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Research Report

The role of active ingredients nanopowder *Stichopus hermanii* gel to bone resorption in tension area of orthodontic tooth movement

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ABSTRACT

Background: *Orthodontic tooth movement is a continual and balanced process between bone deposition and bone resorption in pressure and tension sites. Stichopus hermanii is one of the best fishery commodities in Indonesia. It is natural and contains various active ingredients such as hyaluronic acid, chondroitin sulphate, cell growth factor, eicosa pentaenoic acid (EPA) docosa hexaenoic acid (DHA) and flavonoid that potentially play a role in orthodontic tooth movement.* ***Purpose:*** *The aim of this study was to investigate the active ingredients of nanopowder Stichopus hermanii promoting bone resorption in tension area orthodontic tooth movement.* ***Methods:*** *A quantitative test for active ingredients of stichopus hermanii was conducted. Thirty two male Cavia cobaya were divisibled became four groups. K (–) groups as a negative control group (without treatment), K (+) groups as a positive control group which were provided with a separator rubber for orthodontic tooth movement, and P1, P2 groups, which were treated with 3% and 3.5% stichopus hermanii for orthodontic tooth movement. After treatment the cavia cobaya were sacrificed. TRAP-6 expression as a osteoclast marker was examined by means of an immunohistochemistry method.* ***Results:*** A *one-way Anova test confirmed that TRAP-6 expression was significantly increased with* $p = 0.00$ *(*$p \leq 0{,}05$*) in P2 compared to K (+). P2 to K (–), P2 to P1 and P1 to K (+) had no significant differences* ***Conclusion:*** *Nanopowder Stichopus hermanii 3.5% has an active ingredient that could increase osteoclast activity to resorb periodontal ligament and alveolar bone in tension areas of orthodontic tooth movement.*

Keywords: *Nanopowder; Stichopus hermanii; resorption; TRAP-6; orthodontic tooth movement*

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INTRODUCTION

According to Riskesdas data of 2013, malocclusion is the third most common oral disease.[1] Orthodontic treatment is generally associated with malocclusion that causes esthetic problems.[2] Orthodontic tooth movement involving the use of orthodontic appliances is characterized by remodeling changes of the alveolar bone and a reaction of the periodontal ligament (PDL) to mechanical stimuli. Tooth movement occurs in the direction of force when there is a multifaceted bone remodelling response, with bone resorption on the compression side and bone apposition on the tension side of the periodontal ligament.[3]

Orthodontic treatment for malocclusion correction requires a period of 1–2 years. Mechanically induced periodontal ligament and bone remodelling is still not fully understood. The role of the periodontal ligament has been questioned since tooth movement occurs. As long as an orthodontic appliance is applied, orthodontic movement occurs which can effect sequential reactions as respons of periodontal tissue and alveolar bone in remodeling and releasing of numerous mediators and substances from the periodontal and alveolar tissues and surrounding structures.[4,5] After orthodontic mechanical pressure with both physical and biological characteristics, orthodontic tooth movement can occur either slowly or rapidly depending on its biological response.[3]

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An orthodontic appliance can be defined as a mechanical stimulus resulting in biological celular response. Under orthodontic mechanical force, periodontal ligament responds with subsequent bone resorption in the area of pressure application and, conversely, under tension force will result in bone formation. An early response in the pressure area is one of periodontal ligament inflammation. When inflammation occurs, many cells are produced, including: cytokine, T-cell, B-cell, and matrix metalloproteinases (MMPs).[6] Tissue reactions is also initiated immediately after force application in pressure and tension periodontal ligament. The extravasation and chemoattraction of numerous inflammatory cells begins, and followed by complex process of recruitment of osteoclast and osteoblast progenitors.[3]

Orthodontic appliances with varying degrees of frequency, magnitude, and duration of mechanical loading, result in extensive macroscopic and microscopic changes to the bone adjacent to periodontal tissues. Mechanical loading also alters periodontal tissue vascularity, metabolic process and blood flow resulting in the local synthesis and release of various molecules such as arachidonic acid, cytokines, growth factor, and colony-stimulating factors until tooth movement occurs.[7] The released molecules as cellular responses in the various cell types in and around teeth, such as fibroblasts, osteoblasts, cementoblasts, and vascular cells as a stressor response of mechanical forces, provide a favourable microenvironment for tissue apposition or resorption in orthodontic tooth movement.[3]

When orthodontic forces are applied to the tooth, the resulting pressure will induce fibroblast cells, osteoclasts and osteoblas in periodontal ligament as a response to mechanical pressure. Orthodontic tooth movement is mediated by coupling mechanism between resorption and apposition process in the pressure and tension area of periodontal ligament and alveolar bone.[3] Periodontal ligaments increase widening and induce bone remodeling so that orthodontic tooth movement occurs.[8] Extracellular changes and crevicular gingival fluid as a biomarker orthodontic response occured.[7] Collagen became the most important tissue in periodontal ligament remodeling. The ligament itself undergoes remodeling and the role of MMPs with their natural inhibitors, tissue inhibitors of metalloproteinases (TIMPs), are clearly of importance.[6] During orthodontic tooth movement, periodontal ligament remodeling followed with bone remodeling. Several enzymes such as alkaline phosphatase to induce osteoblast cell, and growth factors such as fibroblast growth factor-2 (FGF-2) to increase fibroblast proliferation, and bone morphogenetic protein-2 (BMP-2) as a mature osteoblast marker were present. Osteoblast plays a direct role in bone formation, especially in bone matrix formation, involving non-collagenous protein and growth factors.[9] Acceleration in bone remodeling will increase orthodontic tooth movement.[10]

Bone resorption is a crucial process during which orthodontic tooth movement by resorbing alveolar bone occurs as orthodontic forces respond. In this process, osteoclast is the most important cell involved in cell mediation. Osteoclasts are found in physiologic periodontal ligaments as mature condition cells. Osteoclasts seems appear within days when orthodontic mechanical force is applied to produce tooth movement.[3] Osteoclasts differentiate from stem cells pathways in haemopoietic lineage and the early precursors of osteoclasts are granulocyte-macrophage colony-forming units. Resorption process cascade involves several steps directed toward removing organic and anorganic structures of alveolar bone matrix by osteoclast so that orthodontic tooth movement occurs. After that, the unmineralized bone surface is replaced by apposition process with lining osteoblasts. Several enzymes such as MMPs, collagenases and gelatinases is produced by osteoblasts which have role in accessing mineralized bone. Local and systemic factors are important for osteoclast activation and induce the production of hydrogen ions, proteolytic enzymes in vacuole under the ruffled border.[3] Tartrate-resistant acid phosphatase (TRAP-6) positive multinuclear cells are an enzyme which is localized within the ruffled border area as a osteoclast mature biomarker.[11]

Orthodontic tooth movement produces both a pressure area and a tension area. In the pressure area, periodontal ligament shows disorganization and diminution of ligament fiber production and vascular contriction. In the tension area, stimulation produced by the stretching of periodontal ligament fiber bundles results in an increase in fiber production.[3,8] Osteoclasts appear in the pressure area and osteoblasts in the tension area. Osteoclasts in the tension area remain poorly understood.[11]

The utilization of marine biota for dental treatment has been developed. *Stichopus hermanii* is well recognized as a human food source. *Stichopus hermanii* contain active ingredients such as proteins 86% (80% collagens), glucosaminoglycans including hyaluronic acid, chondroitin sulphate, cell growth factor, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that are important for tissue regeneration.[12,13]

Previous research showed that 3% *Stichopus hermanii* can decrease relapse biometric and increase FGF-2 and collagen type 1 expression in relapse orthodontic compared with 2.5%. *Stichopus hermanii* can act as an anticandidal.[10,14] Another research showed that *Stichopus hermanii* modulates the inflammatory responses, stimulates the activation and proliferation of fibroblasts and enhances the rapid production of collagen fiber networks with shorter healing times. The level of proinflammatory cytokines; IL-1α, IL-1β, and IL-6, are significantly reduced in *Stichopus hermanii*-treated wounds and stimulation tissue regeneration.[15] The role of *Stichopus hermanii* in bone resorption in tension area orthodontic tooth movement has yet not been fully investigated. The aim of this study was to investigate the active ingredients of nanopowder *Stichopus hermanii* in bone resorption in tension area orthodontic tooth movement of Cavia cobaya.

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MATERIALS AND METHODS

The study was conducted at experimental laboratories with completely randomized control group post-test only design. Ethical permission was obtained from the Ethics and Scientific Research Committee of Experimental Animal Use at the Faculty of Dentistry, Universitas Hang Tuah no 125/KEPK/I/2016. Thirty-two male guinea pigs (*Cavia cobaya*) aged 2.5 months and weighing 200–300 grams, fed a standard pellet diet and tap water *ad libitum*, were randomly divided into four groups each consisting of eight guinea pigs. Based on previous research, the optimum *Stichopus hermanii* concentration used were 3%, while attempts to add concentrations of 3.5% *Stichopus hermanii* were made.[10] A 10% ketamine injection was administered as an anesthetic, with 0.1–0.2 ml/kg for acepromazine 0.5 ml, 10% buffered formalin and cotton.[8]

In this research, *Stichopus hermanii* were taken from Raas Island Sumenep, East Java Indonesia and was cleaned by longitudinal incision using a scalpel without damaging the internal organs. The *Stichopus hermanii* were dried in ovens (U type, Memmert, Wisconsin, USA) at 28 degrees centigrade, blended (model HGBTWT, Waring commercial, USA) and reduced to nanopowder (20 nm) by means of a High Energy Milling (Puspitek, Tangerang, Indonesia) method. Quantitative analysis of *Stichopus hermanii* active ingredients involved UV Vis Transmittance (T872) spectrophotometry (Intertek PTL, Pittsfield, USA) to examine flavonoid, gas chromatography (Agillent GC-MS 5975C, Palo Alto, CA, USA) to examine EPA and DHA and a reversed phase high performance liquid chromatography (HPLC J.T Baker, United States) with UV detection to examine chondroitin sulphate.[10]

To prepare nanopowder, 3% *Stichopus hermanii* gel was made from 0.3 gr *Stichopus hermanii* powder diluted with natrium carboxy methylcellulose (NaCMC) 2% in 10 ml of dimethyl sulfoxide (DMSO) 5%. Nanopowder 3.5% *Stichopus hermanii* gel was made from 0.35 g *Stichopus hermanii* powder diluted with NaCMC 2% in 10 ml of DMSO 5%.[10]

The procedure within this study began with the acclimatization of 32 guinea pigs for 48 hours. The guinea pigs were divisibled became four groups of eight subjects: K(–) group as a negative control group (without treatment), K(+) group as a positive control group whose orthodontic tooth movement was triggered by means of an elastic separator at a force of 0.0474 kN, measured with a gauge name autograph (AGS-X Series, Shimadzu, Kyoto, Japan) during experiment and P1, P2 groups which were given with both orthodontic pressure and *Stichopus hermanii* 3% and 3.5% over 14 days. 0.025 ml of *Stichopus hermanii* gel was applied to the gingival sulcus tension area once per day with an insulin syringe.[10]

The research was conducted at the Biochemistry Laboratory Medical Faculty of Universitas Airlangga. The guinea pigs were monitored during the experiment all of, with all of the groups being sacrificed on the fourteenth day of the experiment. The maxillary incisive teeth were dissected and placed in 10% buffered formaline.[10] Histological sections were subsequently prepared with TRAP-6 immunohistochemistry as an osteoclast marker and then observed by using a light microscope (Nikon optiphot 2, Japan).

The expression of TRAP-6 as osteoclast marker in the periodontal ligament on 1/3 apical in the tension area was observed. Photographs using an optilab advance (Miconos, Yogyakarta, Indonesia) were taken to measure the osteoclasts (TRAP-6) expression seen through a microscope at 400X enlargement. Each histological section was observed and calculated.[10]

Finally, the data was statistically measured using a statistical package for the social science (SPSS) version 20. The resulting research data was tabulated, the statistical hypothesis being conducted with a standard analytic significance of 95% ($p = 0.05$) by one-way Anova test (analysis of variants) to analyze the difference of each variable compared with the control. The data was tested with LSD test ($p < 0.05$).

RESULTS

The data obtained from the quantitative analysis of *Stichopus hermanii* showed that the percentage of flavonoid was higher than the other active ingredients examined (Table 1). The data resulting from orthodontic tooth movement measurement showed that there were differences in orthodontic tooth movement width within each group. In the K(+) group, the mean was 0.45 mm, while the mean of the P1 group was 0.496 mm, and the mean of P2 group was 0.498 mm (Table 2 and Figure 1).

Moreover, the data also showed that the TRAP-6 expression as a osteoclast marker increased in P1 and P2 groups. The highest number found in the P2 group treated

Table 1. Active ingredients of nanopowder *Stichopus hermanii*

No.	Active ingredients	Result	Method
1	Flavonoid (%)	5.3218 (%)	Spectrophotometry (Saura-Calixto,1998; Larrauri et al, 1997)
2	Decosahexaenoic Acid (DHA) (mg/100g)	No Detection (< 1.20)	Gas Chromatography
3	Eicosapentaenoic (EPA) (mg/100g)	17.10 mg/100 g	Gas Chromatography
4	Condroitin sulphate (mg/100g)	706.15 mg/100 g	18-5-56/MU/SMM-SIG, HPLC

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Table 2. Descriptive mean and standard deviation of orthodontic tooth movement maxillary left central incisive (mm)

Group	Mean	Standard Deviation
K(-)	0	0
K(+)	0.45	0.022
P1	0.496	0.008
P2	0.498	0.013

Table 3. Descriptive mean and standard deviation of TRAP-6 expression in tension area (cell/field of view)

Group	TRAP-6 Expression	
	Mean	Standard Deviation
K(-)	9.67	2.73
K(+)	8.5	1.22
P1	11.5	1.87
P2	13.17	2.13

Table 4. One-way Anova test of TRAP-6 expression in tension area

Variable		One-way Anova test	
		F	Sig
TRAP-6 expression	Between group	5.933	0.005
	Within group		
	Total		

Table 5. LSD test of TRAP-6 expression in tension area

Variable			LSD Test
			Sig
TRAP-6 expression	K(-)	K(+)	0.763
		P1	0.434
		P2	0.038
	K(+)	P1	0.087
		P2	0.004
	P1	P2	0.514

with nanopowder *Stichopus hermanii* 3.5% was 13.17 cell/field of view. Meanwhile, in the negative control group, the mean was 9.67, and in K(+) group the mean was 8.5 (Table 3 and Figure 3). TRAP-6 expression is showed in Figure 2. The statistical results with one-way Anova test confirmed that there were significant differences between all groups (pÝ0.05).

The statistical results of one-way Anova and LSD showed that there was a significant difference of TRAP-6 expression as a osteoclast marker in the tension area between K(-), K(+) groups and the P1 and P2 groups. TRAP-6 expression was significantly increased in P2 compare to K(+), P2 to K(-), P2 to P1, but P1 to K(+) had no significantly differences as seen in Table 4.

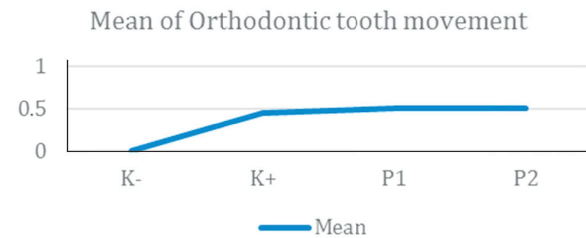

Figure 1. Line chart mean of orthodontic tooth movement.

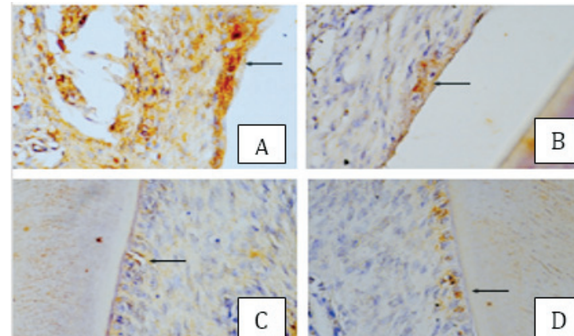

Figure 2. Imunohistological section of TRAP-6 expression in the control group (A), in the orthodontic group (B), in the orthodontic + *Stichopus* 3% group (C),), in the orthodontic + *Stichopus* 3.5% group (D) under a light microscope at 400× magnification.

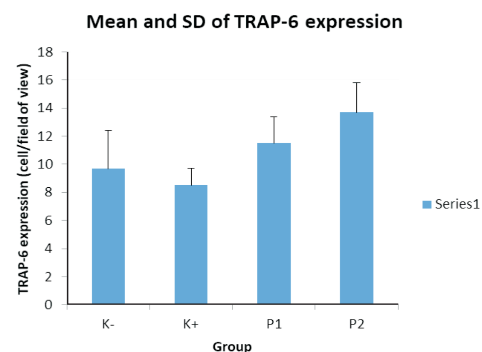

Figure 3. Mean and SD of TRAP-6 in tension area orthodontic tooth movement.

DISCUSSION

The results showed that the P2 group that was administered the orthodontic force separator rubber and *Stichopus hermanii* 3.5% had the widest orthodontic tooth movement (OTM) among the groups. Group P2 also had the highest TRAP-6 expression in the tension site compared to the K(–), K(+) groups.

Orthodontic tooth movement is a useful model for understanding the mechanism of bone remodeling induced by mechanical loading. Osteoclasts play an important role in OTM where osteoclast have a bone resorption function.[11] Osteoclastogenesis mechanisms at a physiologic level have

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a function through the macrophage-colony stimulating factor (M-CSF) that induces osteoclast when there are no growth factor involved.[3]

In the tension area, TRAP-6 is found as positive multinuclear cells are localized within the ruffled border area as a osteoclast mature biomarker which has a relation with matrix remodeling.[16] Osteoclasts which formed by chondrocytes or osteoblasts resorb the bone mineralized matrix. Osteoclasts are multinucleated giant cells dervied from hematopoietic cells. Osteoclast differentiation is dependent on two cytokines, a tumor necrosis factor (TNF) family cytokine, receptor activator of nuclear factor-κB (NF-κB) ligand (RANKL) and M-CSF. M-CSF can stimulate monocyte proliferation. The cytokine receptor activator of nuclear factor-κB ligand (RANKL), which is secreted by mesenchymal stem cell and osteoblasts, stimulates monocyte differentiation into osteoclasts.[16,17] RANKL produced by osteoblasts or their precursors plays a role in osteoclast formation, thereby relation between bone formation to resorption.[17] The interaction of RANKL with its receptor RANK results in a cascade of intracellular events including: NF-κB, mitogen-activated protein kinases (MAPKs), ionic calcium and calcium/calmodulin-dependent kinase by recruiting the adaptor signal protein TNF receptor associated factor (TRAF6). As a result, a number of osteoclast-related marker genes, including: TRAP-6, calcitonin receptor (Ctr), cathepsin K (Ctsk) and nuclear factor of activated T cells (Nfatc1) are upregulated.[16]

Research with a mouse strain without RANKL, which can be conditionally deleted and made a series of Cre-deleter strains to showed that RANKL that controls mineralized bone resorption and bone remodeling produced by hypertrophic chondrocytes and osteocytes, where both embedded in bone matrix. Besides osteoblast, osteocyte RANKL have role for the bone loss associated with unloading.[17] In the research reported here, the highest expression of TRAP-6 occurred in the P2 group that experienced the highest osteoclast activity resulting in matrix remodeling in the group given nanopowder *Stichopus hermanii* 3.5%. The P1 group administered with *Stichopus hermanii* 3% demonstrated no significant relationship with K(-) meaning that osteoclast for matrix remodeling in P1 group approached to normal physiologic condition. Osteoclastogenesis has a crucial role in bone homeostasis. Bone is preserved by active remodeling through the equilibrium between bone resorption by osteoclasts and bone apposition by osteoblasts.[18]

Previous research by Blummer into TRAP deficient mice confirmed that the formation of distinct bone relevant proteins and type I collagen were initiated at an earlier point in time. Osteopontin, another bone specific marker in TRAP deficient mice, directly modulates bone formation in a response to mechanical stress which is independent of its effect on osteoclasts. Runt related transcription factor 2 (Runx2) expression occurred at the same point in TRAP deficient mice and proved crucial for osteoblast differentiation.[19,20] This indicated that TRAP-6 has a role in bone and matrix formation. Bone apposition can occur after TRAP-6 function in bone matrix remodeling.[4]

Nanopowder *Stichopus hermanii* contains various active ingredients such as flavonoid, EPA, DHA, triterpene, and glycosaminoglycans.[13] EPA as a component of: nanopowder Stichopus hermanii is known to have a function in osteoclast differentiation. Osteoclast differentiation takes places through several steps, including: progenitor growth, differentiation to mononuclear pre-osteoclasts, cell fusion to multinuclear osteoclasts and the activation of osteoclasts to unique ability to resorb bone and EPA accelerated osteoclast fusion. In other ways, DHA can prevent osteoclastogenesis is also related to cell-cell fusion, as shown by mononuclear TRAP-positive osteoclasts.[18,21]

Osteoclastogenesis is also regulated by AP-1 as a transcription factor. AP-1 is a cell biosensor that can change extracellular signaling for cell function. When there is no AP-1 expressed in osteoblast, this can induce osteoclastogenesis through TRAP-6.[22] Flavonoid is one active ingredient of *Stichopus hermanii* that can inhibit AP-1 and induce osteoclastogenesis.[23–25]

The application of nanopowder *Stichopus hermanii* in the tension area could induce M-CSF due to the effect of triterpene. Triterpene can induce caspase-3 and caspase-9.[26] Activated caspases play an important role in the degradation of specific nuclear proteins and induce osteoblastic differentiation.[27] Accelerated bone formation in the tension area will induce bone resorption indirectly into the pressure area, thereby increasing biometric orthodontic tooth movement.[28]

Chondroitin sulphate is one glycosaminoglycan which have effect on another functionally with cytokines, kemokins, and growth factors in the alveolar bone element and structures where osteoblasts and osteoclasts cooperate to coordinate the process of bone remodeling. Glycosaminoglycan may help control osteoclastogenesis in microenvironments where osteoblasts/osteoclasts inherent. Glycosaminoglicans-bound RANKL block the interaction between RANKL and RANK.[29] Glycosaminoglycan has affinity for RANKL and significantly prevents RANKL-induced osteoclastogenesis by activating ERK pathway. Local interaction between bone cells is crucial factor for control bone remodeling and formation. The overall effect of glycosaminoglycan on osteoblasts is stimulatory, together with the ability of this glycosaminoglycan to prevent osteoclastogenesis. Glycosaminoglycan shifts the homeostasis of bone remodeling tends towards bone formation by preferencing osteoblastogenesis while antagonizing osteoclastogenesis.[30] The stimulatory effect of bone formation in the tension area can induce bone resorption in the pressure area as a response to physiological mechanical stress.[11]

In conclusion, active ingredients of nanopowder *Stichopus hermanii* gel play a role in bone resorption in tension areas of orthodontic tooth movement. Nanopowder *Stichopus hermanii* 3.5% represented the optimum

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concentration containing the active ingredient flavonoid, EPA that can increase TRAP-6 expression as osteoclast activity to resorb periodontal ligament and alveolar bone in tension area orthodontic tooth movement.

REFERENCES

1. Kementerian Kesehatan Republik Indonesia. Riset kesehatan dasar (RISKESDAS) 2013. Jakarta: Badan Penelitian dan Pengembangan Kesehatan Kementerian Kesehatan RI; 2013. p. 143–5.
2. de Almeida AB, Leite ICG, Melgaço CA, Marques LS. Dissatisfaction with dentofacial appearance and the normative need for orthodontic treatment: determinant factors. Dental Press J Orthod. 2014; 19(3): 120–6.
3. Krishnan V, Davidovitch Z. Cellular, molecular, and tissue-level reactions to orthodontic force. Am J Orthod Dentofac Orthop. 2006; 129(4): 469.e1–32.
4. Ariffin SHZ, Yamamoto Z, Abidin IZZ, Wahab RMA, Ariffin ZZ. Cellular and molecular changes in orthodontic tooth movement. Sci World J. 2011; 11: 1788–803.
5. Proffit WR, Fields HW, Sarver DM. Contemporary orthodontics. 4th ed. St Louis-Missouri: Mosby Elsevier; 2007. p. 75–84.
6. Susilowati S. Peran matriks metaloproteinase-8 pada cairan krevikuler gingiva selama pergerakan gigi ortodontik. Dentofasial. 2010; 9(1): 47–54.
7. Alhadlaq AM. Biomarkers of orthodontic tooth movement in gingival crevicular fluid: a systematic review. J Contemp Dent Pract. 2015; 16(7): 578–87.
8. Prameswari N. The response of periodontal ligament collagen fibres and the thickness of inserting periodontal ligament fibre bundles at cementum pressure sites of fixed orthodontic appliances. Dent J (Maj Ked Gigi). 2007; 40(2): 70–5.
9. Mohamed AM. An overview of bone cells and their regulating factors of differentiation. Malays J Med Sci. 2008; 15(1): 4–12.
10. Prameswari N, Soetjipto S, Rahayu RP. Osteogenesis at tension site by Stichopus hermanii application as relapse orthodontic prevention. Int J ChemTech Res. 2016; 9(6): 686–93.
11. Kitaura H, Kimura K, Ishida M, Sugisawa H, Kohara H, Yoshimatsu M, Takano-Yamamoto T. Effect of cytokines on osteoclast formation and bone resorption during mechanical force loading of the periodontal membrane. Sci World J. 2014; 2014: 1–7.
12. Bordbar S, Anwar F, Saari N. High-value components and bioactives from sea cucumbers for functional foods--a review. Mar Drugs. 2011; 9: 1761–805.
13. Sendih S, Gunawan. Keajaiban teripang: penyembuh mujarab dari laut. Jakarta: Agro Media Pustaka; 2006. p. 13–49.
14. Revianti S, Soetjipto S, Rahayu RP, Parisihni K. Protective role of Sticophus hermanii ethanol extract supplementation to oxidative stress and oral hyperkeratosis in smoking exposed rats. Int J ChemTech Res. 2016; 9(5): 408–17.
15. Zohdi RM, Zakaria ZAB, Yusof N, Mustapha NM, Abdullah MNH. Sea cucumber (Stichopus hermanii) based hydrogel to treat burn wounds in rats. J Biomed Mater Res Part B Appl Biomater. 2011; 98(1): 30–7.
16. Li J, Zeng L, Xie J, Yue Z, Deng H, Ma X, Zheng C, Wu X, Luo J, Liu M. Inhibition of osteoclastogenesis and bone resorption in vitro and in vivo by a prenylflavonoid xanthohumol from hops. Sci Rep. 2015; 5: 1-14.
17. Xiong J, Onal M, Jilka RL, Weinstein RS, Manolagas SC, O'Brien CA. Matrix-embedded cells control osteoclast formation. Nat Med. 2012; 17(10): 1235–41.
18. Akiyama M, Nakahama K, Morita I. Impact of docosahexaenoic acid on gene expression during osteoclastogenesis in vitro--a comprehensive analysis. Nutrients. 2013; 5: 3151–62.
19. Blumer MJF, Hausott B, Schwarzer C, Hayman AR, Stempel J, Fritsch H. Role of tartrate-resistant acid phosphatase (TRAP) in long bone development. Mech Dev. 2012; 129: 162–76.
20. Rodriguez-Carballo E, Gámez B, Ventura F. p38 MAPK signaling in osteoblast differentiation. Front cell Dev Biol. 2016; 4: 1–20.
21. Rahman MM, Bhattacharya A, Fernandes G. Docosahexaenoic acid is more potent inhibitor of osteoclast differentiation in RAW 264.7 cells than eicosapentaenoic acid. J Cell Physiol. 2008; 214: 201–9.
22. Braun T, Zwerina J. Positive regulators of osteoclastogenesis and bone resorption in rheumatoid arthritis. Arthritis Res Ther. 2011; 13: 1–11.
23. Shu S. Immunological effects of complementary and alternative medicine in allergy and astma. Disertation. California: University of California; 2008. p. 1–20.
24. Chi L, Gao W, Shu X, Lu X. A natural flavonoid glucoside, icariin, regulates Th17 and alleviates rheumatoid arthritis in a murine model. Mediators Inflamm. 2014; 2014: 1-10.
25. Osta B, Lavocat F, Eljaafari A, Miossec P. Effects of interleukin-17A on osteogenic differentiation of isolated human mesenchymal stem cells. Front Immunol. 2014; 5: 1–8.
26. Reyes-Zurita FJ, Pachón-Peña G, Lizárraga D, Rufino-Palomares EE, Cascante M, Lupiáñez JA. The natural triterpene maslinic acid induces apoptosis in HT29 colon cancer cells by a JNK-p53-dependent mechanism. BMC Cancer. 2011; 11: 1–13.
27. Bell RAV, Megeney LA. Evolution of caspase-mediated cell death and differentiation: twins separated at birth. Cell Death Differ. 2017; 24: 1359–68.
28. Nimeri G, Kau CH, Kheir NSA, Corona R. Acceleration of tooth movement during orthodontic treatment - a frontier in Orthodontics. Progress in Orthodontics. 2013; 14: 42.
29. Savage JR, Pulsipher A, Rao NV., Kennedy TP, Prestwich GD, Ryan ME, Lee WY. A modified glycosaminoglycan, GM-0111, inhibits molecular signaling involved in periodontitis. PLoS One. 2016; 11(6): 1–20.
30. Ling L, Murali S, Stein GS, van Wijnen AJ, Cool SM. Glycosaminoglycans modulate RANKL-induced osteoclastogenesis. J Cell Biochem. 2010; 109(6): 1222–31.

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