Vegf Regulates Osteoblast Differentiation in Tension and Pressure Regions Orthodontic Tooth Movement Administered with Hyperbaric Oxygen Therapy

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Abstract

Problem in the orthodontic sometimes needs a long-term treatment, about 24 months. One of some efforts that might be useful to accelerate the orthodontic treatment is by adding hyperbaric oxygen (HBO), this HBO is known as a treat that for successfully heal wound in tissue and supports the vascularization. During orthodontic treatment, bone remodeling is a vital process. The aim of this study is to investigate VEGF regulates osteoblast differentiation (osteocalcin parameter) in tension and pressure regions during orthodontic tooth movement administered with HBO. Twenty-four male guinea pigs divided into three equal groups, namely (K0) as the control group, (K1) as the orthodontic group, and (K2) as the HBO group. HBO therapy was used at 2.4 ATA for 90 minutes in 7 days, from day 8 to day 14. VEGF and osteocalcin were examined with immunohistochemistry method. The data were evaluated by using Kruskal Wallis and Mann Whitney tests ($P < 0.05$). Correlation was used Pearson Test. There was increasing VEGF and Osteocalcin giving by HBO, except osteocalcin in pressure regions. Correlation test showed strong correlation between VEGF and osteocalcin with coefficient value 0.643. The provision of HBO therapy can increase VEGF dan osteocalcin expression in the tension region, higher than in the pressure region. VEGF expression and osteocalcin were related simultaneously with strong correlation, when VEGF can induces the expression of osteocalcin, a late marker of differentiation.

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Introduction

Physical appearance has become one of the most important social interaction factors. In modern human life, an attractive physical appearance even can trigger one's confidence. For instance, the condition of teeth, especially anterior teeth, is considered to play a role in affecting the attractiveness or aesthetics of someone's face. If the position or state of the anterior teeth is poor or irregular, the facial attractiveness will decrease.[1] The prevalence of malocclusion in Indonesia remains high at around 80% of the total population.[2] Orthodontic treatment, considered as one of treatments performed in the field of dentistry that aims to get aesthetically pleasing dentofacial appearance by removing crowding teeth, correcting the rotational and apical deviations of the teeth, improving incisor relationship, and creating a good occlusion.[3] The principal purpose of orthodontic treatment is to correct malocclusion, to achieve appropriate occlusion and optimum dentofacial function.[4]

Bone remodeling is a vital process in orthodontic tooth movement. Orthodontic movement is a persistent and steady process considered by bone resorption and bone formation on pressure and tension regions after mechanical forces. Orthodontic forces changing the blood flow and localized environment.[5] These force alterations conduct to the generation and propagation of signaling cascades and associated tissue remodeling by defining biochemical and cellular responses and molecular process, such as signal generation and transduction, cytoskeletal re-organization, gene expression, differentiation, proliferation, synthesis and secretion, and apoptosis occurring in mineralized and nonmineralized tissue including the related blood vessels.[6,7] Osteocalcin are important components in the

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bone remodeling especially in formation periodontal ligament and alveolar bone.[8]

Nevertheless, there is a problem in the orthodontic treatment, especially in the long-term one for about 24 months. Thus, it needs to take various efforts to accelerate the orthodontic treatment process. One of the efforts that may be able to accelerate the orthodontic treatment is by giving hyperbaric oxygen (HBO) therapy, already known as a treatment for healing a wound on tissue. Hyperbaric Oxygen Therapy (HBOT) is a method of treatment by inhaling pure oxygen (100%) continuously in the body with air pressure greater than normal atmospheric pressure.[9,10]

HBO therapy increases oxygen dissolved in the blood and produces a high partial pressure of oxygen (PaO). An increase in pO_2 affects oxygen tension for tissue regeneration, which increases collagen and adeno-sine triphosphate (ATP) synthesis, as well as triggers osteoblastic and osteoclastic activity.[11] Associated with oxygen delivery, vascularization plays an important role in the process of bone remodeling. The administration of orthodontic pressure on the periodontal ligament, consequently, will cause changes in blood vessels in both the diameter and number of blood vessels as well as changes in the endothelium as a signal in tissue remodeling.[7]

In other words, HBO therapy can theoretically increase the amount of oxygen dissolved so that it will be more easily accepted tissue. The provision of HBO therapy can also stimulate neovascularization which will increase the number and diameter of blood vessels for tissue vascularization so that blood flow increases. Besides, one of the HBO effects associated with partial oxygen pressure in plasma is modulating the pressure of oxygen to produce nitric oxide (NO). HBO has a mechanism to modulate nitric oxide (NO) in endothelial cells. Neovascularization then has a major function in the healing process.[12] HBO therapy, therefore, can increase fibroblast activity and collagen formation. Collagen serves as the formation of periodontal ligaments and tissue binders in bone growth.[13]

In vitro studies, NO even can induce synthesis of vascular endothelial growth factor (VEGF) expression and angiogenesis.[12] VEGF will stimulate synthesis collagen in the remodeling process as one of the stages in wound healing.[13] VEGF signaling is also implicated in osteoblast differentiation. Expression analysis on primary human osteoblasts demonstrated that VEGF induces the expression of osteocalcin, a late marker of differentiation, while it has no effect on the early marker. Vascular endothelial growth factor D (HUMAN) VEGF-D in turn acts as a downstream effector of VEGF with autocrine activity on osteoblasts.[14] The aim of this study is to investigate VEGF regulates osteoblast differentiation (osteocalcin parameter) in pressure and tension regions during orthodontic tooth movement administered with hyperbaric oxygen therapy

Materials and Methods

This research was a true experimental laboratory study with completely randomized control group posttest only design. Ethical permission of this research was obtained from the Ethics and Scientific Research Committee of Experimental Animal Use in Dentistry Faculty of Universitas Airlangga. This research used twenty-four male guinea pigs (Cavia cobaya) aged three to four months and weighed 300-400 grams. The guinea pigs, fed with a standard pellet diet and tap water ad libitum, were then randomly divided into three equal groups, namely (K0) as the control group, (K1) as the orthodontic group, and (K2) as the HBO group.

Moreover, materials used in this research were 100% pure oxygen in hyperbaric animal chamber, 10% ketamine injection as anesthetic drug, 0.5 ml acepromazine with a dose of 0.1-0.2 ml / kg, 10% buffered formalin, Betadine solution, and cotton. Next, the working procedure of this research was begun with acclimatization of animals for 48 hours. An orthodontic force triggering orthodontic tooth movement on the maxilla by using elastic separator then was administered to groups K1 and K2. Afterwards, the force (reciprocal) was measured with a gauge during the experiment.

Group K2 was treated with the orthodontic force day for 14 days added with daily HBO therapy at 2.4 ATA for 90 minutes in 7 days, from day 8 to day 14. Hyperbaric oxygen therapy was conducted by using a veterinary hyperbaric chamber model. Those guinea pigs then were monitored during the experiment and sacrificed on the fourteen day of the experiment. Next, the maxillary teeth were dissected and placed in 10% buffered formalin. Afterwards, histological

sections were prepared with Immunohisto-chemistry staining (IHC), and then observed by using a microscope. Photos were taken to measure the expression of VEGF and Osteocalcin seen on the microscope with an enlargement 400x. Each preparat section then was observed and calculated in the field of view.

Figure 1. Cavia Cobaya in Animal Chamber.

After that, data obtained were statistically measured by using Statistical Package for the Social Science (SPSS). Then, the statistically significant differences between the HBO group, the orthodontic group, and the control group were determined and evaluated by using one way Anova and LSD tests ($P < 0.05$).

Results

The variables of this research were the results of the observation and examination of VEGF expression performed with immunohistochemistry technique, using specific antibodies against VEGF (monoclonal anti VEGF). VEGF expression was characterized by a brown color on the vascular endothelial cells in the periodontal ligament tissue in the pressure and tension regions of the tooth movement with 400x magnification as shown in Figure 1 below.

Figures C and F above show positive brown VEGF expressions in the HBO group (given 7 days orthodontic mechanical pressure + 2,4 ATA HBO therapy for 30 x 3 minutes once a day for 7 days), higher than in the positive control group given orthodontic mechanical pressure for 14 days (see Figures B and E), especially in the pressure region of the orthodontic tooth movement. While in the negative control group, Figures A and D illustrate a negative reaction, mark with no color reaction. The data of the examination results of this research variable then were presented in Figure 3.

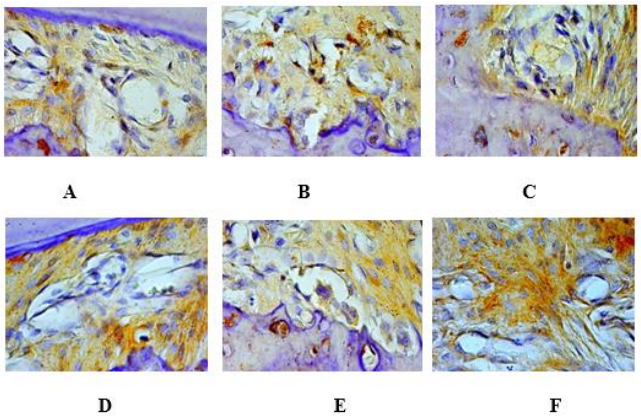

Figure 2. Immunohistochemical Figures of VEGF Expressions Fibroblast Cells of the Periodontal Ligament Tissue of Cavia cobaya. A. K0 Tension Region. B. K1 Tension Region. C. K2 Tension Region. D. K0 Pressure Region. E. K1 Pressure Region. F. K2 Pressure Region with 400X magnification.

Figure 3 depicts the mean (mean) and standard deviation (SD) values of VEGF expression. It indicates that there was increasing VEGF expression in K2 group whether in tension or pressure region. The result from normality test, data in tension and pressure region wasn't have normal distribution so the data were analyzed by using non parametric test, Kruskal Wallis, to know the significant difference of VEGF expression in the pressure and tension regions. The result of Kruskal Wallis test showed in tension dan pressure region there was significant difference with $p = 0.014$ ($p < 0.05$) in pressure region and $p = 0.00$ ($p < 0.05$). Thus, Mann Whitney test was carried out.

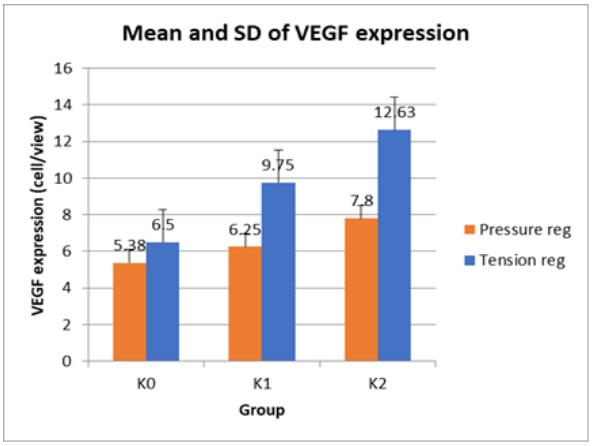

Figure 3. Histogram of the Mean and Standard Deviation Values of VEGF Expression in the Tension and Pressure Regions in Each Research Group.

VEGF in the Pressure Region	K0	K1	K2
K0	-	.161	.007*
K1	.161	-	. 050*
K2	.007*	.050*	-

Note: * There is a significant difference.

Table 1. Results of VEGF Expression of Mann Whitney Test in Pressure Region.

VEGF in the Tension Area	K0	K1	K2
K0	-	.003*	.000*
K1	.003*	-	.010*
K2	.000*	.010*	-

Note: * There is a significant difference.

Table 2. Results of VEGF Expression of Mann Whitney Test in Tension Region.

The Mann Whitney test was performed to evaluate significant differences in the VEGF expression between each group. The results of Mann Whitney indicate a significant difference if the significant value obtained was less than the 0.05 (5%). Every group significant except between K0 and K1 in pressure regions.

Osteocalcin expression was characterized by a brown color on the periodontal ligament tissue in the pressure and tension regions of the tooth movement with 400x magnification as shown in Figure 2 below.

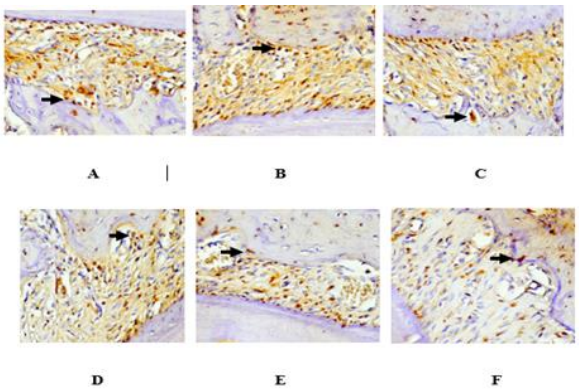

Figure 4. Immunohistochemical Figures of Osteocalcin Expressions of the Periodontal Ligament Tissue of Cavia cobaya. A. K0 Tension Region. B. K1 Tension Region. C. K2 Tension Region. D. K0 Pressure Region. E. K1 Pressure Region. F. K2 Pressure Region with 400X Magnification.

Figures C and F above show positive brown osteocalcin expressions in the K2 group that given 7 days orthodontic mechanical pressure + 2,4 ATA HBO therapy for 30 x 3 minutes once a day for 7 days, compare with K1 especially in the tension region of the orthodontic tooth movement. The data of the examination results of this research variable then were presented in figure 5.

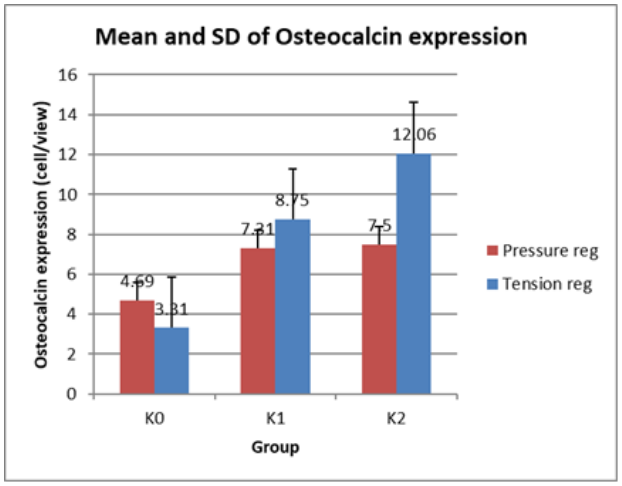

Figure 5. Histogram of the Mean and Standard Deviation Values of Osteocalcin Expression in the Tension and Pressure Regions in Each Research Group.

Figure 5 depicts the mean (mean) and standard deviation (SD) values of osteocalcin expression. It indicates that there was increasing osteocalcin expression in K2 whether osteocalcin expression in tension region higher compare with pressure region. Furthermore, normality test showed the data wasn't distributed normally in pressure and tension region p =0.00 (p > 0,05). Consequently, the data were analyzed by using non parametric test, Kruskal Wallis to know the significant difference of osteocalcin expression in the pressure and tension regions. The result of Kruskal Wallis test showed in tension dan pressure region there was significant difference with p = 0.000 (p < 0.05) in pressure region and p = 0.023 (p < 0.05). Thus, Mann Whitney test was carried out.

Osteocalcin in the Pressure Region	K0	K1	K2
K0	-	.038*	.007*
K1	.038*	-	. 721
K2	.007*	.721	-

Note: * There is a significant difference.

Table 3. Results of Osteocalcin Expression of Mann Whitney Test in Pressure Region.

Osteocalcin in the Tension Area	K0	K1	K2
K0	-	.000*	.000*
K1	.000*	-	.001*
K2	.000*	.001*	-

Note: * There is a significant difference.

Table 4. Results of Osteocalcin Expression of Mann Whitney Test in Tension Region.

The Mann Whitney test was performed to evaluate significant differences in the osteocalcin expression between each group. The results of Mann Whitney indicate a significant difference if the significant value obtained was less than the 0.05 (5%). Every group have significant difference except between group K2 and K1 $p = 0.721$ ($p > 0.05$).

After Mann Whitney Test, Pearson test was used for examine correlation between VEGF expression and Osteocalcin expression, that variables should be linearly. Pearson test has coefficient values 0,643 (strong correlation), it means high degree of correlation indicates that the acceptance of VEGF expression and osteocalcin were related simultaneously.

Discussion

The orthodontic mechanical pressure in this research, the pressure derived from the separator rubber with the strength of 0.48gr / cm^2 was used to move the teeth. The tooth movement can trigger changes in periodontal tissue, alveolar bone, cementum, gingival and dental pulp.[15] The tooth movement is characterized by remodeling changes in the periodontal ligament (PDL) tissues, alveolar bone, pulp, and gingiva. The orthodontic mechanical forces then alter PDL vascularization, resulting in local synthesis of important molecules, such as neurotransmitters, cytokines, growth factors, colony stimulating factor, and arachidonic acid metabolites.[7]

Response triggered by the orthodontic mechanical pressure administered with HBO therapy at 2.4 ATA for 3 x 30 min then was evaluated by molecular changes using VEGF and Osteocalcin parameter with immunohistochemistry method as shown in figure 3 and 5. There is increasing VEGF and osteocalcin expression especially in tension region have higher expression compare with pressure region Next, the data were analyzed with Kruskal Wallis test and the results test showed that there was a significant difference in the orthodontic mechanical process between groups in pressure and tension regions. This indicates that the orthodontic mechanical pressure administered with hyperbaric oxygen therapy at 2,4 ATA 3 x 30 minutes on days 8-14 had certain effects. This result is supporting Sutomo, 2012 study result that HBO have effect on osteoblast cells during orthodontic movement.

Mechanical force from orthodontic appliance activates two different strains in the periodontal ligament: pressure and tension. In pressure region, the force that is generated by the root against the alveolar bone induces bone resorption. In tension regions, periodontal ligament fibers are strained and bone tissue is deposited.[7,16]

The presence of a separator or orthodontic force can produce pressure, resulting in impaired vascularization.[17] The mechanical force can result hypoxia causing cell response by expressions of cell mediators, especially hypoxia inducible factor 1 (HIF-1) consisted of HIF-1α and HIF-1β heterodimers. During hypoxia, HIF-1α binds HIF-1β to elicit an active transcription factor HIF-1 that can increase cell proliferation stimulation, prevent cell death, and activate genes encoding VEGF expressed on very high periodontal ligaments stimulating the occurrence of new blood vessels or angiogenesis.[18-20] Mechanical loading and hypoxia are two permanent stresses that impact severely to the bone. Pressure applied on bone is a potent regulator of matrix genes and chondrocyte physiology through mechano-transduction pathways.[21]

In this research showed that there is increase VEGF, as blood vessel parameter in the periodontal ligaments of pressure and tension regions given hyperbaric oxygen therapy at 2.4 ATA for 3 x 30 minutes with 5 min intervals at normal conditions for 7 from day 8 to 14 day was higher than those not given hyperbaric oxygen therapy. This is because oxygen is one of the essential nutrients needed in the wound healing process since in the proliferation phase of angiogenesis requires more oxygen supply.[22] Particularly in tension area have the higher expression of VEGF so in the 14th days, oxygen and essential nutrient more needed in tension to bone remodeling it was proved that VEGF acts early on bone differentiation by inducing vessel

formation.[14,22,23] Mann Whitney test showed that in pressure area VEGF have no significant difference in K0 and K1 group that means mechanical force wasn't impaired VEGF expression in pressure regions.

This research revealed higher levels of osteocalcin as osteoblast differentiation markers in tension regions compared to K0 and pressure regions. This result parallel with Taddei study, 2012 that in tension regions found osteocalcin more compare with pressure regions. It may be due to the increased promotion of bone deposition in this area by mature osteoblasts.[24] That result was supported with Brito, 2013 study that local application of osteocalcin produced acceleration of orthodontic movement in rats during initial treatment phases.[25] This acceleration was mainly due to an increase of osteoclast recruitment. This had led to suppose that osteocalcin application at initial stages can be sufficient to accelerate orthodontic tooth movement, since it could act as a molecule promoting chemo-attraction for osteoclasts precursor cells, and have role in the remodeling of the matrix as well as favoring a suitable micro-environment for biomineralization.[26] Osteocalcin expression suggested double participation in orthodontic processes: it took part in resorption as well as remodeling processes of the periodontal ligament matrix.[25]

Hyperbaric Oxygen Therapy will modulate Nitric Oxide (NO), which plays an important role in maintaining vascular tone and increasing VEGF. Then VEGF along with fibroblasts will stimulate the synthesis of angiogenesis which is one of the steps in healing the wound. The above mechanism deals with one of the main benefits of HBO for wound healing process. The blood vessels themselves actually play an important role in the administration of oxygen and nutrients and other materials essential for bone synthesis as well as the source of osteoblast cells.[23] Meanwhile, angiogenesis involves the gradual formation of new branches of blood vessels from the existing blood vessels started with the occurrence of vasodilation in response to Nitrogen Oxide (NO) and the increasing of permeability by VEGF.[27]

The beneficial effects of hyperbaric oxygen therapy on bone formation were related to Wnt3a/β-catenin signaling, oxygen availability regulates stem cells via Wnt/β-catenin signaling and has stimulatory effects on cell growth.[29] HBO treatment accelerated the rate of osteoblast differentiation leading to an increase in bone formation.[28] The long-term effects of HBO (14 and 21 day) on the osteogenic differentiation of BMSCs (bone marrow stromal cells) and increased the expression of osteogenic markers such as, osteocalcin.[29] Osteocalcin in this research significantly increase in tension regions in every group, contrast with pressure regions, that there is no osteocalcin increase with HBO administered. Bone resorption occur in pressure regions, where osteoclasts produce protons into the space between the bone surface and cells using vacuolar H^+ATPase, and the acidification results in the dissolution of inorganic mineral. Organic bone matrix degradation is mediated by proteolytic enzymes such as primarily cathepsin K. Because osteocalcin is rather having a tendency to proteolysis *in vitro*, acids and proteases may also attack osteocalcin during bone degradation. Osteocalcin should rather be considered an indicator of bone turnover and not just a marker of bone formation.[30]

VEGF expression and osteocalcin were related simultaneously with strong correlation. VEGF is involved in both angiogenesis and osteogenesis.[31] Orlandini study, 2006 showed involvement of VEGF-D in maturation and regulation of osteoblastic activity via vascular endothelial growth factor receptor 3 (VEGFR-3) is a potent and specific endothelial cell mitogen that regulates blood vessel development. VEGFR-3 is expressed in primary human osteoblasts and VEGF-D stimulates their differentiation measured as osteocalcin induction and mineralized nodule formation. Expression analysis on primary human osteoblasts demonstrated that VEGF-D induces the expression of osteocalcin, a late marker of differentiation, while it has no effect on the early marker such as RUNX2. This is also confirmed by the fact that VEGF-D induced mineralization in these cells.[14]

Conclusion

The provision of HBO therapy at 2.4 ATA for 30 x 3 minutes once a day for 7 days in days 14th can increase VEGF dan osteocalcin expression in the tension region, higher than in the pressure region. Osteocalcin increase in tension regions in every group, contrast with pressure regions, that there is no osteocalcin

increase with HBO administered osteocalcin is rather having a tendency to proteolysis during bone resorpsion. VEGF expression and osteocalcin were related simultaneously with strong correlation, when VEGF can induces the expression of osteocalcin, a late marker of differentiation.

Declaration of Interest

No conflict of interest for this article.

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References

1. Zhang M. The Impact of Malocclusion and its Treatment on Quality of Life. Int J Pediatr Dent 2006;16:381-7.
2. Purwanegara MK, Wulandari NYS, Purbiati M. Effect of Various Motivation Methods on Oral Hygiene Index Scores of Fixed Orthodontic Patients. J Int Dent Med Res 2018;11(2):390-7.
3. Proffit WR, Fields HW. Sarver DM. Contemporary Orthodontic. 4th edition. Mosby Elsevier. Missouri 2007;55.
4. Farmasyanti CA, Dewi INK, Alhasyimi AA. Potency of Bilimbi Fruit (Averrhoa Bilimbi L.) Leaf Extract as Corrosion Inhibitors of Stainless Steel Orthodontic Wires. J Int Dent Med Res 2018;11(2):634-8.
5. D'Apuzzo DF, Cappabianca S, Ciavarella D, Monsurrò A,Silvestrini-Biavati A, Letizia Perillo L. Biomarkers of Periodontal Tissue Remodeling during Orthodontic Tooth Movement in Mice and Men: Overview and Clinical Relevance. Sci World J 2013;319-23.
6. Prameswari N, Brahmanta A, Mulawarmanti D. Bone-immune interaction in osteogenesis Relapse Orthodontic after Nanopowder Stichopus hermanii Application. J Int Dent Med Res 2018;11(1):323-9.
7. Krishnan V, Davidovitch Z. Cellular, Molecular, and Tissue-level reactions to Orthodontic Force. Am J Orthod Dentofac Orthop 2006;129(4):469.
8. Soeroso Y, Bachtiar EW, Boy B, Sulijaya B, Prayitno SW. The Prospect of Chitosan on The Osteogenesis of Periodontal Ligament Stem Cells. J Int Dent Med Res 2012;5:(2):93-7.
9. Duvallet AL, Duvallet E, Lhuissier F, Constantin P, and Beaudry M. Can hyperoxia and hyperbaric conditions induce a increase in systemic pressures (SBP, DBP, MAP) to the breathing of 100 percent oxygen at normal and hyperbaric barometric pressure? Faseb J 2017; 31(1):1-1091.
10. Bhutani S, Vishwanath G. Hyperbaric oxygen and wound healing. Indian J Plast Surg 2012;45(2):316–24.
11. Okubo Y, Bessho K, Fujimura K, Kusumoto K, Ogawa Y, Iizuka T. Effect of Hyperbaric Oxygenation on Bone Induced by Recombinant Human Bone Morphogenetic Protein-2. Br J Oral Maxillofacial Surg 2001;39(2):91–5.
12. Susilo I, Devi A, Purwandhono A, Warsito SH. Effects of hyperbaric oxygen therapy in enhancing expressions of e-NOS, TNF-a, and VEGF in wound healing. J Phys 2017;Conf. Ser. 853012030.
13. Brahmanta A, Soetjipto, Narmada IB. The Expression of Collagen Type- I In the Tension Region of Orthodontic Tooth Movement with Adjuvant Of Hyperbaric Oxygen Therapy. Int J Chem Tech Res 2016;9(7):199-204.
14. Orlandini M, Spreafico A, Bardelli M, Rocchigiani M, Salameh A, Nuccioti S, Capperucci C, Frediani B, Salvatore Oliviero. Vascular Endothelial Growth Factor-D Activates VEGFr-3 Expressed in Osteoblasts Inducing Their Differentiation. J Biol Chem 2006;281(26):17961-7.
15. Sutomo S, Pambudi R, Achmad S. The effect of Hyperbaric Oxygen in Increasing the Amount of Osteoblast Cells on Remodeling Process During Tooth Movement on Male Adult Cavia Cobaya. Orthod Dent J 2012;3(1):22-32.
16. Masella RS, Meister M. Current concepts in the biology of orthodontic tooth movement. Am J Orthod Dentofac Orthop 2006;129(2006):458-68.
17. Niklas A, Proff P, Gosau M, Romer P. The Role of Hypoxia in Orthodontic Tooth Movement. Int J Dent 2013;1-7.
18. Jan A. Effects of Hyperbaric Oxygen on Healing of Bone, Bone Grafts and Bone Graft Substitutes in Calvarial Defects. Dissertation. University of Tampere: Finland 2010:103-10.
19. Kawata T, Kohno S, Kaku M, Fujita T, Ohtani J, Motokawa M, Tanne K. Expression of Vascular Endothelial Growth Factor on Neovascularization during Experimental Tooth Movement by Magnet. Biomed Res 2011;22(2):249-54.
20. Plank MJ, Sleeman BD. Tumour-induced Angiogenesis: A Review. J Theor Med 2003;5(3–4):137–53.
21. Lafont JE. Lack of oxygen in articular cartilage: consequences for chondrocyte biology. Int J Exp Pathol 2010;91(2):99-106.
22. Jennifer F, Sean M, Robert L. The use of hyperbaric oxygen therapy to treat chronic wounds: A review. Wound Repair Regen 2008;16(3):321-30.
23. Domenico MD, D'Apuzzo F, Feola A, Cito L, Monsurro A, Pierrantoni GM. Cytokines and VEGF Induction in Orthodontic Movement in Animal Models. J Biomed Biotechnol 2012;2012:1-4.
24. Taddei SRA, Moura AP, Andrade I Jr, Garlet GP, Garlet TP, Teixeira MM, Da Silva TT. Experimental model of tooth movement in mice: A standardized protocol for studying bone remodeling under compression and tensile strains. J Biomech 2012;45(2012):2729–35.
25. Brito MV, Pérez MAA,Rodríguez FJM. Osteocalcin expression in periodontal ligament when inducing orthodontic forces. Rev Odontológica Mex 2013;17(3):150-3.
26. Hashimoto F, Kobayashi Y, Mataki S, Kobayashi K. Administration of osteocalcin acelerates orhodontic tooth movement induced by close coil spring in rats. Eur J Orthod 2001;23 (5):535-45.
27. Mitchell L. Introduction to Orthodontics. 4th ed. Oxford 2013;2.
28. Al-Hadi. The effect of hyperbaric oxygen therapy on osteoclast and osteoblas function. Thesis.Plymouth University: England 2013;97-104.
29. Lin SS, Ueng SWN, Niu CC, Yuan LJ, Yang CY, Chen WJ, Lee MS, Chen JK. Hyperbaric oxygen promotes osteogenic differentiation of bone marrow stromal cells by regulating Wnt3a/β-catenin signaling- An in vitro and in vivo study. Stem Cell Res 2014;12(1):260-74.
30. Ivaska KK, Hentunen TA, Vääräniemi J, Ylipahkala H, Pettersson K, Väänänen HK. Release of Intact and Fragmented Osteocalcin Molecules from Bone Matrix during Bone Resorption in Vitro. J Biol Chem 2004; 279:18361-9.
31. Tang W, Yang F, Li Y, de Crombrugghe B, Jiao H, Xiao G, Zhang C. Tr anscriptional regulation of Vascular Endothelial Growth Factor (VEGF) by osteoblast-specific transcription factor Osterix (Osx) in osteoblasts. J Biol Chem 2012; 287(3):1671-8.