

Cellular Effects Related to the Clinical Uses of Laser in Orthodontics

Angela Camacho Dominguez^a, Paola Zúñiga Castro^b, Mónica Cardona Morales^c

^a *Specialist in Orthodontics, CIEO, Bogotá, Colombia; Professor, Orthodontics Department, Faculty of Dentistry, Universidad del Valle, Cali, Colombia.*

^b *Specialist in Orthodontics, Universidad del Valle, Cali, Colombia.*

^c *Third-year Resident, Orthodontics Graduate Program, Universidad del Valle, Cali, Colombia.*

Abstract: Some orthodontic procedures are improved by the therapeutic use of laser, bringing some advantages for both patient and practitioner, by either reducing the time of procedures or pain induced by the activation of devices during the treatment. This therapy deserves further analysis of the biological principles, to provide insight into the effects of laser on different cell lineages involved.

The aim of this article is to provide a review of the literature about laser effects on fibroblasts, osteoblasts and osteoclasts, studied in animal models and in human subjects. The review indicates that the application of low energy laser during orthodontic movement is safe in terms of the cellular effects, and there is a strong basis to explain the significant favorable clinical results reported.

Keywords: orthodontics, laser therapy, oral cell biology, fibroblasts, osteoblasts, osteoclasts.

J Oral Laser Applications 2009; 9: 199-203.

Submitted for publication: 04.04.09; accepted for publication: 25.09.09.

In terms of clinical evidence, *in vitro* studies are not usually taken into account, as it is often argued that a clinical decision cannot be supported on the effects observed in cell cultures. It is acknowledged that a direct extrapolation of culture results to the human being is at least uncertain, and probably incomplete. Nevertheless, laser therapy in dentistry has been extensively used without previous *in vivo* evidence. The following review includes updated publications (cited in Pubmed and reported in Colombia in English and Spanish from 1980 to 2008) reporting the therapeutic effects of laser in each cell-line relevant to orthodontically induced dental movement.

Background

Many cellular reactions occur in order to make an induced dental movement possible,¹⁻⁴ and there are many reports indicating that the application of low energy laser beams accelerate critical cellular events necessary to attain the dental movement.⁵ For many years now, laser systems have been therapeutically used, but without the support of scientific evidence about the effects in the cells involved in dental movement.

The purpose of this systematic review of the literature is to update and bring together the information about laser effects on fibroblasts, osteoblasts, and osteoclasts.

In Vitro Studies on Different Cell Lineages

Fibroblasts

Gingival fibroblasts were irradiated in a study published by Almeida Lopez,⁶ with a laser energy of 2 J/cm². The cell proliferation was higher than in the nonirradiated control group. This in vitro study also related the duration of exposure to the rate of fibroblast proliferation and compared infrared with visible laser wavelengths. The authors concluded that there was a negative correlation between duration of exposure and rate of proliferation, with the infrared laser being more effective than visible laser. The effects on fibroblast rate of proliferation were equal for a similar dose of energy, independent of the wavelength.

Pereira et al⁷ evaluated the synthesis of pro-collagen after irradiation of gingival fibroblast cultures. This group used Ga-As laser in a range of energy between 3 to 5 J/cm², 120 mW of power and a wavelength of 904 nm, measuring pro-collagen immunoprecipitation and determining growth curves. They concluded that laser light enhances cell proliferation without a significant effect on pro-collagen synthesis.

Marques and Pereira⁸ analyzed protein synthesis and fibroblast morphology in human gingival fibroblasts irradiated with a low energy Ga-Al-As laser, at a wavelength of 904 nm, 120 mW and 3 J/cm². They reported a significant reduction in the amount of protein synthesized and changes in the structure of cytoplasm organelles in the cells treated. The authors related the observed ultrastructural changes in irradiated human gingival fibroblasts to changes in collagen metabolism.

Kreisler⁹ evaluated the potential stimulation of low energy laser irradiation on periodontal ligament fibroblasts. It is accepted that periodontal fibroblast cultures are quite different from gingival fibroblast cultures,¹⁰ but Kreisler found that laser enhanced the rate of proliferation compared to the nonirradiated control, as in gingival fibroblasts.

There are also reports on fibroblasts irradiated with LEDs (light emitting diodes) and low energy laser,¹¹ which confirm stimulation of cell proliferation.

These studies are difficult to compare, as they used different emission sources, arbitrary distances, and nonstandardized manual techniques. Timberlake¹² emphasized the need of using standard parameters of irradiation for cultured cells. Therefore, in 2008, a pilot study¹³ was carried out to develop a protocol for use in experimental studies with human gingival and periodontal fibroblast, osteoblasts, and pre-osteoclasts. The protocol was developed using the As-Ga-Al laser

set to 37 mW power, 830 nm wavelength and 3.75 J/cm² of energy. The rate of cell proliferation was measured after 24, 48, 72, 120 and 144 hours of irradiation. It was proved that gingival fibroblast rate of proliferation was indeed higher than the control, but the difference was not significant. On the other hand, irradiated periodontal fibroblasts showed no difference to the control,^{14,15} which indicates that previous results might be due to the heterogeneity of the fibroblast subpopulations studied.

Young et al¹⁶ studied macrophages irradiated at 660 nm, 820 nm and 870 nm wavelengths and reported that the 870 nm wavelength stimulated the release of factors that are known to stimulate fibroblast rate of proliferation, whereas other wavelengths inhibited the release of the same factors or encouraged the release of some inhibitory factors. These results taken together only suggest that at certain wavelengths there is some stimulating effect, while for other wavelengths the effect might be to inhibit cell proliferation. Therefore, laser therapy could be used as necessary, either to stimulate or to inhibit fibroblast proliferation.

Osteoblasts

Osteoblasts are the essential bone regeneration cells, and are derived from specialized fibroblasts. The first study¹⁷ about irradiated osteoblast cultures was performed in rat calvaria cells at different stages of cell growth, with Ga-As-Al laser at 780 nm and 500 mW. Besides cell proliferation, bone nodule formation, alkaline phosphatase activity and osteocalcin expression were also examined. It was found that laser phototherapy enhances cell proliferation at initial stages of cell growth, and increases alkaline phosphatase activity, osteocalcin gene expression and bone nodule number. The net result is that laser stimulates the proliferation and differentiation of osteoblast precursors involved in bone formation.

Coombe and Darendeliev¹⁸ studied the effects of laser irradiation on a line of osteoblastic cell from human osteosarcoma (SAOS-2). They used Ga-Al-As laser in continuous mode at 830 nm wavelength, 90 mW and 0.3, 0.5, 1.2 or 4 J/cm². Cell proliferation under these conditions was not significantly affected, but an increased concentration of intracellular calcium was observed, which could be relevant to orthodontic movement and deserving of further study.

Using Ga-Al-As laser (780 nm, 10 mW and 3 J/cm²), Fujihara et al¹⁹ irradiated rat calvaria osteoblast-like cells, previously treated or not treated

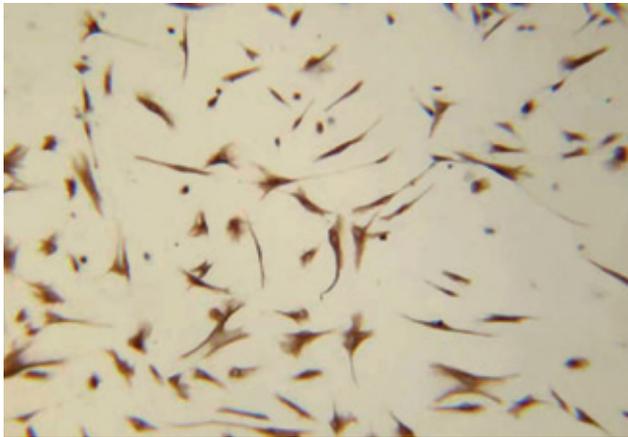


Fig 1 Human osteoblasts from PromoCell Bioscience alive© and Cambrex BioScience, Walkersville Inc, and cultured in the BD, BioCoat™ Osteologic™ Bone Cell Culture system (4X).

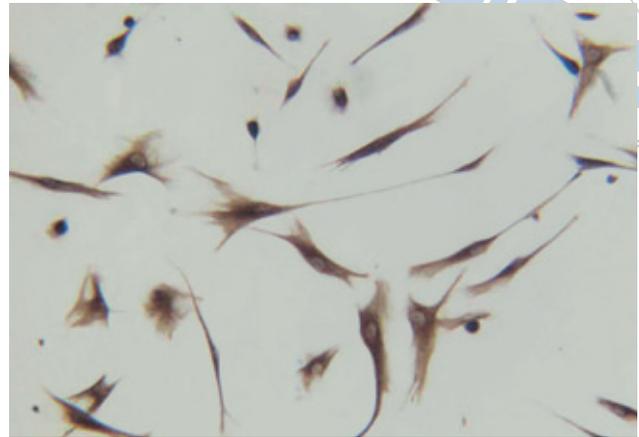


Fig 2 Human osteoblasts from PromoCell Bioscience alive© and Cambrex BioScience, Walkersville Inc, and cultured in the BD, BioCoat™ Osteologic™ Bone Cell Culture system (10X).

with dexamethasone. This study analyzed the adhesion, proliferation and synthesis of osteonectin. The study concluded that laser therapy induced cell proliferation independent of the presence of dexamethasone.

Dominguez et al²⁰ irradiated normal human osteoblasts (NHOst) with low energy laser (As-Ga-Al, 3.75 J/cm² and 36.7 mW), for periods of 24, 48, 72, 96, 120 or 148 hours. The results indicate that NHOst are sensitive to low level laser irradiation while in early stages of development in culture. Cell proliferation was enhanced from the first day on, until there was contact inhibition (Figs 1 and 2). This finding could be clinically applicable in orthodontics and periodontal surgery, when bone neoformation is required.

Preosteoclasts and Osteoclasts

Osteoclasts are highly differentiated members of the macrophage/monocyte lineage, derived from hemopoietic precursors. The expression of tartrate-resistant acid phosphatase (TRAP) is characteristic of this lineage and it is frequently used as a biochemical marker of this cell proliferation.²² In vitro osteoclastic activity is measured by the lacunae excavation in bone or dentin layers and this activity identifies active and mature functional osteoclasts.^{23,24}

Tsay et al,²⁵ using an adult rat model, reported that osteoclasts involved in orthodontic remodeling derive from periodontal ligament precursors and when prolonged strong forces are applied, they originate from

bone marrow precursors. They quantified the osteoclast activation cycle, lasting approximately four weeks, while the active life of the osteoclast was 9 or 10 days. This explains why it is so difficult to study and analyze the in vitro behavior of preosteoclasts and osteoclasts.

In an in vivo rat model, Saito and Shimizu²⁶ studied the stimulating effect of low energy laser irradiation on bone regeneration in the midpalatal suture during rapid maxillary expansion. They used Ga-Al-As laser (100 mW) modifying the exposure time, and found out that the accelerated rate of bone regeneration in the irradiated group was dose dependent.

Kawasaki and Shimizu²⁷ reported that low level laser stimulates both the rate of dental movement and osteoclast differentiation on the pressure side during experimental dental movement in vivo. Altogether, the reported results agree with the well-accepted model of bone remodeling as a physiologic process involving bone resorption by osteoclasts and bone synthesis by osteoblasts.²⁸⁻³²

Sun et al³³ performed an experiment in which they applied an orthodontic force of 80 g in rabbit maxillary first molars. They found out a difference in the number of osteoclasts between the experimental side laser irradiated and the control, after 3, 5 or 7 days of treatment. The authors concluded that low energy laser promotes dental movement by enhancement of alveolar bone remodeling.

Aihara and Yamaguchi³⁴ performed studies on the system OPG/RANKL/RANK (Osteoprotegerin/Receptor activator NK ligand and Receptor activator NK

during orthodontic dental movement, finding it to be an important molecular regulatory system to balance the alveolar bone resorption in periodontal tissue. Those authors reported that after low-energy laser irradiation applied to rat osteoclast precursor cells for 1, 3, 6, or 10 min at 24-h intervals during the culture period, the laser irradiation groups showed upregulated expressions of RANK. In the pit formation assay, resorption pits were significantly more abundant in the laser irradiation groups than in the controls.³⁴ This research suggests that low-energy laser irradiation facilitates differentiation and activation of osteoclasts via RANK expression.

Human Studies

Studies performed in human subjects have shown an effect of laser on post adjustment pain.^{36,37} The randomized controlled study performed at the CIEO Foundation in 1996⁵ to study the effects on pain during the alignment and leveling stage of treatment, detected not only a pain relief effect but a reduction in the total duration of treatment, as compared to the control group.

Meguro³⁸ found an inhibitory effect of low energy laser irradiation on relapse and opening of the gingival embrasure space. Using Ga-Al-As laser, this group irradiated an area of 0.5 cm² in the vestibular and lingual papillae between canines and observed a reduction in gingival inflammation.

Cruz et al³⁹ studied 11 patients during the orthodontic distalization of canine teeth for 60 days. The experimental group was irradiated with therapeutic laser (wavelength 780 nm, power 20 mW, energy 5 J/cm²) for 10 s per month. The rate of canine distalization movement was enhanced in the irradiated group versus the control.

Beyond the scope of this review of literature, additional applications of laser in orthodontics are being developed, for instance, to etch enamel for orthodontic bonding⁴⁰ and for soft-tissue management.⁴¹

CONCLUSIONS

The reports reviewed here on laser effects on fibroblasts agree that there is an enhancement of the proliferation in irradiated cultures. Periodontal fibroblasts apparently are not activated in the same proportion as gingival or dermal fibroblasts.

Normal osteoblasts irradiated with therapeutic laser also increase the rate of proliferation, which allows them to perform the bone synthesis required in orthodontic and periodontal surgical clinical situations.

Animal studies indicate that the number of osteoclasts in irradiated sites during orthodontic movement is increased. Human studies indicate a significant increase in orthodontic movements and reduction of post-adjustment pain.

According to the updated review of reliable studies, there appears to be strong evidence to support the use of low energy laser as a therapeutic tool in orthodontics, safe at the cellular level and clinically efficient.

REFERENCES

1. Roberts WE, Goodwin WC Jr, Heiner SR. Cellular response to orthodontic force. *Dent Clin North Am* 1981;25:3-17.
2. Meikle MC. The tissue, cellular, and molecular regulation of orthodontic tooth movement: 100 years after Carl Sandstedt. *Eur J Orthod* 2006;28:221-240.
3. Krishnan V, Davidovitch Z. Cellular, molecular, and tissue-level reactions to orthodontic force. *Am J Orthod Dentofacial Orthop* 2006;129:469.
4. Masella RS, Meister M. Current concepts in the biology of orthodontic tooth movement. *Am J Orthod Dentofacial Orthop* 2006;129:458-68.
5. Abello Hilda Marcela, Valbuena Diana. Efecto del láser blando (ir) en la velocidad de alineación del tratamiento ortodóncico. Tesis de postgrado Fundación C.I.E.O Bogotá, Colombia 1996.
6. Lopes A. Comparison of the low level laser therapy effects on cultured human gingival fibroblasts proliferation using different irradiance and same fluence. *Lasers Surg Med* 2001;29:179-184.
7. Pereira AN. Effect of low-power laser irradiation on cell growth and procollagen synthesis of cultured fibroblasts. *Lasers Surg Med* 2002;31:263-267.
8. Marques M, Pereira A. Effect of low-power laser irradiation on protein synthesis and ultra structure of human gingival fibroblasts. *Lasers Surg Med* 2004;34:260-265.
9. Kreisler M. Effect of low-level GaAlAs laser irradiation on the proliferation of human periodontal ligament fibroblasts: an in vitro study. *J Clin Periodontol* 2003;30:353-358.
10. Giannopoulou C. Functional characteristics of gingival and periodontal ligament fibroblasts. *J Dent Res* 1996;75:895-902.
11. Vinck EM. Increased fibroblast proliferation induced by light emitting diode and low power laser irradiation. *Lasers Med Sci* 2003; 18:95-99.
12. Timberlake GT. An inexpensive, automated instrument for laser irradiation of cultured cells. *Photomed Laser Surg* 2004;22:233-239.
13. Domínguez A, Clarkson A, López R. An in vitro study of the reaction of periodontal and gingival fibroblasts to low-level laser irradiation: A pilot study. *J Oral Laser Applic* 2008;8:235-244.
14. McCulloch CA, Bordin S. Role of fibroblast subpopulations in periodontal physiology and pathology. *Perio Res* 1991;26:144-154.
15. Soto JE, Parra B, Contreras A. Cultivo y caracterización in vitro de una línea celular de fibroblastos. *Revista Estomatología* 1999; 8:27-32.

16. Young S, et al. Macrophage responsiveness to light therapy. *Lasers Surg Med* 1989;9:497-505.
17. 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;22:347-354.
18. Coombe AR, Darendeliler N. The effects of low level laser irradiation on osteoblastic cells. *Clin Orthod Res* 2001;4:3-14.
19. Fujihara NA, Hiraki KR, Marques MM . Irradiation at 780nm increases proliferation rate of osteoblasts independently of dexamethasone presence. *Lasers Surg Med* 2006;38:332-336.
20. Dominguez A, Castro P, Morales M. An in vitro study of the reaction of human osteoblasts to low-level laser irradiation. *J Oral Laser Applic* 2009;9:21-28.
21. Roodman GD. Advances in bone biology: the Osteoclast. *Endocr Rev* 1996;17:308-332.
22. Roodman GD, Ibbotson KJ, MacDonald BR, Kuehl TJ, Mundy GR. 1,25-dihydroxyvitamin D3 causes formation of multinucleated cells with several osteoclast characteristics in cultures of primate marrow. *Proc Natl Acad Sci USA* 1985;82:8213-8217.
23. Chambers TJ, Revell PA, Fuller K, Athanasou NA. Resorption of bone by isolated rabbit osteoclasts. *J Cell Sci* 1984;66:383-399.
24. Aihara N, Yamaguchi M, Kasai K. Low-energy irradiation stimulates formation of osteoclast-like cells via RANK expression in vitro. *Lasers Med Sci* 2006;21:24-33..
25. Tsay TP, Chen MH, Oyen OJ. Osteoclast Activation and Recruitment After Application of Orthodontic Force. *AJO-DO*. 1999; 115:323-330.
26. Saito S, Shimizu N. Stimulatory effects of low-power laser irradiation on bone regeneration in midpalatal suture during expansion in the rat. *Am J Orthod Dentofac Orthop* 1997;111:525-532.
27. Kawasaki K, Shimizu N. Effects of low-energy laser irradiation on bone remodeling during experimental tooth movement in rats. *Lasers Surg Med* 2000;26:282-291.
28. King GJ, Keeling SD, Wronski TJ. Histomorphometric study of alveolar bone turnover in orthodontic tooth movement. *Bone* 1991;12:401-409.
29. King GJ, Latta L, Rutenberg J, Ossi A, Keeling SD. Alveolar bone turnover and tooth movement in male rats after removal of orthodontic appliances. *Am J Orthod Dentofac Orthop* 1997; 111:266-275.
30. Rody WJ Jr, King GJ, Gu G . Osteoclast recruitment to sites of compression in orthodontic tooth movement. *Am J Orthod Dentofac Orthop* 2001;120:477-489.
31. Lemaire V, Tobin FL, Greller LD, Cho CR, Suva LJ. Modeling the interactions between osteoblast and osteoclast activities in bone remodeling. *J Theor Biol* 2004 229:293-309.
32. Garlet TP, Coelho U, Repeke CE, Silva JS, Cunha Fde Q, Garlet GP. Differential expression of osteoblast and osteoclast chemottractants in compression and tension sides during orthodontic movement. *Cytokine* 2008;42:330-335.
33. Sun X, Zhu X, Xu C, Ye N, Zhu H. Effects of low energy laser on tooth movement and remodeling of alveolar bone in rabbits. *Hua Xi Kou Qiang* 2001;19:290-293.
34. Nishijima Y, Yamaguchi M, Kojima T, Aihara N, Nakajima R, Kasai K. Levels of RANKL and OPG in gingival crevicular fluid during orthodontic tooth movement and effect of compression force on releases from periodontal ligament cells in vitro. *Orthod Craniofacial Res* 2006;9,63-70.
35. Aihara N, Yamaguchi M, Kasai K. Low-energy irradiation stimulates formation of osteoclast-like cells via RANK expression in Vitro. *Lasers Med Sci* 2006;21:24-33.
36. Lim HM. A clinical investigation of the efficacy of low level laser therapy in reducing orthodontic postadjustment pain. *Am J Orthod Dentofacial Orthop* 1995;108:614-622.
37. Harazaky M. Soft laser irradiation effects on pain reduction in orthodontic treatment. *Bull Tokyo Dent Coll* 1997;38:291-295.
38. Meguro D. Laser irradiation inhibition of open gingival embrasure space after orthodontic treatment. *Aust Orthod J* 2002;18:53-63.
39. Cruz DR, Kohara EK, Ribeiro MS, Wetter NU. Effects of low-intensity laser therapy on the orthodontic movement velocity of human teeth: a preliminary study. *Lasers Surg Med* 2004;35:117-120.
40. Ozer T, Basaran G, Berk N Laser etching of enamel for orthodontic bonding. *Am J Orthod Dentofacial Orthop* 2008;134:193-197.
41. Kravitz ND, Kusnoto B. Soft-tissue lasers in orthodontics: an overview *American J.Orthod Dentofacial Orthop* 2008;133: 5110-5114.

Contact address: Angela Camacho Dominguez, Universidad de Valle, Calle 4B No 36-00 Cali, Columbia. Fax: +57-2-321-2100. e-mail: angela.domiguez@gmail.com