Effect of carbonated hydroxyapatite synthesis from cuttlefish shells on orthodontic relapse prevention: in silico study

Bondan Setyoko[*], Aufa Lufhf Ambar Verisandri[*], Adelia Tantri Damayanti[*], Firda Ayu Fitriana[*], Berliant Salsabila Julieta[**], Paramita Noviasari[**], Ananto Ali Alhasyimi[***]

[*]Faculty of Dentistry, Universitas Gadjah Mada, Indonesia,
[**]Faculty of Math and Science, Universitas Gadjah Mada, Indonesia,
[***]Department of Orthodontics, Faculty of Dentistry, Universitas Gadjah Mada, Indonesia

Corresponding author: anantoali@ugm.ac.id

Received 24 january 2023; 1st revision 31 May 2023; 2nd revision 31 June 2023; Accepted 11 July 2023; Published online 31 July 2023

Keywords:

Relapse; orthodontic; cuttlefish shell; molecular docking

ABSTRACT

Background: *Relapse occurs frequently, 70–90% of the time, and typically compromises the outcome of orthodontic therapy. Calcium carbonate ($CaCO_3$), which is found in cuttlefish shells, can be used to make a better biomaterial. One example is carbonated hydroxyapatite, which is very similar to human bone tissue and can stop osteoclast activity on the pressure side of the retention phase. This is a factor in orthodontic relapse, which is when the bone doesn't remodel properly. In this study, a test was done to see if carbonated hydroxyapatite (CHA) could be used as an alternative material to stop orthodontic relapse. The test was based on how the RANK-RANKL, OPG, and TGF-β proteins interacted with each other.*

Method: *CHA extracted from cuttlefish shells after 6 hours of calcination at 1000°C. RANK-RANKL, OPG, and TGF-β interactions were investigated in silico using molecular docking.*

Result: *A cuttlefish shell extract containing CHA has the potential to be used as an alternate material to prevent orthodontic recurrence. CHA chemicals can disrupt the link between RANK and RANKL and enhance OPG and TGF-β expression. This induces enhanced proliferation, which increases the number of osteoblasts and osteoblast differentiation while decreasing the rate of osteoclast activity.*

Conclusion: *Cuttlefish shell with CHA extract has the potential to be used as an alternative material to prevent orthodontic relapse.*

doi: http://dx.doi.org/10.30659/odj.10.1.19-27
2460-4119 / 2354-5992 ©2022 National Research and Innovation Agency
This is an open access article under the CC BY-SA license (https://creativecommons.org/licenses/by-sa/4.0/)
Odonto : Dental Journal accredited as Sinta 2 Journal (https://sinta.kemdikbud.go.id/journals/profile/3200)
How to Cite: Setyoko *et al*. Effect of carbonated hydroxyapatite synthesis from cuttlefish shells on orthodontic relapse prevention: in silico study. Odonto: Dental Journal, v.10, n.1, p. 19-27, July 2023.

INTRODUCTION

Dental and oral health is one of the societal requirements for living a healthy life and achieving well-being. The general public's awareness of the need to enhance dental health has grown in recent years. The current trend is no longer about cavities, but rather about dental aesthetics or beauty, as many individuals have mentioned. However, after aesthetic dental treatment, problems such as improper occlusion (malocclusion) and functional abnormalities that might become impediments to physical health frequently occur. Orthodontic treatment, which attempts to enhance both the dentofacial appearance and the function of the bite, can be used to address malocclusion.[1] Orthodontic relapse is the most prevalent issue orthodontists face during orthodontic therapy.[2] In circumstances of orthodontic treatment, relapse refers to a return to the original form of malocclusion, which is a loss of the achieved correction. Imperfect alveolar bone remodeling is typically the cause of relapse. Alveolar bone remodeling is a type of bone compensation against pressure and tension caused by orthodontic tooth movement, resulting in the formation of new sockets that alter the position of the teeth in accordance with orthodontic treatment. Orthodontic relapse can be prevented by injection of carbonated hydroxyapatite (CHA).[3,4] However, the market price of carbonated hydroxyapatite in Indonesia is 1.5 million Indonesian rupiahs per 5 milligrams, and its availability is still dependent on imported supplies.

CHA has been developed as a dental material that is biocompatible with human tissues and has a chemical composition comparable to that of bone.[5] CHA is the main inorganic component in bones and teeth[6]. CHA materials have a composition remarkably close to that of human bone tissue, which can suppress osteoclast activity in the pressure side of the retention phase and contribute to imperfect bone remodelling in orthodontic relapse.[3] The cuttlefish (*Sepia sp*) fishing sector in Indonesia has increased. According to the Ministry of Maritime Affairs and Fisheries (2018), cuttlefish exports amounted to 25,92 tons in 2016 and 33,16 tons in 2017. Every year, there is a growth in the number of exports. This has an effect on the growth of the agro-industry for processing fishing goods, although only the meat and head of cuttlefish are utilized in processing, while the shells and offal are regarded by-products.[7] It is common knowledge that waste cuttlefish shells contain inorganic components comprising between 75 and 90 percent, the majority of which is calcium carbonate.[8] Calcium carbonate, which is found in cuttlefish shells, can be used to make better orthopedic materials. One such material is CHA.[9]

Molecular modeling refers to the process of utilizing computational chemistry and graphical visualization tools to present three-dimensional images of chemical systems in order to investigate the structure and characteristics of molecules. These approaches allow for the display of molecular models. Computational methods are utilized in the processes of molecular docking, which involves the observation of the three-dimensional geometry of compounds that are bound to the active site of a protein; predicting the affinity and activity of a molecule, and predicting the binding of a molecule to the protein that serves as its target, and predicting the affinity and activity of a molecule.[10] As a result, it is essential to conduct research in order to ascertain whether or not carbonated hydroxyapatite has the potential to serve as an alternate material for the purpose of preventing orthodontic relapse, based on the interaction of receptor activator of NF-kappa β (RANK) and RANK ligand (RANKL), osteoprotegerin (OPG),

transforming growth factor beta (TGF-β) proteins using the molecular docking method.

RESEARCH METHOD

This study employed the in silico method to investigate the influence of CHA formation in cuttlefish shells on osteoclasts and osteoblasts during the process of alveolar bone remodeling. Then, for macroscopic observations, literature studies are utilized. Mortar, analytical balance, measuring cup, beaker glass, stative, waterbath, dropper pipette, porcelain cup, micropipette, microtip, magnetic stirrer, oven, vacuum oven, calcination tool, furnace tool, hot plate, water bath, 10 ml scale pipette, burette, FTIR were used in the experiment. Asus laptop has 7th generation Core i3 processor, 8GB Nvidia gforce RAM, 1TB HDD, and 240GB SSD. Discovery Studio 2021 Client App, Hex 8.0.0, the RCSB PDB Website, and MN-AM were utilized for in-silico research. It also utilized cuttlefish shells, a solution of 96% ethanol, a solution of 85% phosphoric acid, sodium citrate, distilled water, and calcium hydroxide. RANK-RANKL (4GIG), OPG (3URF), TGF-β (5E8S) proteins.

a. Study in silico

Receptor target preparations in humans were obtained from the Protein Data Bank (PDB) https://www.rcsb.org with codes for analyzing the effect on osteoclasts and osteoblasts which are RANK-RANKL (4GIG), OPG (3URF), TGF-β (5E8S), which is then separated by water and unused cargo. The ligand structure was created using the MN-AM website. Furthermore, the ligand and receptor protein were docked using the Hex 8.0.0 Cuda application. Then it was visualized using Discovery Studio 2021 Client.

b. Taxonomic identification of cuttlefish shells

The method used in collecting sample data was in two stages, which were sampling and observing the obtained samples. Sampling location for cuttlefish was from PPI Tambak Lorok Semarang.

c. Production of Cuttlefish Shell CHA

The reaction was carried out using the co-precipitation method. In the first stage, the cuttlefish shells were washed using distilled water to remove the sticky dirt and then boiled at 100°C for 5 hours. Afterwards, put it in the oven with a temperature of 70°C to dry, then reduce the size using a mortar. In the second stage, the calcination process of cuttlefish shells was carried out at 1000°C for 6 hours. The results of functional group analysis determine the selected calcium oxide extract. The third stage of CHA synthesis was obtained by reacting 0.5 M $Ca(OH)_2$ suspension (calcium precursor) plus distilled water (according to the stoichiometric amount with CaO). The solution of 0.3 M H_3PO_4 (phosphate precursor) was dripped into $Ca(OH)_2$ suspension with Ca/P molar concentration of 1.67. In the fourth stage, the final solution was stirred for 24 hours and the temperature was maintained at 90°C, then settled for another 24 hours. The precipitated CHA was washed centrifugally, then dried at 80°C for 24 hours. It was then sintered at 400°C for 2 hours.

c. Test of CHA Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is used to describe the CHA functional groups. The results were viewed by observing the infrared spectra on the CHA hydrogel samples. In general, the spectra of phosphate group (PO_4^{3-}) have absorption bands *v1* (wavenumber molecular vibration) and *v2* in the wavenumbers area of 960-1100 cm^{-1} and 550-610 cm^{-1}, while absorption bands of *v1* and *v2* of carbonate group (CO_3^{2-})

appeared to be around wavenumbers of 865-875 cm^{-1} and 1400-1460 cm^{-1}.

RESULTS

A. Identification of Cuttlefish

Cuttlefish have an oval-shaped wide mantle. Wide fins almost as long as the mantle. The end of the tentacular (sucker) is quite long. Protective membrane does not meet at its base, 8 suckers in transverse rows with 5 or 6 medians (3rd and 4th series) are moderately large. They have left IV hectoctoyl arm: 12 quadriceal rows is normal, next 10 bars with ventral suckers (2 rows) are normal but dorsal of 2 rows small and are separated from ventral row by on a fleshy back with transversely grooved ridge. Colour in fresh condition, there is a clear transverse stripe pattern on the dorsal side of the mantle and head (especially in males); a narrow, faint dotted line along the base of the fin (Figure 1).

= 

Figure 1. Identification of Cuttlefish

B. Test of FTIR CHA

Observing the infrared spectra of a carbonated hydroxyapatite sample from cuttlefish shells reveals that the phosphate group (PO_4^{3-}) exhibits an absorption band in the precise range, 602.62 cm^{-1}, and in the exact wavenumber region with a value of 1034.86 cm^{-1}(Figure 2).

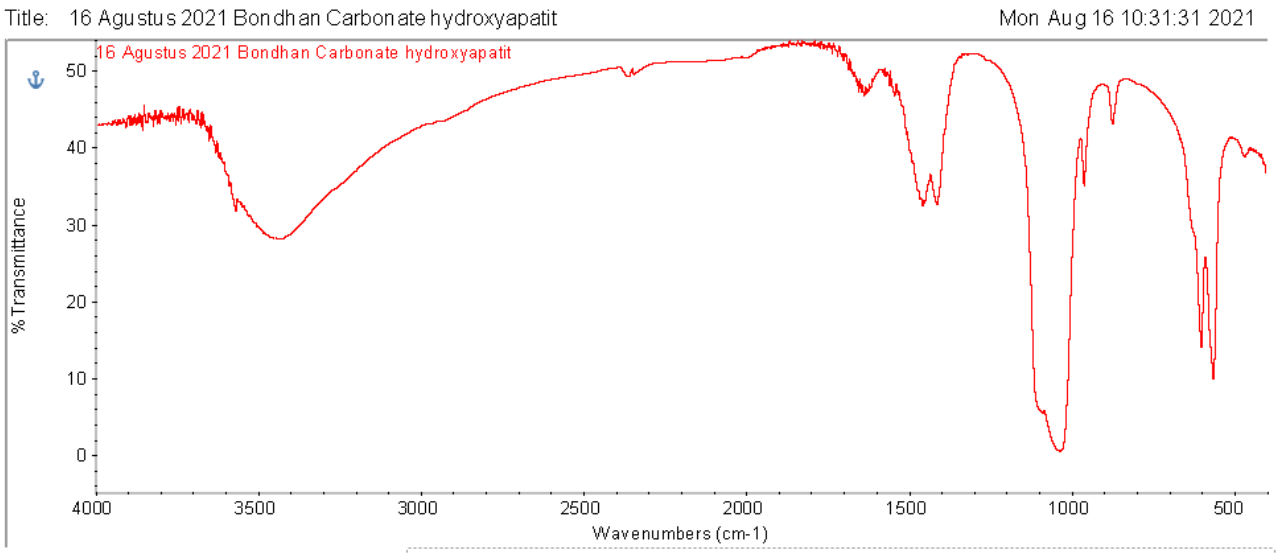


Figure 2. FTIR CHA Test Results for Cuttlefish Shells

Table 1. FTIR Test Results

No	Functional groups	Wavenumber (cm^{-1}) CHA	Wavenumber (cm^{-1})
1.	O – H	3403.32	3700-3000 cm^{-1}
2.	CO_3^{2-}	1054.82	1400-1460 cm^{-1}
3.	PO_4^{3-}	V1 = 602.62	V1 = 550-610 cm^{-1}
		V2 = 1034.86	V2= 960-1100 cm^{-1}

C. Results of In silico Analysis: Molecular Docking of CHA on RANK, RANKL, OPG, and TGF-β

Table 2. Total energy from docking results

No	Target Protein	Total energy (kcal/mol)
1.	RANK-RANKL	-206.27
2.	OPG	-236.72
3.	TGF-β	-266.56

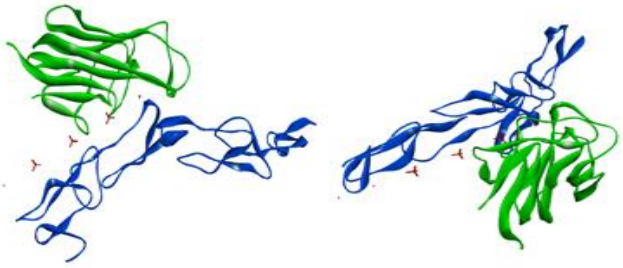


Figure 3. Visualization of CHA molecular docking on RANK RANKL

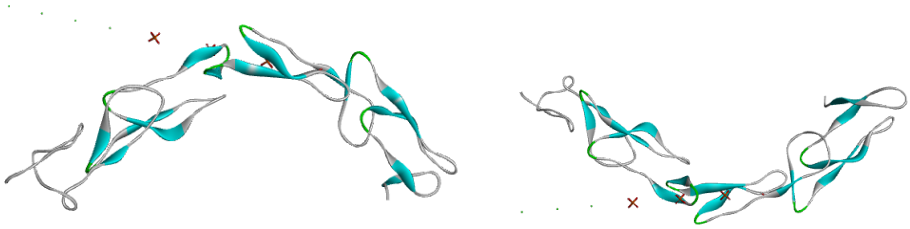


Figure 4. Visualization of CHA Docking on OPG

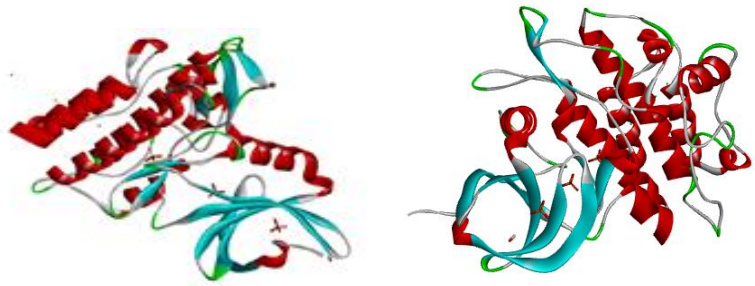
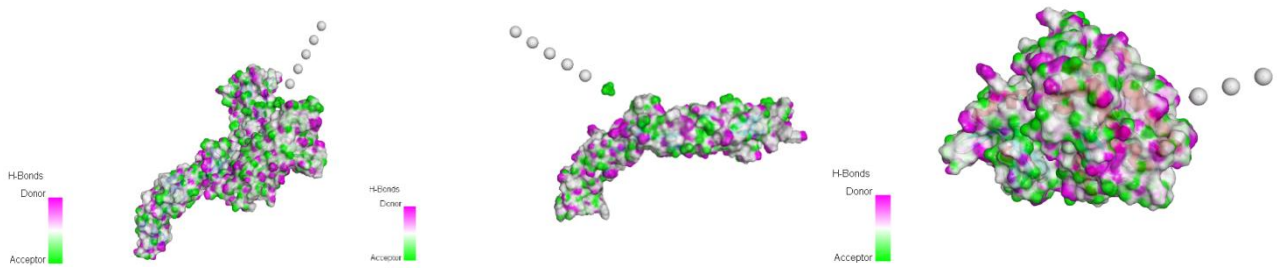


Figure 5. Visualization of CHA Docking on TGF-β



(a) (b) (c)

Figure 6. Hydrogen bond interactions (a) CHA with RANKL-RANK (b) CHA with OPG (c) CHA with TGF-β

DISCUSSION

In the process of characterizing a sample using FTIR, the goal is to locate certain groups or components within the sample that are indicated by the existence of a peak at a specific wave number. This characterization was carried out in order to gain correct information regarding the vibrations of hydroxyl, phosphate, and carbonate compounds. This was done in order to ensure the preparation of carbonated hydroxyapatite compounds without the association of organic groups.[11] According to Ahmiatri and Djarwani[12], the spectra of phosphate group (PO_4^{3-}) generally have an absorption band of *v1* (wavenumber molecular vibration) and *v2* in the area of wavenumber of 960 - 1100 cm^{-1} and 550 - 610 cm^{-1}, while the absorption band of carbonate group (CO_3^{2-}) is viewed around wavenumber of 1400-1460 cm^{-1}. From the observation of the infrared spectra of carbonated hydroxyapatite sample of cuttlefish shells, it can be viewed that phosphate group (PO_4^{3-}) indicates an absorption band in the exact range, which is 602.62 cm^{-1} and is in the exact wavenumber area with a value of 1034.86 cm^{-1}.

Following the docking of ligands and receptor proteins with the application Hex 8.0.0 Cuda, these structures were brought into Discovery Studio 2021 Client for visualization. During the process of preparing the protein, water molecules (H_2O) that were around the structure of the protein were also eliminated. This was done with the intention of preventing the water molecule from interfering with the docking process, which would allow it to be established beyond a reasonable doubt that the ligand is the only substance that interacts with the protein.[13] The docking method is stated to be good if it results in root mean square deviation value of ≤ 2 Å.[14] The affinity value of the binding energy and the number of interactions that take place between the ligand and the active site of the receptor are two factors that can be used to evaluate the influence that carbonated hydroxyapatite has on the target protein when molecular docking is performed. The amount of energy required to form a binding between a ligand and its receptor is referred to as the bond energy affinity. The lower the amount of energy required, the more stable the enzyme-ligand complex should be.[15] The lower the bond energy value indicates the stronger the affinity of the compound for target protein.[16]

From the docking results in Figure 3, it can be viewed that CHA is located between RANKL-RANK so that CHA prevents RANKL from binding to RANK. Prevention of bonding between RANKL and RANK will reduce the formation of new osteoclasts and existing osteoclasts.[17] This is due to the fact that carbonated hydroxyapatite has a structure that is comparable to that of cartilage. The rate of osteoclast activity can be decreased while an increase in osteoblast proliferation and differentiation is possible because to CHA's abilities. The protein RANK that is found in osteoclasts is involved in the process of inhibiting osteoclastogenesis. The death of osteoclasts, known as apoptosis, can be triggered by the inhibition of RANKL binding to RANK by OPG. This results in a reduction in the process of bone resorption. This material may also sustain bone cell metabolism and viability in an appropriate manner[18], which enables it to promote the process of new bone formation and can be utilized to avoid orthodontic relapse.

The docking results indicates that CHA binds to OPG (Figure 4). According to Kanayama et al.[19], CHA has the potential to significantly increase OPG expression. Kanzaki et al.[20] reported that local OPG gene transfer in periodontal tissue was proven to inhibit osteoclastogenesis activity by RANKL resulting in orthodontic tooth movement inhibition. After having orthodontic treatment, OPG local

injection has been demonstrated to have the potential to act as a stabilizing agent. In experimental animals whose teeth were relocated orthodontically, the local injection of OPG was able to restore the volume and density of bone tissue as well as lower the relapse rate.[21-22] Therefore, increased OPG expression is able to inhibit osteoclast differentiation and induce osteoclast apoptosis.

On the results of such docking, CHA was able to bind to TGF-β (Figure 5). Based on the results of Setiawatie et al.[23] research, CHA was able to significantly increase TGF-β expression so that it can increase the number of osteoblasts. TGF-β was produced by osteoblasts and stored in sufficient quantities in the bone matrix and is an important regulator of homeostatic processes of bone development and bone metabolism. Increased TGF-β can upsurge the process of bone remodelling. TGF-β plays a major role in the development and treatment affecting cartilage and bone metabolism, including alveolar bone. The TGF-β-induced bone formation process is driven by the chemotactic attraction of osteoblasts and increased osteoblast proliferation through protein production.[24]

Interaction between the ligand and the protein consists of various bonds (Figure 6). It can be viewed that interactions between the native ligand and the target protein include hydrogen bonds (green cylinders), van der Waals forces (striped balls), and electrostatic interactions. The volume of compound is shown with a grey circle. The number of hydrogen bonds formed in protein-ligand interactions contributes to the stability of complex structure. In other words, the greater the number of hydrogen bonds formed, the more complex the structure will be. If there are enough hydrogen bonds, it can be stated that the interaction between the two molecules is fairly strong.[15]

Interaction of the CHA ligand with the RANK-RANKL receptor has many hydrogen bond interactions. The bonding occurs between residues with proteins HIS223, GLU225, THR232, GLU268, ASN294, ASP45, LYS56, LEU59, HIS60, HIS77. CHA ligand interactions with OPG receptors have many hydrogen bond interactions, which are LYS67, GLN70, CYS84, GLU95, PRO64, and CYS 66. While the interaction of CHA ligands with TGF-β receptors has many hydrogen bond interactions, which are GLY198, THR200, ILE201, ALA202, LEU207, SER210, LYS213, GLY214, PHE216, GLU218, PAL219, LYS223, and GLN448.

CONCLUSION

Synthesis of Cuttlefish shell CHA has potential as an alternative material for preventing orthodontic relapse. CHA compounds are able to break the bond between RANK and RANKL and are able to increase the expression of OPG and TGF-β. This results in an increase in proliferation which causes an increase in the number and differentiation of osteoblasts and a decrease in the rate of osteoclast activity. This process will cause the results of orthodontic treatment to be stable so that orthodontic relapse does not occur.

ACKNOWLEDGMENT

This data source from Bondan Setyoko and Firda Ayu Fitriana's thesis from Faculty of Dentistry, Universitas Gadjah Mada.

REFERENCE

1. Alhasyimi AA, Pudyani PS. *Effect of cocoa administration during orthodontic tooth movement on RUNX2, calcium levels, and osteoclast bone-resorbing activity in rats*. J Pharm Pharmacogn Res 2022; 10(5): 857–864.

2. Alhasyimi AA, Rosyida NF, Rihadini MS. *Postorthodontic relapse prevention by administration of grape seed (vitis vinifera) extract containing cyanidine in rats*. Eur J Dent. 2019;13(4):629-634.
3. Alhasyimi AA, Suparwitri S, Christnawati C. *Effect of carbonate apatite hydrogel-advanced platelet-rich fibrin injection on osteoblastogenesis during orthodontic relapse in rabbits*. Eur J Dent 2021;15(3):412-419.
4. Alhasyimi AA, Pudyani PP, Asmara W, Ana ID. *Enhancement of post-orthodontic tooth stability by carbonated hydroxyapatite-incorporated advanced platelet-rich fibrin in rabbits*. Orthod Craniofac Res 2018;21(2):112-118.
5. Mondal S, Mondal B, Dey A, Mukhopadhyay SS. *Studies on processing and characterization of hydroxyapatite biomaterials from different bio wastes.* J Met Mater Miner. 2012; 11(1):56-67.
6. Dorozhkin SV. *Calcium orthophosphates (CaPO4): occurrence and properties*. Prog Biomater 2016;5:9-70.
7. Venugopal V. *Valorization of seafood processing discards: bioconversion and bio-refinery approaches*. Front. Sustain. Food Syst 2021; 5: 611835.
8. Cho ML, Heu MS, Kim JS. *Food component characteristics of cuttle bone as a mineral source*. J Fish Aquat Sci 2021; 34(5):478-482.
9. Cadman J, Zhou S, Chen Y, Li Q. *Cuttlebone: characterisation, application and development of biomimetic materials*. J Bionic Eng 2012;9: 367–376.
10. Venkatesan J, Rekha PD, Anil S. et al. *Hydroxyapatite from cuttlefish bone: isolation, characterizations, and applications.* Biotechnol Bioproc E 2018;23: 383–393.
11. Pratama AA, Yusnita R, Asnah M. *Docking Molekuler Senyawa 5,5' - Dibromometilsesamin*. Majalah Farmasi dan Farmakologi. 2017; 21(3):67-69
12. Ahmiatri SS, Djarwani SS. *Pengaruh ion karbonat dalam proses presipitasi senyawa kalsium fosfat*. Jurnal Makara Sains 2012. 6(2): 55-58.
13. Lemmon G, Meiler J. *Towards ligand docking including explicit interface water molecules*. PLoS One 2013; 8(6):e67536.
14. Puratchikody A, Sriram D, Umamaheswari A, Irfan N. *3D Structural interactions and quantitative structural toxicity studies of tyrosine derivatives intended for safe potent inflammation treatment*. Chem. Cent. J 2016; 10(24): 1–19.
15. Pantsar T, Poso A. *Binding affinity via docking: fact and fiction*. Molecules. 2018;23(8):1899.
16. Carlson HA, Smith RD, Khazanov NA, Kirchhoff PD, Dunbar JB Jr, Benson ML. *Differences between high- and low-affinity complexes of enzymes and nonenzymes*. J Med Chem 2008;51(20):6432-6441.
17. Alhasyimi AA, Rosyida NF. *Cocoa administration may accelerate orthodontic tooth movement by inducing osteoclastogenesis in rats*. Iran J Basic Med Sci. 2019;22(2):206-210.
18. Germaini MM, Detsch R, Grunewald A, Magnaudeix A, Lalloue F, Boccaccini AR, Champion E. *Osteoblast and osteoclast responses to A/B type carbonate-substituted hydroxyapatite ceramics for bone regeneration*. Biomedical Materials 2017; 12(3): 123-137.
19. Kanayama K, Sriarj W, Shimokawa H, Ohya K, Doi Y, Shibutani T. *Osteoclast and osteoblast activities on carbonate apatite plates in cell cultures*. J Biomater Appl. 2011;26(4):435-49.
20. Kanzaki H, Chiba M, Takahashi I, Haruyama N, Nishimura M, Mitani H. *Local OPG gene transfer to periodontal tissue inhibits orthodontic tooth movement*. J Dent Res 2004; 83(12):920-5.
21. Dolci GS, Portela LV, Onofre de Souza D, Medeiros Fossati AC. *Atorvastatin-induced osteoclast inhibition reduces orthodontic relapse*. Am J Orthod Dentofacial Orthop. 2017;151(3):528-538.
22. Hudson JB, Hatch N, Hayami T, Shin JM, Stolina M, Kostenuik PJ, Kapila S. *Local delivery of recombinant osteoprotegerin enhances postorthodontic tooth stability*. Calcif Tissue Int. 2012; 90(4):330-42.
23. Setiawatie EM, Widiyanti P, Ryan M, Rubianto M. *Carbonate hydroxyapatite-hyaluronic acid as bone healing accelerator: in-vitro and in-vivo studies on the alveolar bone of wistar rats*. J. Int. Dent. Medical Res. 2019; 12(4): 1280-1286.
24. Kresnoadi U, Prabowo, TSY. *Expression of Interleukin-1β and TGF-B due to induction with natural propolis extract and bovine bone graft*

combination in tooth extraction sockets leading to alveolar bone regeneration. J. Int. Dent. Medical Res. 2020;13(3): 935-939.