Laser. *Lasers Surg Med.* 2002;31:322-332.
20. Buchelt M, Kutschera HP, Katterschafka T, et al. Erb:YAG and Hol:YAG laser osteotomy: the effect of laser ablation on bone healing. *Lasers Surg Med.* 1994;15:373-381.
21. Lewandrowski KU, Lorente C, Schomacker KT, Flotte TJ, Wilkes JW, Deutsch TF. Use of the Er:YAG laser for improved plating in maxillofacial surgery: comparison of bone healing in laser and drill osteotomies. *Lasers Surg Med.* 1996;19:40-45.
22. Sasaki KM, Aoki A, Ichinose S, Yoshino T, Yamada S, Ishikawa I. Scanning electron microscopy and Fourier transformed infrared spectroscopy analysis of bone removal using Er:YAG and CO<sub>2</sub> lasers. *J Periodontol.* 2002;73:643-652.
23. Mester E, Mester AF, Mester A. The biomedical effects of laser application. *Lasers Surg Med.* 1985;5:31-39.
24. Abergel RP, Meeker CA, Lam TS, Dwyer RM, Lesavoy MA, Uitto J. Control of connective tissue metabolism by lasers: recent developments and future prospects. *J Am Acad Dermatol.* 1984;11:1142-1150.
25. Kesler G, Romanos G, Koren R. Use of Er:YAG laser to improve osseointegration of titanium alloy implants: a comparison of bone healing. *Int J Oral Maxillofac Implants.* 2006;21:375-379.
26. Pourzarandian A, Watanabe H, Ruwanpura SM, Aoki A, Noguchi K, Ishikawa I. Er:YAG laser irradiation increases prostaglandin E production via the induction of cyclooxygenase 2 mRNA in human gingival fibroblasts. *J Periodontol Res.* 2005;40:182-186.
27. Lubart R, Kesler G, Lavie R, Friedmann H. Er:YAG laser promotes gingival wound repair by photo-dissociating water molecules. *Photomed Laser Surg.* 2005;23:369-372.
28. Hollinger JO, Hart CE, Hirsch SN, Lynch S, Friedlaender GE. Recombinant human platelet derived growth factor: biology and clinical applications. *J Bone Joint Surg Am.* 2008;90(suppl 1):48-54.
29. Nelson JS, Orenstein A, Liaw LH, Berns MW. Mid infrared erbium:YAG laser ablation of bone: the effect of laser osteotomy on bone healing. *Lasers Surg Med.* 1989;9:362-374.
30. Belal MH, Watanabe H, Ichinose S, Ishikawa I. Effect of Er:YAG laser combined with rhPDGF BB on attachment of cultured fibroblasts to periodontally involved root surfaces. *J Periodontol.* 2007;78:1329-1341.
31. Burchardt H, Enneking WF. Transplantation of bone. *Surg Clin North Am.* 1978;58:403-427.
32. Clark RA. Cutaneous tissue repair: basic biologic considerations. I. *J Am Acad Dermatol.* 1985;13:701-725.
33. Frost A, Jonsson KB, Ridefelt P, Nilsson O, Ljunghall S, Ljunggren O. Thrombin, but not bradykinin, stimulates proliferation in isolated human osteoblasts, via a mechanism not dependent on endogenous prostaglandin formation. *Acta Orthop Scand.* 1999;70:497-503.
34. Bazzoni G, Dejana E, Del Maschio A. Platelet neutrophil interactions. Possible relevance in the pathogenesis of thrombosis and inflammation. *Haematologica.* 1991;76:491-499.
35. Gentry PA. The mammalian blood platelet: its role in haemostasis, inflammation and tissue repair. *J Comp Pathol.* 1992;107:243-270.
36. Zuo ZJ, Zhu JZ. Study on the microstructures of skull fracture. *Forensic Sci Int.* 1991;50:1-14.
37. Barnes GL, Kostenuik PJ, Gerstenfeld LC, Einhorn TA. Growth factor regulation of fracture repair. *J Bone Miner Res.* 1999;14:1805-1815.
38. Joyce ME, Roberts AB, Sporn MB, Bolander ME. Transforming growth factor beta and the initiation of chondrogenesis and osteogenesis in the rat femur. *J Cell Biol.* 1990;110:2195-2207.
39. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet rich plasma: growth factor enhancement for bone grafts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 1998;85:638-646.
40. Antoniades HN. Human platelet derived growth factor (PDGF): purification of PDGF I and PDGF II and separation of their reduced subunits. *Proc Natl Acad Sci U S A.* 1981;78:7314-7317.
41. Ross R, Raines EW, Bowen Pope DF. The biology of platelet derived growth factor. *Cell.* 1986;46:155-169.
42. Bowen Pope DF, Vogel A, Ross R. Production of platelet derived growth factor like molecules and reduced expression of platelet derived growth factor receptors accompany transformation by a wide spectrum of agents. *Proc Natl Acad Sci U S A.* 1984;81:2396-2400.
43. Knighton DR, Oredsson S, Banda M. Regulation of repair: hypoxic control of macrophage mediated angiogenesis. In: Hunt TK, Haepfenstahl RB, Pines E, eds. *Soft Tissue Repair.* New York, NY: Praeger; 1984:41-49.