Biostimulation Effects of Superpulsed, High-Intensity, Low-Average Power Laser Application on the Timing of Orthodontic Aligner Sequencing of the Invisalign® System

Nelson Marquina, MSc, PhD, DC[1]; Fred Stalley, DDS[2]

[1]USA Laser Biotech Inc., Richmond, Virginia, United States

[2]Redondo Beach Dental Group, Redondo Beach, California, United States

J Laser Dent 2009 17(3):139-145

ABSTRACT

Purpose: The aim of this study was to ascertain the biostimulatory effects of a superpulsed, high-intensity laser on the bone remodeling cycle under load of orthodontic forces. Furthermore, this study tested whether the bone remodeling process can be accelerated enough in order to show a clinically significant reduction of the timing between aligner changes of the Invisalign® system (Align Technology, Inc., Santa Clara, Calif.), referred to below as 'the orthodontic system.'

Background: It is common knowledge that bone, as an organ, will respond to pressure above a certain threshold with remodeling. In orthodontic applications, the type of remodeling response will depend on whether bone is under compression (direction of tooth movement) or under tension (the side opposite the direction of movement). On the compression side, the periodontal ligament (PDL) fibers are compressed, which initiates a signaling cascade leading to osteoclastic resorption of bone. On the tension side, the stretching of the periodontal ligament fibers appears to promote osteoblast-mediated bone proliferation. Research has also shown that biostimulation light energy from a low-average power laser can have a stimulating and acceleratory effect on tissue regeneration by promoting an increase in cell populations and signaling molecules responsible for the tissue regeneration and repair cycle. With this information, it stands to reason that laser-induced accelerated bone remodeling will also effectively accelerate the orthodontic movement of teeth without an increase of the orthodontic force applied.

Methods: Forty patients undergoing orthodontic system treatment were selected for this study. These patients were randomly divided into two groups of 20 patients: GL, which received laser treatment, and GC, which served as a control. Each patient was instructed to wear the aligners for a minimum of 20 hours per day. Each patient in GL presented for phototherapy twice a week with at least two days between each of the phototherapy sessions. GC presented for progress checks once a week. Phototherapy was conducted with a superpulsed, high pulse power, and low-average power 910-nm GaAs laser. Tracking progress of all moving teeth for both groups was evaluated at every appointment, during which the computerized progression model was compared to the actual *in vivo* alignment. Once the *in vivo* alignments of the teeth matched the computer model for a particular aligner, the aligner was switched to the next in sequence.

Results: At the termination of the study GL had statistically significant fewer days between aligner exchanges (mean = 9.6 days) than GC (mean = 14.6 days).

INTRODUCTION

The scientific rationale behind orthodontic movement of teeth began more than100 years ago with a book by Sandstedt that was subsequently published as a three-part article in 1904 and 1905.[1-2] Sandstedt constructed a unique experimental model in which the six maxillary incisors of a dog were attached to an appliance and moved 3 mm into the lingual direction within a three-week period. His histological analysis revealed that bone had formed on the alveolar wall of the tension side of the tooth, where the newly formed bone spicules were in the same alignment as the periodontal ligament fibers. These findings were consistent with the application of both light and heavy orthodontic forces. On the pressure side, however, he found that bone was resorbed by osteoclastic activity (as evidenced by the presence of Howship lacunae) with light orthodontic forces, and found certain cell-free areas (as a result of capillary thrombosis and cell death) which he referred to as hyalinization zones under heavy orthodontic forces (Figures 1-2). It was hypothesized that some form of molecular signaling mechanism was responsible for the mechanotransduction which stimulates osteoblasts to generate newly formed bone and osteoclasts to resorb bone. The necessity of mechanical stimuli for the proper maintenance of bone in

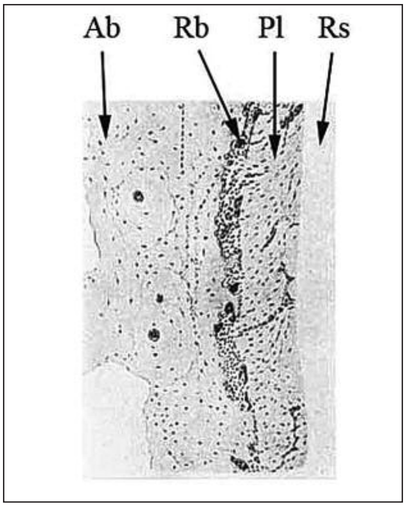

Figure 1: Sandstedt's histological section showing the pressure side after mild force application in the canine model. **Ab**, active bone; **Rb**, resorptive bone; **Pl**, periodontal ligament; **Rs**, root surface. One can clearly recognize numerous osteoclasts in multiple Howship lacunae. (From Sandstedt CE. *Några bidrag till tandregleringens teori [Some contributions to the dental association rules theory].* Stockholm: Kungle Boktrycheriet/Nordstedt & Söner, 1901)

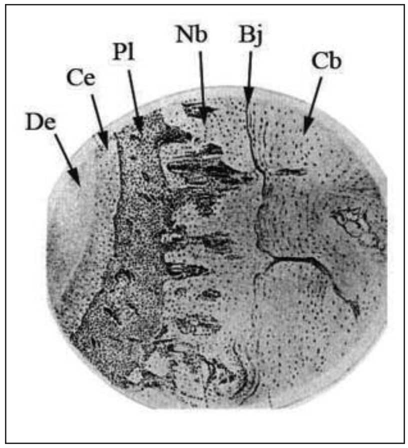

Figure 2: Sandstedt's histological section of the tension side. **De**, dentin; **Ce**, cementum; **Pl**, periodontal ligament; **Nb**, new bone; **Bj**, old bone junction; **Cb**, compact bone. One can clearly see how the newly formed osteoblasts are oriented along the stretched fibers of the ligament. (From Sandstedt CE. *Några bidrag till tandregleringens teori [Some contributions to the dental association rules theory]*. Stockholm: Kungle Boktrycheriet/Nordstedt & Söner, 1901)

the skeletal system had already been recognized since the middle of the 19th century.[3-4] Moreover, Frost reported that the bone tissue within the human skeleton is continuously being remodeled by the tightly orchestrated interaction of osteoclasts resorbing old bone and osteoblasts forming new bone.[5]

More than 100 years after Sandstedt's publication, more detailed knowledge has been gained of the signaling cascades which lead from the mechanical deformation of the periodontium to either osteogenesis or bone resorption. An examination of the basic molecular architecture of cell attachments will help illuminate this pathway. Cells such as osteoblasts or fibroblasts have integral membrane proteins which interact with the extracellular matrix (ECM) and mediate a variety of intracellular signals or attach to other cells. These integral membrane proteins are thus classified as cell surface receptors and collectively referred to as integrins.

It has been shown that numerous signaling pathways can be activated by integrins, which "sense" a mechanical distortion between the cytoskeletal elements they are attached to the inside of a cell and the ECM attachment outside the cell. Basdra *et al*. showed that concentration levels of integrin-mediated signaling proteins (rab and rho guanosine triphosphatases) were altered in mechanically stretched PDL fibroblasts,[6] and Peverali *et al*. have shown similar results for the mitogen-activated protein kinase family in osteoblasts.[7] Harell *et al*. suggested in 1977 that a specific sequence of molecular events is initiated in osteoblast-like cells undergoing mechanical deformation in an *in vitro* environment.[8] During this sequence of events adelynate cyclase is activated, leading to a transient increase of cyclic adenosine monophosphate (cAMP), an increase intracellular [Ca++], and the initiation of DNA synthesis and mitosis,[9] thereby initiating a repair process, as Ngan *et al*. reported in their *in vitro* study involving human gingival fibroblasts.

In summary, DNA synthesis, mitosis, and cell differentiation of fibroblasts and osteoblasts are a direct result of a shift in mechanical pressures within the PDL space.

Furthermore, recent *in vitro* studies have shown that low-average power laser light can stimulate and enhance the proliferation and differentiation of bone marrow stem cells (BMSCs) as well as increase the secretion of growth factors.[10-16] The biostimulatory effects of certain nonablative lasers have been reported in the literature of the late 1960s and early 1970s.[17-18] Since then, numerous publications have elevated phototherapeutic laser applications into mainstream medicine and dentistry.[19-25] Laser energy interacts with tissues through chromophores. Chromophores are parts of the molecule responsible for its color. In biological molecules that serve to absorb or detect light energy, the chromophore is the moiety that causes a conformational change of the molecule when hit by light. Water is one of the most predominant chromophores in human tissue, capable of absorbing light in various amounts throughout the infrared spectrum where dental lasers operate.[26] However, between approximately 600 and 1000 nm, there is a narrow bandwidth in which the absorptive power of water is greatly reduced, and infrared light can penetrate deeply into tissues.[27-28]

Virtually all phototherapeutic lasers today have emissions within the 600-to-1000 nm spectral range. The basis of the biostimulatory effects of phototherapeutic lasers is actually a synergistic amalgamation of two separate effects:

- The photochemical effect, in which the photon energy of the laser is absorbed by cytochrome-*c* oxidase, a large transmembrane protein found in the mitochondrial cell wall. This will cause a short-term activation of the respiratory chain in which the resulting changes of redox states of the mitochondrial plasma and membrane help establish a chemiosmotic potential that the adenosine triphosphate (ATP)-synthase enzyme then uses to synthesize ATP.[29] This sudden increase in ATP within cells will lead to the ability to not only initiate but also accelerate the physiology of irradiated tissues.

- The photomechanical effect, in which the photons from a high-intensity, low-average power, pulsed laser interact with the target tissues to promote gene expression.[16, 30] Gene expression is an important step during the transformatory phase of mesenchymal stem cells into osteoprogenitor cells such as osteoblasts.

When these biochemical effects of laser therapy are considered, it stands to reason that, with the correct laser dosage and wavelength, bone remodeling is one of the cellular processes that can be accelerated in an *in vivo* environment. Bone remodeling is the cornerstone of the translation of teeth in the orthodontic process. Consequently, our hypothesis was that properly dosed laser therapy can accelerate the bone remodeling, initiated through mechanical force changes around teeth, and thus accelerate the orthodontic movement of teeth, given a constantly applied pressure.

SELECTION CRITERIA

This investigation was characterized as a Phase I study with less than 50 subjects. Selection criteria included men and women between the ages of 20 and 40 who were candidates for the orthodontic movement of teeth utilizing the specific orthodontic system identified above. Participation was strictly voluntary. The Institutional Review Board from Texas Applied Biomedical Services (Houston, Texas) approved the study protocol.

Patients who qualified for this research project were properly informed of the system's procedure, biostimulatory laser therapy, as well as the associated risks and benefits. In addition, alternative treatment modalities and their associated risks and benefits were discussed. A brief synopsis of the research project – its goals, expectations, and possible benefits – was also given to each patient.

Exclusion criteria were as follows:

- Patients under the age of 20 and over the age of 40 were not considered in order to obtain a more homogeneous population sample.
- Patients with potential neoplasms in the head-and-neck region were excluded, since a possibility exists that laser application might accelerate carcinogenesis in patients suffering from such pathologies, although there is currently no research demonstrating such clinical effects.
- Patients who were smokers were excluded because nicotine has a very strong vasoconstricting effect on microvasculature and may interfere with the normal regeneration process of bone.
- Patients who were undergoing bisphosphonate therapy were excluded since studies are emerging that indicate cancer patients being treated with bisphosphonates may be at risk for osteonecrosis of the jaw.[31]

MATERIALS AND METHODS

Each potential research subject was screened and categorized as "eligible" or "not eligible" based on the inclusion and exclusion criteria identified above.

The orthodontic system takes advantage of the dramatic developments in CAD/CAM technology in recent years.[32-35] A patient's impression is scanned to produce a computerized three-dimensional image of the initial alignment of teeth. The desired final alignment is done virtually with computer software and then translated into a sequence of aligner trays which are necessary to achieve that goal. The number of aligners necessary for a case will vary from patient to patient and can be as few as 10 and as many as 40. The Invisalign protocol calls for aligner change every 14 days, if the alignment of the teeth *in vivo* matches the alignment of the teeth on the computer model (ClinCheck® 2.6 software, Align Technology, Inc., Santa Clara, Calif.).

Those subjects who were selected were randomly assigned to one of two groups of 20 individuals each:

- **Group L (GL):** Patients received the orthodontic system and also presented twice a week for laser treatments and progress checks, with at least two days between laser treatments.
- **Group C (GC):** Patients received the orthodontic system only, and presented weekly for progress checks only.

Each patient was instructed to wear the aligners for a minimum of 20 hours per day.

Progress checks were performed with ClinCheck, which displays a three-dimensional image of the dentition within each aligner sequence. Individual tooth positions were measured in relation to adjacent teeth with the help of a millimeter grid overlay in the software (Figure 3).

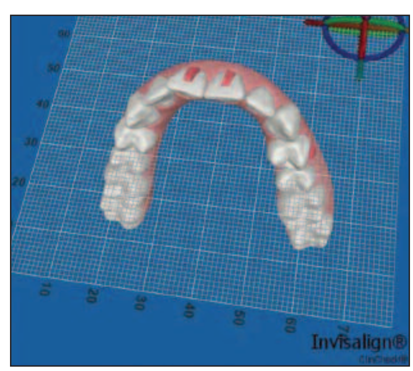

Figure 3: A typical example of an Invisalign case analysis. The overlay grid is used to measure distances and angulations of individual teeth to each other, which is then compared to the intraoral environment

These measurements were synchronized *in vivo* with a periodontal probe (Hu-Friedy, Chicago, Ill.) for each tooth that was being moved orthodontically. The computer model measurements were then compared to the intraoral measurements. If the intraoral alignments of the teeth matched the computer model, the patient was given the next aligner in the sequence. These progress checks were conducted for both groups.

Laser treatments were performed with a free-running, pulsed 910-nm GaAs laser (Lumix 2, USA Laser Biotech Inc., Richmond, Va.). The laser was used at 45 Watts peak power at 30 KHz for this study. The diameter of the emitter tip is 8 mm and has a divergence angle of 12 degrees. Each arch was divided into six "illumination spots," three on each side of the arch, so that there was slight overlap between the illuminated areas to cover the whole arch (Figure 4). Each of the six spots was illuminated for 90 seconds, for a total duration of 9 minutes per arch. This translates into 27 joules of energy per spot and 162 joules per arch. The emitter tip was held at approximately 5 mm from the buccal surfaces, just apical to the cementoenamel junction (CEJ) (Figure 5). Once the patients completed their aligner sequence, the following data were collected:

- Total number of aligners used
- Average number of days between aligner switches
- Number of midcourse corrections (additional trays used to correct abnormal tracking of teeth in midcourse).

Statistical analysis was done with the SPSS statistical software (PASW Statistics 18.0, SPSS Inc., Chicago, Ill.).

RESULTS

Both, the laser group (GL) and the control group (GC) samples consisted of 20 patients each. The longest and shortest treatment course for GL was 240 days and 99 days, respectively. The longest and shortest treatment course for GS

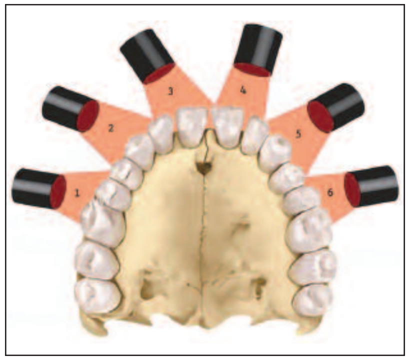

Figure 4: Six areas of laser illumination were used to cover the entire maxillary arch, and six areas were also used for the mandibular arch. Each of these areas received 90 seconds worth of exposure. This image shows the illumination pattern for the maxillary arch

was 425 days and 143 days, respectively. The average number of aligners needed to complete the case was 18.1 for GL and 19.85 for GC, which was not statistically different at a significance level alpha of 0.05. The average number of days between aligner exchanges was 9.57 for GL and 14.63 for GC, which produced statistically highly significant results using a one-tailed t-test P value of 0.000 at an alpha level of 0.01. Therefore, the laser group had a 34.6% faster aligner sequencing. Furthermore, of the 20 subjects in the control group, 5 needed midcourse corrections, whereas only 3 subjects needed midcourse corrections in the laser group (Table 1). On a subjective side note, patients in the laser treatment group reported fewer complaints of moderate-to-severe soreness the first two days after a new aligner had been fitted than the patients in the no laser treatment (control) group.

DISCUSSION

Teeth move in a certain direction by applying pressure on the teeth in the direction of translation. This will cause a deformity of the PDL on the pressure side as well as the tension side of the individual teeth. The deformation of certain cells in the PDL environment (fibroblasts and

Figure 5: The emitter portion of the laser is directed toward the buccal surfaces of the roots just apical to the cemento-enamel junction

osteoblasts) is "sensed" by the integrins in their cell wall, which then release specific signaling molecules to activate the remodeling process. This process is called "mechanotransduction." Some of these integrin-mediated signaling proteins (rab and rho guanosine triphosphatases, and mitogen-activated protein kinase) cause the proliferation of osteoclasts on the pressure side of the teeth and proliferation of osteoblasts on the tension side of the teeth. Furthermore, other signaling molecules will initiate an inflammatory reaction and mesenchymal stem cell division and transformation as part of the healing response.

All of these cellular processes are energy-intensive activities for cells, especially DNA synthesis, mitosis, and transformation of stem cells into new fibroblasts or osteoblasts. The basic energy molecule for all cells is adenosine triphosphate (ATP).[36] Cells use this molecule to power their various activities. If a particular cell lacks sufficient quantities of ATP, certain cellular activities cannot take place. A sufficient quantity of ATP is therefore the prerogative for various cellular activities, especially in the repair and remodeling process of bone.

Phototherapy laser energy has been shown to increase the rate of cell division, cell transformation (mesenchymal stem cells), production of signaling molecules, and development of osteoblasts, fibroblasts, and osteoclasts.[37] All of these cellular

Orthodontic System Data with Biostimulatory Laser Application				Orthodontic System Data without Biostimulatory Application			
Subject	Total Number of Aligners	Average Days Between Aligners	Number of Midcourse Corrections	Subject	Total Number of Aligners	Average Days Between Aligners	Number of Midcourse Corrections
S1	15	11.5		S1	28	15.2	
S2	22	8.7		S2	19	13.6	
S3	25	9.6		S3	10	14.3	
S4	20	10.2	1	S4	16	12.8	1
S5	15	8.9		S5	17	15.9	
S6	19	11.6		S6	20	19.5	
S7	16	9.2		S7	22	13.1	
S8	10	9.9		S8	32	12	
S9	22	8	1	S9	16	17.4	1
S10	17	8.8		S10	18	16.2	
S11	14	10.2		S11	14	14.6	
S12	10	8.3		S12	25	16.2	
S13	13	9.5		S13	18	11.5	2
S14	17	7.9		S14	26	14.2	
S15	22	12.2		S15	13	15.1	
S16	14	9		S16	18	14.9	
S17	26	8.7		S17	20	14	1
S18	18	9.4		S18	11	13.7	
S19	23	9.7	1	S19	31	12.6	1
S20	24	10.1		S20	23	15.8	
Mean	9.57 Days			Mean	14.63 Days		

Table 1: This table shows the data distribution of all 40 subjects in both groups. Group GL is the table on the left side and group GC is on the right side. The results show a statistically significant difference between GL and GC with respect to the actual days between aligner exchanges and the recommended 14 days.

events are involved in the bone remodeling processes of orthodontic movement of teeth. It is therefore a reasonable assumption that a low-average power laser can also accelerate the rate of tooth movement in the orthodontic process.

As previously mentioned, of the two groups that were studied, the laser group showed a faster aligner sequencing. One can speculate, based on the information given above, that the chromophores of the cells in question were able to absorb the photon energy of the laser and convert this energy into their own usable energy in the form of ATP. This process is governed by very specific pathways; in particular, the main chromophore in the mitochondria is the protein molecule cytochrome-*c* oxidase, which is a component of the respiratory chain. It is the terminal enzyme that mediates the transfer of electrons from cytochrome-*c* to molecular oxygen. Additionally, ferrocytochrome-*c* is oxidized, dioxygen is reduced, and protons are pumped from the mitochondria to the cytosol.[36] The electrochemical potential generated across the inner membrane of the mitochondrion by this redox drives the oxidative photophosphorylation of ADP into ATP.[36] Once the concentration of ATP had increased inside the cells, they were able to initiate and execute the remodeling cascade with more efficiency and speed. It is our hypothesis that these were the most predominant precipitating factors in the accelerated tray sequencing we observed in the laser group. Indeed, there are studies using rats[38-39] as well as one using a group of 15 patients[40] that demonstrate how a low average power laser can contribute to a more rapid movement of teeth.

CONCLUSION

Within the confines of this study we have shown that low-average power, high-pulse intensity laser phototherapy can potentially accelerate orthodontic movements of teeth for the Invisalign platform. Future studies should compare these results with variations in wavelength, energy deliverance, and exposure durations. The limitation of this study was the small sample size. Larger-scale studies need to be done, perhaps on a multicenter level, in order to confirm and expand these results. It is our opinion that this study is synergistic to the ever-growing body of evidence showing that low-power laser phototherapy may have a positive effect on overall healing, since the molecular healing cascade is similar for various tissue types in the human body.

AUTHOR BIOGRAPHIES

Dr. Nelson Marquina is the president of USA Laser Biotech Inc., a medical device developer focused on lasers and bioelectromagnetic devices for USA and Canadian markets. He has earned a Master of Science degree in statistics from Worcester Polytechnic Institute and doctoral degrees in systems engineering from the University of Houston and in chiropractic medicine from Logan University. He is an adjunct associate professor of biophysics at Virginia State University and a consultant to the National Foundation for Alternative Medicine (Washington, D.C.). Dr. Marquina is a former senior scientist at NASA/Johnson Space Center (Texas) and Director of Research at Logan University (Chesterfield, Mo.). He was Director of Information Systems in Mars, Inc. (New Jersey) and former partner in Coopers & Lybrand's Information Technology Consulting Services (New York, N.Y.). He is an executive, consultant, and educator with more than 25 years of combined management, teaching, and technical experience in information systems, statistical analysis, and bioelectrical and bioelectromagnetic systems. Dr. Marquina is also a developer of bioelectrical, biophotonics, and bioelectromagnetic systems and their treatment protocols. He has chaired national conferences in computer expert systems, high technology in alternative medicine, and computer vision. He can be reached at Marquina@comcast.net.

Disclosure: *Dr. Marquina is a paid consultant to Harris Medical Resources and BEMER USA. He is a stockholder in USA Laser Biotech Inc.*

Dr. Fred Stalley is a private practitioner based in Redondo Beach, Calif. He received his dental degree in 1979 from the University of Southern California. He specializes in cosmetic and implant dentistry and is a graduate of the Las Vegas Dental Institute. He is also member of the Academy of Osseointegration, the American Academy of Implant Dentistry, the International Congress of Oral Implantologists, and the American Academy of Cosmetic Dentistry. He has been utilizing laser technology since 1994. Dr. Stalley has lectured nationwide as well as abroad. He can be reached at rbdg@redondobeachdentalgroup.com.

Disclosure: *Dr. Stalley has nothing to disclose relative to this manuscript.*

REFERENCES

1. Sandstedt CE. *Några bidrag till tandregleringens teori [Some contributions to the dental association rules theory].* Stockholm: Kungle Boktrycheriet/Nordstedt & Söner, 1901. Swedish.
2. Sandstedt C. Einige beiträge zur theorie der zahnregulierung. *Nord Tandläk Tidskr* 1904;5:236-256; 1905;6:1-25; 1905;6:141-168. German.
3. Meyer GH. Die architektur der spongiosa. (Zehnter beitrag zur mechanic des menschlichen knochengerüstes.) *Arch Anat Physiol Wiss Med* 1867;47:615-628. German.
4. Wölff JD. Das gesetz der transformation der knochen. Berlin: Verlag von August Hirschwald, 1892. German. Also see Julius Wolff. *The law of bone remodeling.* Maquet P, Furlong R, translators. Berlin: Springer-Verlag, 1986.
5. Frost HM. *Bone remodeling dynamics.* Springfield, Ill.: Charles C Thomas, 1963.
6. Basdra EK, Papavassiliou AG, Huber LA. Rab and rho GTPases are involved in specific response of periodontal ligament fibroblasts to mechanical stretching. *Biochim Biophys Acta* 1995;1268(2):209-213.
7. Peverali FA, Basdra EK, Papavassiliou AG. Stretch-mediated activation of selective MAPK subtypes and potentiation of AP-1 binding in human osteoblastic cells. *Mol Med* 2001;7(1):68-78.
8. Harell A, Dekel S, Binderman I. Biochemical effect of mechanical stress on cultured bone cells. *Calcif Tissue Res* 1977;22(Suppl 1):202-207.
9. Ngan PW, Crock B, Varghese J, Lanese R, Shanfeld J, Davidovitch Z. Immunohistochemical assessment of the effect of chemical and mechanical stimuli on cAMP and prostaglandin E levels in human gingival fibroblasts *in vitro. Arch Oral Biol* 1988;33(3):163-174.
10. Mvula B, Mathope T, Moore T, Abrahamse H. The effect of low level laser irradiation on adult human adipose derived stem cells. *Lasers Med Sci* 2008;23:277-282.
11. Mvula B, Moore TJ, Abrahamse H. Effect of low-level laser irradiation and epidermal growth factor on adult human adipose-derived stem cells. *Lasers Med Sci.* Epub 2009 Jan 27. DOI 10.1007/s10103-008-0636-1.
12. Hou J-F, Zhang H, Yuan X, Li J, Wei Y-J, Hu S-S. *In vitro* effects of low-level laser irradiation for bone marrow mesenchymal stem cells: Proliferation, growth factors secretion and myogenic differentiation. *Lasers Surg Med* 2008;40(10):726-733.
13. Eduardo Fde P, Bueno DF, de Freitas PM, Marques MM, Passos-Bueno MR, Eduardo Cde P, Zatz M. Stem cell proliferation under low intensity laser irradiation: A preliminary study. *Lasers Surg Med* 2008;40(6):433-438.
14. de Souza SC, Munin E, Alves LP, Salgado MAC, Pacheco MTT. Low power laser radiation at 685 nm stimulates stem-cell proliferation rate in *Dugesia tigrina* during regeneration. *J Photochem Photobiol B* 2005;80(3):203-207.
15. Tuby H, Maltz L, Oron U. Low-level laser irradiation (LLLI) promotes proliferation of mesenchymal and cardiac stem cells in culture. *Lasers Surg Med* 2007;39(4):373-378.

16. Stein E, Koehn J, Sutter W, Wendtlandt G, Wanschitz F, Thurnher D, Baghestanian M, Turhani D. Initial effects of low-level laser therapy on growth and differentiation of human oseoblast-like cells. *Wien Klin Wochenschr* 2008;120(3-4):112-117.

17. Carney SA, Lawrence JC, Ricketts CR. The effect of light from a ruby laser on the metabolism of skin in tissue culture. *Biochim Biophys Acta* 1967;148(2):525-530.

18. Mester E, Spiry T, Szende B, Tota JG. Effect of laser rays on wound healing. *Am J Surg* 1971;122(4):532-535.

19. Wang GH, Jiang SJ, Hu SJ, Zheng GC, Peng SY. A study of the analgesic effect of low power He-Ne laser and its mechanism by electrophysiological means. In: Yamamoto H, Atsumi K, Kusakari H, editors. *Lasers in dentistry. Proceedings of the International Congress of Laser in Dentistry,* August 5-6, 1988, Tokyo, Japan. Amsterdam: Excerpta Medica, 1989:277-281.

20. Walsh LJ. The current status of low level laser therapy in dentistry. Part 1. Soft tissue applications. *Aust Dent J* 1997;42(4):247-254.

21. Walsh LJ. The current status of low level laser therapy in dentistry. Part 2. Hard tissue applications. *Aust Dent J* 1997;42(5):302-306.

22. Hopkins JT, McLoda TA, Seegmiller JG, David Baxter G. Low-level laser therapy facilitates superficial wound healing in humans: A triple-blind, sham-controlled study. *J Athl Train* 2004;39(3):223-229.

23. Hawkins D, Houreld N, Abrahamse H. Low level laser therapy (LLLT) as an effective therapeutic modality for delayed wound healing. *Ann N Y Acad Sci* 2005;1056:486-493.

24. Khadra M. The effect of low level laser irradiation on implant-tissue interaction. *In vivo* and *in vitro* studies. *Swed Dent J Suppl* 2005;172:1-63.

25. Parker S. Low-level laser use in dentistry. *Br Dent J* 2007;202(3):131-138.

26. Manni JG. *Dental applications of advanced lasers* (DAAL™). Burlington, Mass.: JGM Associates, Inc., 2004:2-17.

27. Karu T. *The science of low-power laser therapy.* Amsterdam: OPA (Overseas Publishers Association), 1998:3.

28. Peavy GM. Lasers and laser-tissue interaction. *Vet Clin North Am Small Anim Pract* 2002;32(3):517-534, v-vi. Erratum in: *Vet Clin North Am Small Anim Pract* 2002;32(6):ix.

29. Hamblin MR, Demidove TN. Mechanisms of low level light therapy. In: Hamblin MR, Waynant RW, Anders J, editors. *Mechanisms for Low-Light Therapy*, January 22 and 24, 2006, San Jose, Calif. Proc. SPIE 6140. Bellingham, Wash.: SPIE – The International Society for Optical Engineering, 2006:614001-1 – 614001-12.

30. Moriyama Y, Nguyen J, Akens M, Moriyama EH, Lilge L. *In vivo* effects of low level laser therapy on inducible nitric oxide synthase. *Lasers Surg Med* 2009;41(3):227-231.

31. Maerevoet M, Martin C, Duck L. Osteonecrosis of the jaw and bisphosphonates [correspondence]. *N Eng J Med* 2005;353(1):99-102; discussion 199-102.

32. Bishop A, Womack WR, Derakhshan M. An esthetic and removable orthodontic treatment option for patients: Invisalign®. *Dent Assist* 2002;71(5):14-17.

33. Melkos AB. Advances in digital technology and orthodontics: A reference to the Invisalign® method. *Med Sci Monit* 2005;11(5):PI39-P142.

34. Wong BH. Invisalign A to Z. *Am J Orthod Dentofacial Orthop* 2002;121(5):540-541.

35. Norris RA, Brandt DJ, Crawford CH, Fallah M. Restorative and Invisalign®: A new approach. *J Esthet Restor Dent* 2002;14(4):217-224.

36. Karu T. Primary and secondary mechanisms of action of visible to near-IR radiation on cells. *J Photochem Photobiol B* 1999;49(1)1-17.

37. Karu T. *Ten lectures on basic science of laser phototherapy.* Grängesberg, Sweden: Prima Books, 2007.

38. Kawasaki K, Shimizu N. Effects of low-energy laser irradiation on bone remodeling during experimental tooth movement in rats. *Lasers Surg Med* 2000;26(3):282-291.

39. Ozawa Y, Shimizu N, Kariya G, Abiko Y. Low-energy laser irradiation stimulates bone nodule formation at early stages of cell culture in rat calvarial cells. *Bone* 1998;22(4):347-354.

40. Youssef M, Ashkar S, Hamade E, Gutknecht N, Lampert F, Mir M. The effect of low-level laser therapy during orthodontic movement: A preliminary study. *Lasers Med Sci* 2008;23(1):27-33.