Celluler Analysis In Orthodontic Tooth Movement Post Robusta Coffee Extract Administration

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Abstract

This study aimed to analyze the number of osteoclasts, osteoblasts, fibroblasts, and the improvement of dental movement post Robusta coffee extract administration.

10 guinea pigs were divided into 2 groups i.e. group C: guinea pigs were applied using orthodontic force, and group T: guinea pigs were applied using orthodontic force and 87.5 mg / 500 g BW of Robusta coffee extract. Orthodontic force in guinea pigs was carried out using a orthodontic bracket and open coil spring with strength of 52.5 grams. Observations were conducted on the day 15 by performing histological processing to calculate of the number of osteoblasts, osteoclasts and fibroblasts. The improvement of tooth movement was measured by an X-Ray µ-CT device.

Robusta coffee extract increased the number of osteoclasts, osteoblasts, and fibroblasts. The number of osteoclasts in the compression area was greater than that in the tension area ($p < 0.05$). The number of osteoblasts and fibroblasts in the tension area was greater than that in the compression area ($p < 0.05$). The Robusta coffee extract also improved the movement of the teeth ($p < 0.05$).

Robusta coffee extract increased the number of osteoclasts, osteoblasts, fibroblasts and improved orthodontic tooth movement.

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Introduction

The theory of tooth movement states that in a few seconds post force application, a tooth shifts its position in the space of periodontal ligament resulting in compression in several places and tension in the other sides of periodontal ligament[1]. In the tooth movement, orthodontic force will alter blood flow in the periodontal ligament and local hypoxia occurs. The O2 tension reduction stabilizes HIF-1(hypoxia inducible factor-1), a transcription factor that activates vascular RANKL (Receptor activator of Nuclear Factor-igB Ligand) and expression of VEGF (Vascular endothelial growth factor) in osteoblasts and fibroblasts; osteoclast differentiation increases, causing resorption in the compression area.[2,3,4] Hypoxia and fluid flow in the same time will promote bone remodeling and periodontal ligaments. The compressive force causes the compression area and the tension area in the periodontal ligament and is closely related to nerve and blood vessel tips. When the nerve tips are distorted, they release vasoactiveneurotransmitters e.g. CGRP (calcitonin gene-related peptide) and SP (substance P), which interact with vascular endothelial cells causing vasodilatation and increases permeability with plasma leakage.[5.6] Activated endothelium binds and recruits circulatin leukocytes macrophages, and monocytes to the periodontal ligament, indicating acute inflammation.[7.8] Leukocytes release growth factors, prostaglandins, cytokines and colony-stimulating factors that promote remodeling of tissue.[9,10] After a few days, inflammation changes from acute to chronic and the process of proliferation involves osteoblasts, fibrosblasts,

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osteoclasts, and endothelial cells.[1]

Osteoclasts are bone degrading cells.[11] Active osteoclasts are filled with mitochondria in the cytoplasm to provide the necessary energy for bone resorption. Active osteoclasts will result in bone resorption.[12,13] Bone resorption will subsequently lead to tooth movement of orthodontic followed by remodeling of periodontal ligament and alveolar bone.[6] Differentiation of osteoclasts is regulated by cytokines i.e. RANKL and M-CSF (Macrophage colony-stimulating factor), which are important roles responsible for the survival and proliferation of precursor cells of osteoclast. It also causes expression of RANK in precursor cells of osteoclast generate an efficient response to signal path of the RANKL-RANK.[11,13]

Osteoblasts are cells that bone form and have several important roles in remodeling of bone: expression of osteoclastogenic factors, production of bone matrix proteins, and mineralization of bone.[14] New bone is formed in the tension area as a result of orthodontic force during orthodontic treatment.[15] Osteoblasts are "non-core" and special bone-forming cells. Osteoblasts can synthesize type I collagen matrix and express Runx2 (Runt-related transcription factor-2)/Cbfa1(Core Binding Factor a-1), a master switch for differentiation of osteoblast from mesenchymal progenitor cells.[16] Osteoblasts that function in bone formation are needed for remodeling in the resorption sites of compression area, and form new bone in the compression area and tension area.[17]

Fibroblasts are important cells that may carry out the function of collagen formation as well as resorption function or collagen destruction. In the tension area, during ortgodontic tooth movement, the periodontal ligament will extend and fibroblast proliferation occurs. The fibroblasts synthesize extracellular matrix proteins including glycosaminoglycan, fibronectin, and collagen which are protein structures of periodontal connective tissue. Subsequently, it occurs bone remodeling and re-formation of periodontal cords.[18] In the orthodontic tooth movement, collagen cords in the ligament of periodontal are required to continue undergoing remodeling in order to adapt to the changes of tooth position.[19]

The success of orthodontic treatment is influenced by many factors, including oral hygiene, periodontal health, and orthodontic strength.[20] A number of studies on the effects of materials and medicines on orthodontic tooth movement have been carried out for instance medicines demonstrating the effects that inhibit orthodontic tooth movement e.g. osteoprotegerin and bisphosphonate (Pamidronat)[21.22,] and those which may accelerate orthodontic tooth movement e.g. caffeine.[23,24] Application of injection or topical PGE2 (Prostaglandin E2) may also improve tooth movement.[25,26,27]

Coffee is widely consumed as beverage which has a high antioxidant content.[28] Coffee contains caffeine which may increase osteoclast formation through increased RANKL.[23,29] Some compounds in coffee have antioxidant effects i.e. chlorogenic acid, ferulic, caffeic and n coumaric.[30] In roasted coffee, melanoidins or brown pigments will be formed which consider as strong antioxidants.[31] Caffeic and chlorogenic acids which have antioxidant effects, may reduce oxidative stress in osteoblasts.[32] The studies showed chlorogenic acid promotes osteogenesis in hAMSCs (human adipose-derived mesenchymal stem cells).[33] Caffeic acid accelerates wound healing by stimulating collagen synthesis by fibroblasts.[34]

This study aimed to analyze the Robusta coffee extract effects on the number of osteoblasts, osteoclasts and fibroblasts, as well as the improvement of tooth movement using animal experiment i.e. guinea pigs.

Materials and methods

This experimental laboratories research used 10 guinea pigs with following criteria: male, healthy, aged 10-12 months, and weighing about 500 grams. The guinea pigs were divided randomly into 2 groups: control group (C): the guinea pigs were applied using orthodontic force and treatment group (T): the guinea pigs were applied using orthodontic force and Robusta coffee extract of 87.5 mg/500 g of BW (equivalent with that contained in 1 cup of coffee). The orthodontic force applied to guinea pigs was performed by means of anesthetizing guinea pigs using ketamine, afterward each mandible incisor of the animals was installed using a band matrix and orthodontic bracket (Ortho-technology, USA) and moved to the distal using an open coil spring (Ortho-technology, America) with strength of 52.5 grams. (Figure 1).

Figure 1. Installation of an orthodontic appliance in the guinea pigs. A band matrix and orthodontic bracket (yellow arrow) were installed on the mandible incisors, and moved distally using an open coil spring (red arrow)

Observations were made by means of sacrificing guinea pigs on the day 15, and taking their right and left mandible incisors and their periodontal tissues. The tissue was subsequently processed histologically and HE staining was performed. Calculation of the number of osteoclasts, osteoblasts and fibroblasts was conducted using a light microscope (Olympus, Tokyo, Japan). The measurement of improved tooth movement in the animals was performed by measuring from the mesial of right mandible incisor passing the top of the alveolar bone between the two teeth to the mesial left mandible incisor using Micro-Computed Tomography (μ-CT) Bruker SkyScan 1173 High X-Ray device. Energy Micro-CT at FMIPA ITB Micro-CT Laboratory. The initial processing was repositioning of objects in 3D room, and object analysis in the form of length measurements was performed using Data Viewer software (Bruker Micro-CT, Belgium).

The data was analized using independent t test and paired t test with 95% level of significance (α = 0.05). This research has been approved by the Ethical Research Committee of the Medical Faculty, Jember University, Number: 1150/H25.1.11/KE/2017.

Results

The effect of Robusta coffee extract on the number of osteoclasts, osteoblasts, fibroblasts and the improvement of right and left mandible incisor movement were shown in the Tables 1,2,3 and 4 and the Figures 2,3,4, and 5.

The mean and standard deviation of the number of osteoclasts in the control and the treatment groups in the compression and tension areas are shown in Table 1. The data shows that Robusta coffee extract administration increased the number of osteoclasts in the compression area (p <0.05) and the tension area (p> 0.05), Robusta coffee extract increased the number of osteoclasts in the compression area greater than that in the tension area (<0, 05). The histology of osteoclast in the compression and the tension areas in the Control (C) and Treatment (T) groups shown in Figure 2.

Groups	N	∑ Osteoclasts (Mean ± Standard Deviation)		p
		Compression	Tension	
Control	5	2,140 ± 0,114	0,220 ± 0,130	0,000**
Treatment	5	3,940 ± 0,114	0,240 ± 0.114	0,000**
p		0,000*	0,803*	

Table 1. The mean and standard deviation of the number of osteoclasts and the results of different tests between the compression areas and the tension areas in the control group and treatment group

Notes : p < 0,05 = significant
p > 0,05 = non significant
* Based on t test
** Based on paired t test

Groups	N	∑ Osteoblasts (Mean ± Standard Deviation)		p
		Compression	Tension	
Control	5	12,320 ± 0,277	12,420 ± 0,311	0,606**
Treatment	5	13,320 ± 0,277	15,380 ± 0.349	0,000**
p		0,000*	0,000*	

Table 2. The mean and standard deviation of osteoblasts and the results of different tests between the compression areas and the tension areas in the control group and treatment group.

Notes : p < 0,05 = significant
p > 0,05 = non significant
* Based on t test
** Based on paired t test

Groups	N	∑ Fibroblasts (Mean ± Standard Deviation)		p
		Compression	Tension	
Control	5	20,060 ± 0,207	24,000 ± 0,158	0,000**
Treatment	5	22,900 ± 0,339	28,580 ± 0.192	0,000**
p		0,000*	0,000*	

Table 3. The mean and standard deviation of fibroblasts, and different test results between the compression area and the tension area in the control group and treatment group

Notes : p < 0,05 = significant
p > 0,05 = non significant
* Based on t test
** Based on paired t test

Groups	n	∑ Tooth movement (mm)
Control	5	2,967 ± 0,005
Treatment	5	4,450 ± 0,002
p		0,000*

Table 4. The mean and standard deviation of the distance between the mesial of the right mandible incisor and the mesial of the left mandible incisor which shows the right and left mandible incisor movement, and the different test results between the control group and the treatment group.

Notes : p < 0,05 = significant
* Based on t test

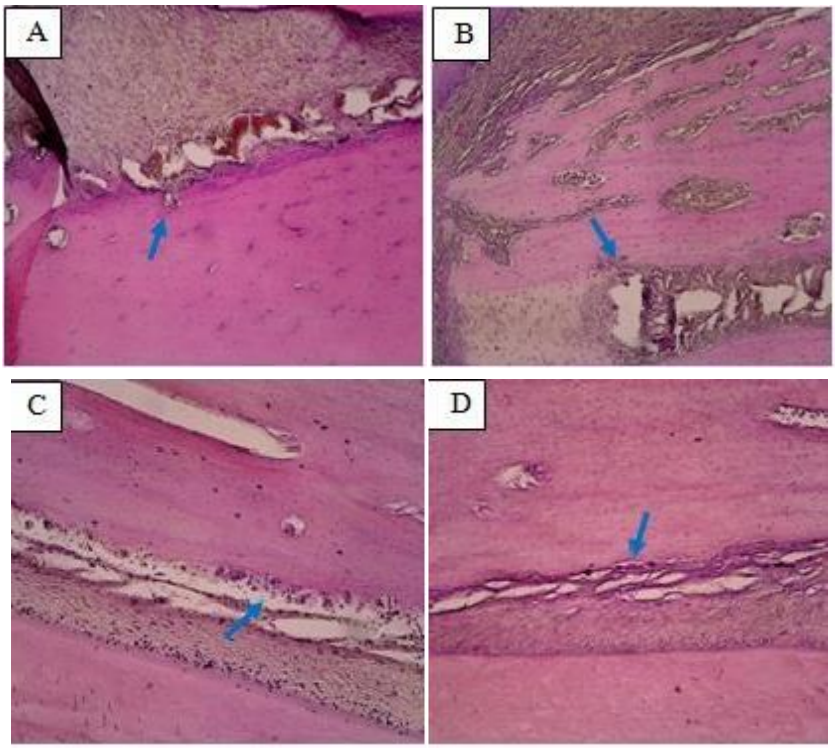

Figure 2. Osteoclasts in in periodontal ligament are marked with blue arrows: in compression area of control group (A), tension area of control group (B), compression area of treatment group (C) and tension area of reatment group (D) (HE, 100x magnification).

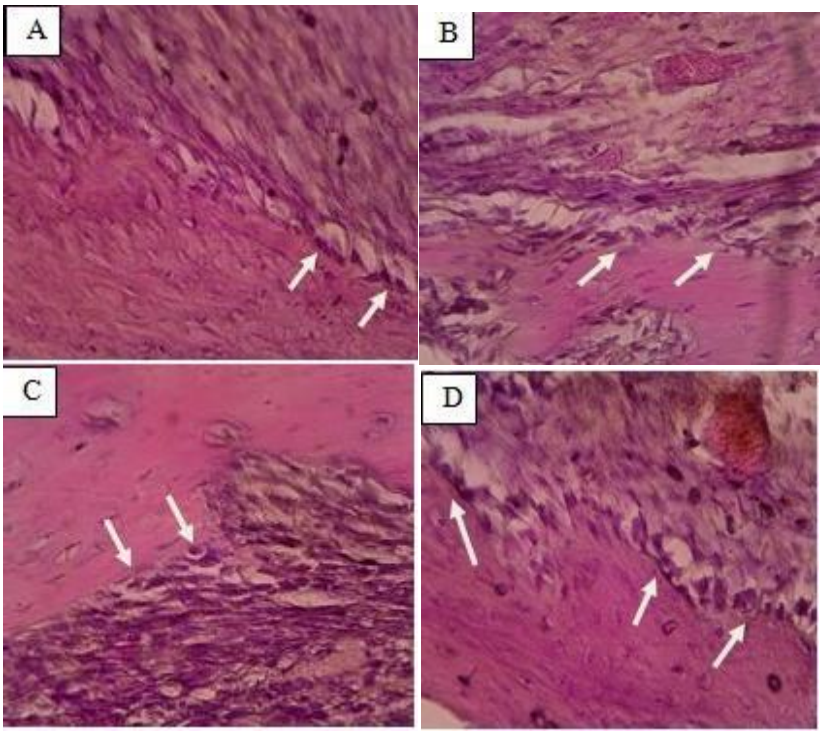

Figure 3. Osteoblasts in periodontal ligament are marked with white arrows: in compression area of control group (A), tension area of control group (B), compression area of treatment group (C) and tension area of treatment group (D) (HE, 400x magnification).

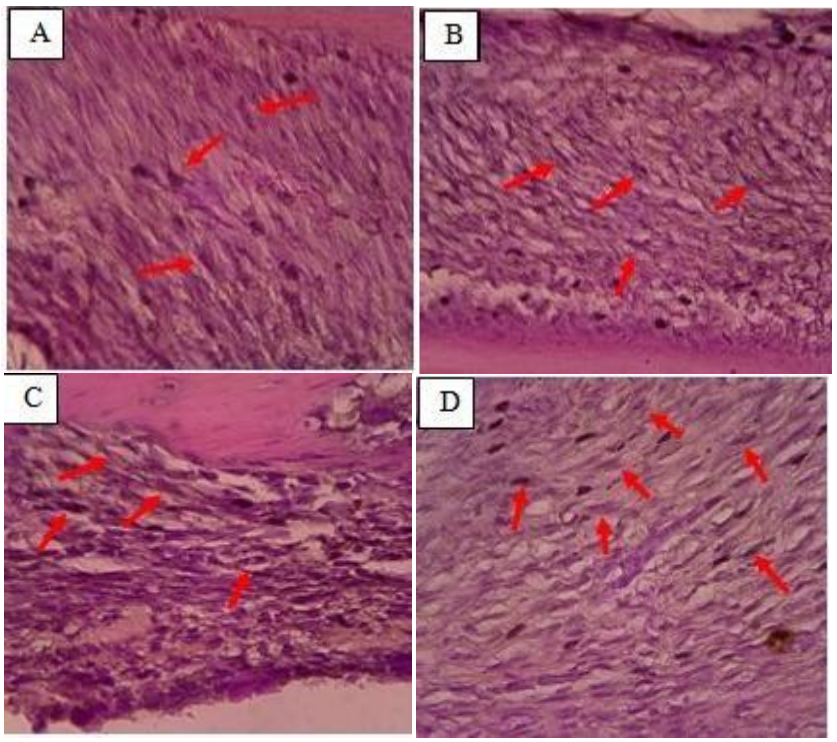

Figure 4. Fibroblasts in in periodontal ligament are marked with red arrows: in compression area of control group (A), tension area of control group (B), compression area of treatment group (C) and tension area of treatment group (D) (HE, 400x magnification).

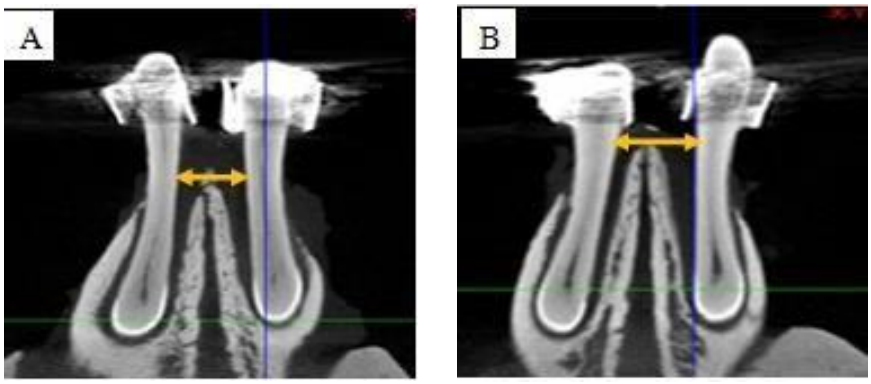

Figure 5. Improved right and left mandible incisor movement to distal which is the distance between the mesial of the right mandible incisor and the mesial left mandible incisor using X-Ray Micro-Computed Tomography (µ-CT) in the control group (A) and the treatment group (B) indicated by yellow arrow.

The mean and standard deviation of the number of osteoblasts in the control and the treatment groups in the compression and the tension areas are shown in Table 2. The data show that Robusta coffee extract administration increased the number of osteoblasts in the compression area ($p < 0.05$) and the tension area ($p < 0.05$). Robusta coffee extract increased the number of osteoblasts in the tension area greater than that in the compression area (p <0 , 05).The histology of osteoblasts in the compression and the tension areas in the Control (C) and Treatment (T) groups shown in Figure 3.

The mean and standard deviation of the number of fibroblasts in the control and the treatment groups in the compression and the tension areas are shown in Table 3. The data show that Robusta coffee extract increased the number of fibroblasts in the compression area ($p < 0.05$) and the tension area ($p < 0.05$). Robusta coffee extract administration increased the number of fibroblast in the tension area greater than that in the compression area (p <0,05). The histology of fibroblast in the compression and the tension areas in the Control (C) and Treatment (T) groups shown in Figure 4.

The improvement of right and left mandible incisor movement using X-Ray Micro-Computed Tomography (µ-CT) was obtained by measuring the distance from the mesial of the right mandible incisor passing through the alveolar bone to the distal of the left mandible incisor in the Control (C) and Treatment (T) groups. (Figure 5). The measurement results were a distance between the mesial of the right mandible incisor and the mesial of the left mandible incisor are shown in Table 4.

Table 4 shows the mean and standard deviation of the distance between the mesial of the right mandible incisors and the mesial of the left mandible incisors indicating the movement of the right and left mandible incisors to the distal in the control group and treatment group. The test results showed that administration of the Robusta coffee extract improved the distance between the mesial of the right mandible incisors and the mesial of the left mandible incisors compared to the control group ($p < 0.05$)

Discussion

The ability of the tooth to move through the bone depends on the periodontal ligament attached to the tooth to the adjacent bone. The periodontal ligament is a structure of solid fibrous connective tissue consisting of cells, collagen cords, nerve and the components of vascular, and tissue fluid. Cells contained in the periodontal ligament include: 1) synthetic cells i.e. fibroblasts ranged 50-60%, osteoblasts, cementoblasts, 2) resorptive cells i.e. osteoclasts, fibroblasts and cementoclasts, 3) progenitor cells including undifferentiated mesenchymal cells, 4) defense cells i.e. macrophages, lymphocytes and mast cells, and 5) epithelial cells which are remnants of the epithelial root sheath of Hertwig.[1]

Robusta coffee extract increases the number of osteoclasts in the compression and the tension areas compared to the control, while in the compression area the number of osteoclasts is more than that in the tension area. This is because coffee contains caffeine which may increase osteoclast formation through an increase in RANKL.[23] RANKL which is a

regulator of bone remodeling during tooth movement,[35] subsequently binds to RANK on precursors of osteoclast, which promotes osteoclast differentiation and proliferation allowing osteoclasts become active and result in increased resorption of bone.[12,13] Bone resorption will shortly cause orthodontic tooth movement, followed by remodeling of alveolar bone and periodontal ligament.[6] Increased osteoclasto-genesis in the compression area of periodontal ligament that causes an increase in tooth movement is also evident in this study which showed an increase in the right and left mandible incisor movement towards distal.

Differentiation of osteoclasts is regulated by the transcription factor NFATc1 (Nuclear Factor of Activated T-cells 1)[11]. NFATc1 is the main regulator that induces osteoclast differentiation and plays an important role in fusion and activation of osteoclast through increased regulation of various genes responsible for osteoclast attachment, migration, acidification, degradation of inorganic and bone organic matrix [36] The research showed that Robusta coffee extract increased NFATc1 in rats induced by orthodontic forces.[37]

Osteoblasts function to form bone, necessary to remodeling the resorption area in the compression area and forming new bone in the compression area and tension area.[17] Differentiation of osteoblast in alveolar bone involves an increase in expression of Runx2/ Cbfa1.[38] Runx2 induces differentiation of multipotent mesenchymal cells into immature osteoblasts.[39]

Robusta coffee extract increases the number of osteoblasts in compression and tension areas due to the content of chlorogenic, caffeic ferulic, and n coumaric acids which has antioxidant properties.[33] ROS may increase peroxidation of lipid which is the main cause of damage to cell membranes, the structure and function of osteoblast cells. The results studies have also shown that oxidative stress reduces the rate of bone formation by decreasing the osteoblasts differentiation and life.[40] Chlorogenic acid and caffeic acid convert free radicals into stable products[41], may reduce oxidative stress in osteoblasts,[33] thus increasing differentiation and the life of osteoblasts, as well as important for stimulating osteoblast activity.[42] The results showed that Robusta coffee extract increased the Runx2 in rats induced with orthodontic force.[37]

Application of orthodontic force may emerge hyalinization, cause necrosis of periodontal ligament cells, and bone resorption, resulting in damage to the periodontal cord structure [43]. Fibroblast cells experience swelling in the endoplasmic reticulum, vacuole formation, fibroblast cells rupture and cytoplasmic loss.[6]

Robusta coffee extract increased the number of fibroblasts on the compression and tension area compared to those not administered with Robusta coffee extract. It occurs because the content of coffee is chorogenic acid and caffeic acid may increase the number of fibroblasts [44]. Other studies have also shown that topical application of chorogenic acid may increase TGF β1 expression on days 6 to 15 [45] , which functions as a factor that stimulates fibroblasts and osteoblasts in the periodontal ligament and proliferates to improve protein matrix, in addition TGFβ1 also helps mediate macrophages to clear tissue debris, spread angiogenesis, help recrystallize and increase collagen deposition in the proliferation phase [46]. Topical application of caffeic acid found in coffee may increase collagen and fibroblast cell proliferation on days 10 and 15.[47]

The number of osteoblasts and fibroblasts in the tension area is greater than that in the compression area. It occurs because the large areas in tension area required more osteoblasts and fibroblasts for the formation and repairing of bone tissue and periodontal ligaments.

Conclusions

The administration of Robusta coffee extract increases the number of osteoblasts, osteoclasts and fibroblasts. The Robusta coffee extract improves the movement of mandible incisors to distal direction.

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References

1. Li Y, Jacox LA, Little SH, Ko CC . Orthodontic tooth movement: The biology and clinical implications. The Kaohsiung Journal of Medical Sciences Type: Review, Journal Article 2018; 34(4):207-214.
2. Dandajena TC, Ihnat MA, Disch B, Thorpe J, Currier G.F. Hypoxia triggers a HIF-mediated differentiation of peripheral blood mononuclear cells into osteoclasts. Orthod Craniofac Res 2012;15: 1-9.
3. Park HJ, Baek KH, Lee HL, Kwon A, Hwang H, Qadir AS., *et al.* Hypoxia inducible factor-1alpha directly induces the expression of receptor activator of nuclear factor-kappaB ligand in periodontal ligament fibroblasts. Mol Cells 2011;31: 573-578.
4. Huang H, Williams RC, Kyrkanides S. Accelerated orthodontic tooth movement: molecular mechanisms . Am J Orthod Dentofac Orthop, 2014; 146: 620-632.
5. Wise GE, King GJ. Mechanisms of tooth eruption and orthodontic tooth movement. J Dent Re. 2008; 87: 414-434.
6. Krishnan V and Davidovitch Z. 2006. Cellular, molecular, and tissue-level reactions to orthodontic force. American Journal of Orthodontics and Dentofacial Orthopedics, 129(4):469-e1-32.
7. Krishnan V and Davidovitch Z. On a path to unfolding the biological mechanisms of orthodontic tooth movement. J Dent Res. 2009; 88: 597-608
8. Middleton J, Patterson AM, Gardner L, Schmutz C, Ashton BA. Leukocyte extravasation: chemokine transport and presentation by the endothelium. Blood 2002);100: 3853-3860.
9. Ren Y, Vissink A. Cytokines in crevicular fluid and orthodontic tooth movement. Eur J Oral Sci, 2008; 116:89-97.
10. Yamaguchi M, Kojima T, Kanekawa M, Aihara N, Nogimura A, Kasa K. Neuropeptides stimulate production of interleukin-1 beta, interleukin-6, and tumor necrosis factor-alpha in human dental pulp cells. Inflamm Res. 2004;53:199-204.
11. Kim JH and Kim N. Regulation of NFATc1 in Osteoclast Differentiation. J Bone Metab 2014;21(4):233-241.
12. Meikle CM, 2006. The tissue, cellular, and molecular regulation of orthodontic tooth movement: 100 years after Carl Sandstedt,Europian Journal of Orthodontics 28, pp. 221-40.
13. Yamaguchi M, RANKL/OPG Duri**ng** Orthod Craniofal Res , vol.12, pp 113-119.
14. Karsenty G. Transcriptional Control of Skeletogenesis. Annu. Rev. Genomics Hum. Genet 2008; 9: 183-196.
15. Sprogar S, Vaupotic T, Cör A, Drevenšek M, and Drevenšek G. The endothelin system mediates bone modeling in the late stage of orthodontic tooth movement in rats. Bone 2008;43(4):740-747**.**
16. Ducy P, Schinke T, Karsenty G. The Osteoblast: A Sophisticated Fibroblast under Central Surveillance. Science 2000; 289 (5484): 1501-1504
17. Kawakami M and Yamamoto TT. Local injection of 1,25-dihydroxyvitamin D3 enhanced bone formation for tooth stabilization after experimental tooth movement in rats . J Bone Miner Metab 2004;2:541-546
18. Meng Y, Han X, Huang L, Bai D, Yu H, He Y. 2010. Orthodontic mechanical tension effects on the myofibroblast expression of alpha-smooth muscle actin. The Angle Orthodontist 2010; 80(5): 912-918
19. Apajalahti S, Peltola JS. Apical root resorption after orthodontic treatment. A. Restrospective Study. Eur J of Orthod, 2007; 29: 12-408
20. Cardaropoli D and Gaveglio L. “The influence of orthodontic movement on periodontal tissues level,” Seminars in Orthodontics 2007; 13(4): 234–245
21. Matthew D.Dunn MD, Park CH, Kostenuik PJ, Kapila S, and Giannobile WV. Local delivery of osteoprotegerin inhibits mechanically mediated bone modeling in orthodontic tooth movement. Bone 2007;41(3):446-455.
22. Venkataramana V, Rajasigamani K, Nirmal Madhavan, S.N.Reddy, Karthik, and Kurunji Kumaran N. Inhibitory effect of bisphosphonate [pamidronate] on orthodontic tooth movement in newzealand albino rabbits. Journal of International Dental and Medical Research 2012;5(3): 136-142.
23. Jianru Y, Boxi Y, and Meile L “et al”. Caffeine may enhance orthodontic tooth movement through increasing osteoclastogenesis induced by periodontal ligament cells under compression. Archives of Oral Biology 2016;64:51-60
24. Herniyati, Narmada IB, and Soetjipto. The Role of Rankl and Opg in Alveolar Bone Remodeling and Improvement of Orthodontic Tooth Movement Post Coffee Brew Administration. Journal of International Dental and Medical Research 2017;10(1):84-88.
25. Kale S, Kocadereli I, Atilla P, and Aşan E. Comparison of the effects of 1,25 dihydroxycholecalciferol and prostaglandin E_2 on orthodontic tooth movement. American Journal of Orthodontics and Dentofacial Orthopedics 2004;125(5):607-614.
26. Seifi M, Eslami B, and Saffar AS. The effect of prostaglandinE 2 and calcium gluconate on orthodontic tooth Movement and root resorption in rats. Eur J Orthod 2003;25:199 - 204
27. Widayati R, Suniarti DF, Poerwaningsih EH, and Iskandriati D. Root Resorption and RANKL Concentration in Orthodontic Tooth Movement Accompanied by Topical PGE2 Gel Application. Journal of International Dental and Medical Research 2016;9(3):228-232.