

Exposure of Gel Emulsion Zoledronate Bisphosphonate Olive Oil Increase Osteoclast Apoptosis

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Abstract

Several researchers reported that injection of Zoledronate Bisphosphonate (ZOL) could inhibit orthodontic tooth movement by osteoclasts apoptosis, but the administration by injection is painless. Use of ZOL emulsion with olive oil in a gel (Ge-ZOL) is expected to overcome the lack of administration by injection. To proved the effects of exposure of Ge-ZOL with olive oil on the number of osteoclasts apoptosis. This research was an in vivo laboratory experiment using 27 Sprague Dawley rats consist of 9 rats experimental group received Ge-ZOL, 9 rats control group receive Ge-without ZOL and 9 rats of normal groups. Gel with 40 µg of ZOL and gel without ZOL were applied on oral mucosa on beginning, hours to four to eight for two minutes on days 1, 2, 3 and 4. Rats were sacrificed on days 1, 3, 5 and then made preparation histological staining with IHK Caspase-3.

Ge-ZOL could increase the number of osteoclasts apoptosis on day 1 and 3 as well as the highest increase on the 3rd day. Ge-ZOL with olive oil dose of 40 µg proven to increase the number of osteoclast apoptosis.

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Introduction

Orthodontics is the speciality of dentistry concerned with the management and treatment of malocclusion to achieve good occlusion.¹ Principles of orthodontic treatment is to move teeth into the desired position and hold the unwanted tooth movement, termed anchorage. The challenge is to design a force system that maximizes desirable tooth movement and simultaneously minimizes anchorage loss.² Some experts have proposed various mechanical appliances to prevent anchorage loss, both extra and intra oral. However, these anchorage loss

and other unexpected side effects such as root resorption, white spot lesion, caries, gingivitis and compromised treatment results.³⁻⁵

Besides the use of mechanical tools, pharmacological methods also has the potential to minimizes anchorage loss. Recently, a class of drugs called Bisphosphonates hold great potential for reducing anchorage loss by inhibit bone resorption.⁶⁻⁸ Bisphosphonates are drugs used to treat bone metabolism disorders such as osteoporosis, Paget's disease, osteogenesis imperfecta, fibrous dysplasia, Gaucher's disease, malignant hypercalcemia and bone pain from some types of cancer. Bisphosphonate can bind to hydroxyapatite crystals in a mineralized bone matrix and make the bone more resistant to osteoclasts, inhibit differentiation of bone marrow precursors into osteoclasts, inhibit osteoclast function by interfering with the mevalonate pathway of cholesterol biosynthesis and induce apoptosis of osteoclasts.⁹ Bisphosphonates are divided into 2 families: nonnitrogen-containing Bisphosphonate and nitrogen-containing

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Biphosphonate. Zoledronate (ZOL), a third-generation, nitrogen containing heterocyclic imidazole Bisphosphonate, has been found to be a more potent inhibitor of bone resorption than other Biphosphonates that are currently available.⁶

The studies have shown that Bisphosphonate injection could inhibit tooth movement and decrease anchorage loss in rats. Although Bisphosphonate injection could inhibit tooth movement, but there is adverse effect such as pain during needle infiltration and systemic effects.⁶⁻⁸ To overcome those effects, it is needed to develop a new kind of Bisphosphonate in a form of gel emulsion. Gel emulsion has an advantage in simple usage. It could be applied on oral mucosa without pain, and in sequence, so that the effects are expected to be better.^{10,11} Olive oil and carboxymethylcellulose (CMC) are the chosen gel emulsion. Olive oil in gel emulsion has advantages such as olive oil has a distinctive aroma and has a function as a softener.^{12,13} CMC is known as one of mucoadhesive polymers which are capable of attaching to oral mucosa.¹⁴ The purpose of this study was to prove that Zoledonate gel emulsion (Ge-ZOL) could inhibit bone resorption based on the observation on apoptosis osteoclasts cell - count in vivo in oral mucosa of rats, using immunohistochemical Caspase-3.

Materials and methods

Twenty seven rats of Sprague-Dawley, under supervision of LITBANGKES DEPKES RI veterinarian with criteria of male, 3 months old, 180-200 g, were in good condition to be studied. Twenty seven rats were divided into 3 groups: 9 rats with Ge-ZOL application (experimental), 9 rats receive Ge-without ZOL(control) and 9 rats without any application (normal). Rats in normal group were used as a validity to rats in control group. This study had been approved by Ethical Commission of Faculty of Medicine, University of Indonesia No. 256/UN2.F1/ETIK/2015.

Ge-ZOL was made recenter paratus which consisted of 40 µg ZOL based on the preliminary study.25 mg of Ge-ZOL were applied on mesial area of buccal mucosa using cotton bud with circular movement. Nine rats of experimental and 9 rats of control were applied in sequence of 0 hour, 4th hour, 8th hour on days 1, 2, 3 and 4. Rats were sacrificed on the 1st day, 3rd day and 5th day.

Furthermore, the mucosa and the bone of oral tissue were cut transversal on the mesial area of mandible first right molar. Histological preparations was done on Histology Laboratory of Faculty of Medicine, University of Indonesia. Tissue then processed into formalin-fixed paraffin-embedded (FFPE) and then tissue was cut with the thickness of ± 4 µm, and used for immunohistochemistry staining using Caspase-3, Rabbit monoclonal caspase-3 p12 antibody (Abcam).

Apoptosis osteoclasts were counted using Olympus IX73 research inverted microscope (UI-Olympus Bioamaging Center) with 20x magnification. Calibration test was done on 20 % samples between histological expert of Faculty of Medicine University of Indonesia and researchers.

Statistical Analysis

Interobserver reliability test using Bland Altman test. Data were analyzed using One-way ANOVA. Differences were considered statistically significant when $p < 0.05$. All data were tabulated and statistical test were performed with SPSS V 18.

Results

Interobserver reliability test with Bland Altman test showed there was no difference which meant the reability test was good. The result of One-way ANOVA test showed that there were statistical significant differences in apoptosis osteoclast cell-count on experimental groups compare to control and normal groups on 1st day and 3rd day, $p = 0.000$, $p < 0.05$ (Figure 1).

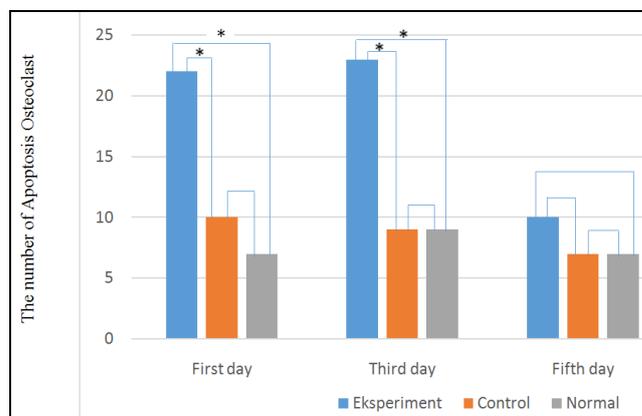
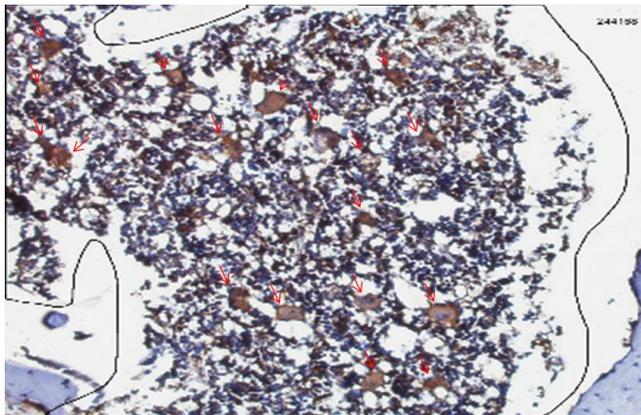


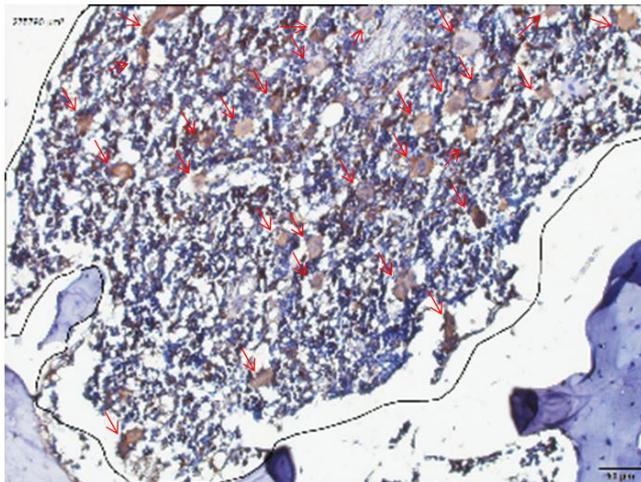
Figure 1. The difference of amount apoptosis osteoclasts cells - count on 1st day, 3rd day and 5th day between experimental, control and normal group, using one-way ANOVA. * $p < 0.05$.

Histological examination result using immunohistochemical Caspase-3 from each group between experimental, control and normal group on 1st day, 3rd day and 5th day is presented on figure 2, 3 and 4. The figure in experimental groups on 1st day and 3rd day showed increase apoptosis osteoclasts compared on 5th day.

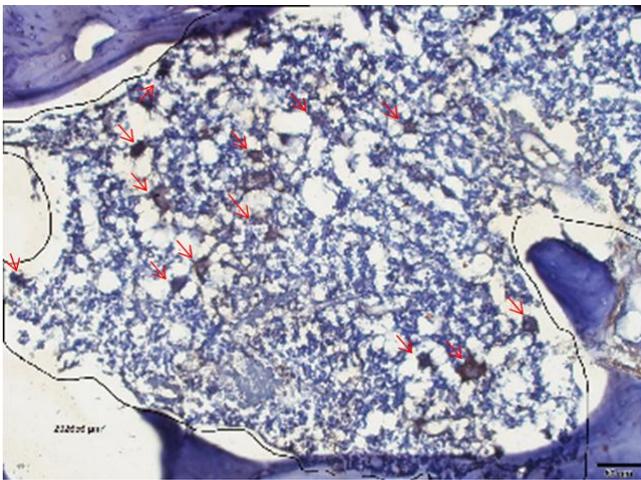
buccal's Sprague Dawley on experimental group, 20 x magnification, bar 50 μ m. Arrow showed apoptosis osteoclasts. (A) Experimental group on 1st day; (B) Experimental group on 3rd day; (C) Experimental group on 5th day. The picture showed increase apoptosis osteoclasts on 1st day and 3rd day compared on 5th day.



A

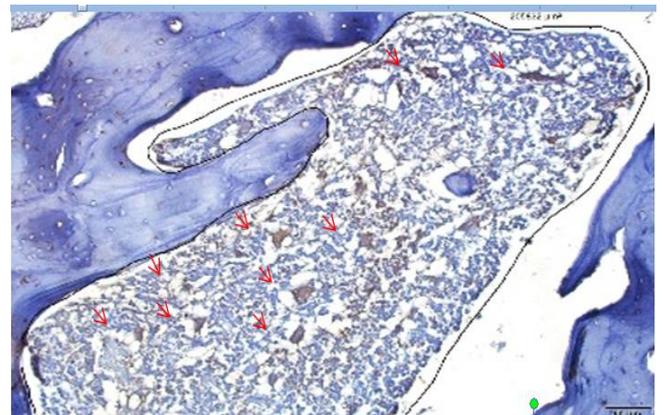


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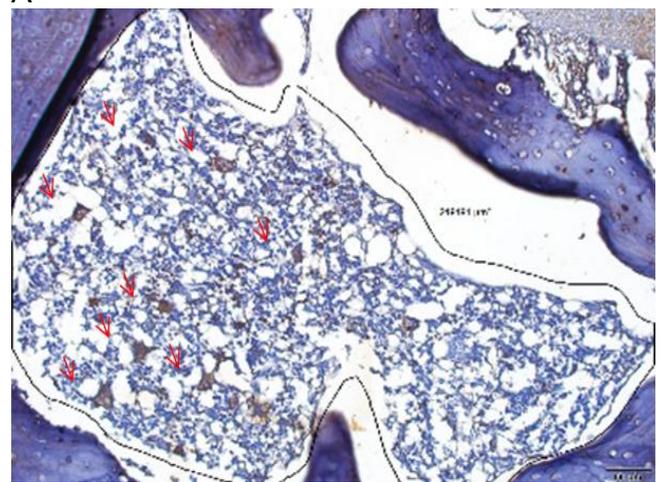


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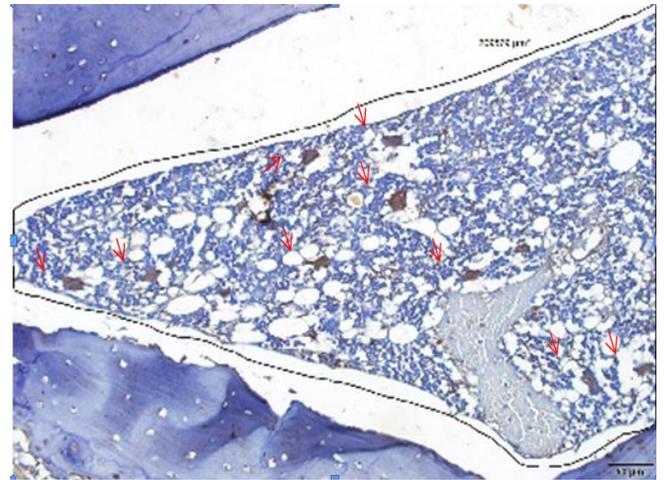
Figure 2. Alveolar bone on mesial area of 46



A



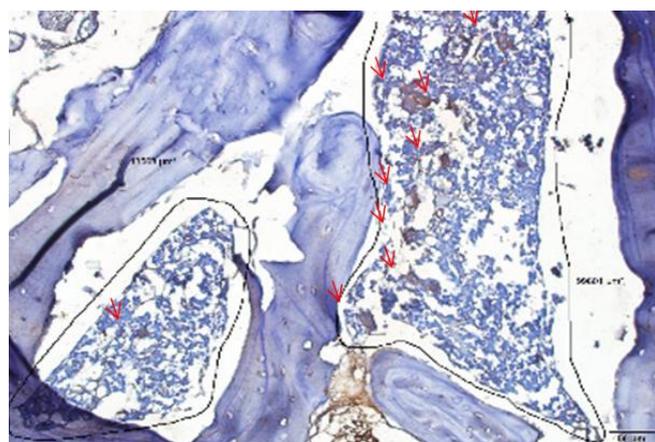
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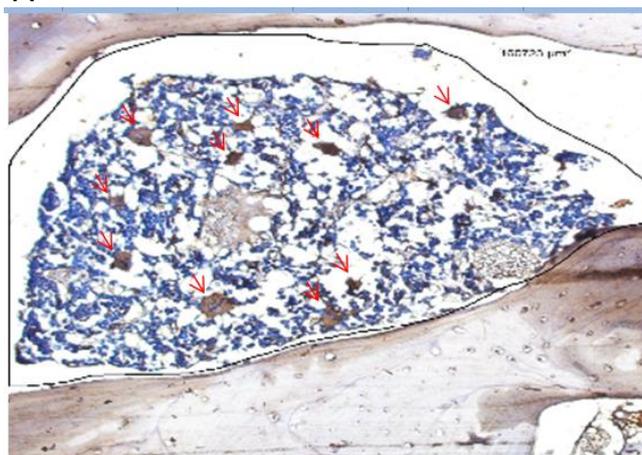
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Figure 3. Alveolar bone on mesial area of 46

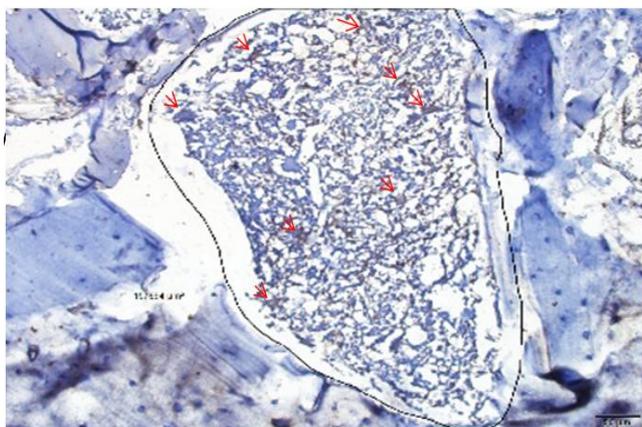
buccal's Sprague Dawley on control group using Olympus IX73 research inverted microscope, 20 x magnification, bar 50 μ m. Arrow showed apoptosis osteoclasts. (A) Control group on 1st day; (B) Control group on 3rd day; (C) Control group on 5th day. The picture showed some apoptosis osteoclasts on 1st day, 3rd day and 5th day.



A



B



C

Figure 4. Alveolar bone on mesial area of 46 buccal's Sprague Dawley on normal group using

Olympus IX73 research inverted microscope, 20 x magnification, bar 50 μ m. Arrow showed apoptosis osteoclasts. (A) Normal group on 1st day; (B) Normal group on 3rd day; (C) Normal group on 5th day. The picture showed some apoptosis osteoclasts on 1st day, 3rd day and 5th day.

Discussion

Several studies showed that ZOL injection on buccal mucosa combined with orthodontic force could inhibit tooth movement, although it has disadvantages such as a pain and systemic effects.⁶⁻⁸ To overcome those effects, it is needed to develop a new kind on Zoledronate Bisphosphonate in form of gel emulsion. The form of gel was chosen because its usage in oral medication had been wellknown. Compared to injection, gel application was not painful and *in vivo* it had good biocompatibility and could release medicine from gel to mucosa. Gel emulsion has an advantage in simple usage in oral mucosa without pain.^{10,11} *Carboxy Methyl Cellulose (CMC)* was chosen due to its mucoadhesiveness in oral, such as toothpaste and topical anaesthetics, CMC and olive oil gel are the chosen emulsion gel. CMC has stability on storage, good tolerance of water miscible solvents and good adhesive strength.¹⁴ Olive oil in gel emulsion has advantages such as its has a distinctive aroma and has a function as a softener.^{12,13} In dentistry, until recently there is no Ge-ZOL.

Currently, buccal mucosa area is a specific location that was developed to conduct medicine. Research showed that this pathway had been used to conduct many kinds of medicine.¹⁴ To determine the effect of Ge-ZOL used rats as an experimental animals. Rats oral mucosa structure is not different from epithel layer of human oral mucosa, but the thickness of rat's oral mucosa is less than human, about 40-140 μ m.¹⁵ To be able to make the small dosage of ZOL, which is 40 μ g and could penetrate into mucosa layer, the application of Ge-ZOL could be done in sequence, 3 times of 0, 4, 8 hours on 1, 2, 3, 4 days. The active accumulation could continue to penetrate into deeper mucosa tissue until the alveolar bones. This study showed the effect of gel emulsion zoledronate bisphosphonate olive oil were increased osteoclast apoptosis. It could be well said that

zoledronate bisphosphonate was proven active and had a potential to decreased bone resorption through osteoclast apoptosis and inhibit orthodontic tooth movement.

Furthermore, Ortega et al (2012) reported a single, small and locally applied dose of Zoledronate injection was sufficient to increase apoptosis osteoclasts and inhibit orthodontic tooth movement.⁶ However, they used 16 µg Zoledronate that 3 times smaller than the dose of our study (40 µg). It is based on topical administration that there is a possibility that not all the drug into the mucosal tissues and alveolar bone.

In this research, histological preparation was using immunohistochemical Caspase-3. Immunohistochemical Caspase-3 contain antibodies that can detect and bind to antigen Caspase-3 in osteoclasts undergoing apoptosis. Apoptosis osteoclasts, giving rise “brown” color that are seen on immunohistochemical Caspase-3.¹⁶ Furthermore, apoptosis osteoclasts cell-count could be done through Olympus IX73 research inverted microscope with 20 x magnification. Immunohistochemical Caspase-3 staining showed that apoptosis osteoclasts on experimental group on 1st and 3rd day higher than control group. It means that Ge-ZOL could penetrate into alveolar bone and stimulate apoptosis osteoclasts. Our results are consistent with Benford et al (2011) studied that investigated effects of Bisphosphonate in apoptosis osteoclasts using cell culture. They found that Bisphosphonates could increase apoptosis osteoclasts after 24 hours and will continue to rise after 48 hours.¹⁶ However, in our study on day 5 the number of apoptosis osteoclasts decreased. This is probably due to the mechanism of the balance of osteoblast-osteoclast. Increasing apoptosis osteoclasts would trigger parathyroid hormone (PTH) and receptor activator of nuclear factor kappa-B ligand (RANKL) that inhibit apoptosis osteoclasts.¹⁷ Figure 1 showed that pressure during application could not increase apoptosis osteoclasts on control group. So control group had a good validity as compared to the experimental group.

This study reports that Ge-ZOL could penetrate into alveolar bone rats and increase apoptosis osteoclasts based on apoptosis osteoclasts cell-count. For the next study, we suggest to determine the effect of Ge-ZOL on orthodontic tooth movement.

Conclusions

It is concluded that Ge-ZOL could penetrate into alveolar bone rats based on the observation of apoptosis osteoclasts cell-count. After 1st and 3rd day of Ge-ZOL application, there was a significant difference increasing of the apoptosis osteoclasts cell-count compared to control.

Declaration of Interest

The authors report no conflict of interest and the article is not funded or supported by any research grant.

References

1. Couborne MT, Dibiase AT. Handbook of Orthodontics. 1st ed. Philadelphia: Mosby Elsevier. 2011:107–12.
2. Graber LW, Vanarsdall RL, Vig KWL. Orthodontics Current Principles and Techniques. 5th ed. Philadelphia: Mosby Elsevier. 2012:253–62.
3. Roberts Harry D, Sandy J. Orthodontics. Part 9: Anchorage control and distal movement. Br Dent J. 2004;196(5):255–63.
4. Keles A, Erverdi N, Sezen S. Bodily distalization of molars with absolute anchorage. Angle Orthod. 2003;73(4):471–82.
5. Bondemark L, Thorne J. Anchorage provided during intra-arch distal molar movement: A comparison between the nance appliance and a fixed frontal bite plane. Angle Orthod. 2005;75(3):437–43.
6. Ortega AJ, Campbell PM, Hinton R, Naidu A, Buschang PH. Local application of Zoledronate for maximum anchorage during space closure. Am J Orthod Dentofacial Orthop. 2012;142(6):780–91.
7. Ozturk F, Babacan H, Inan S, Gumus C. Effects of bisphosphonates on sutural bone formation and relapse: a histologic and immunohistochemical study. Am J Orthod Dentofacial Orthop. 2011;140(1):31–41.
8. Iglesias-Linares A, Yanez-Vico RM, Solano-Reina E, Torres-Lagares D, Gonzalez-Moles MA. Influence of bisphosphonates in orthodontic therapy: Systematic review. J Dent. 2010;38(8):603–11.
9. Fujimura Y, Kitaura H, Yoshimatsu M, et al. Influence of bisphosphonates on orthodontic tooth movement in mice. Eur J Orthod. 2009;31(6):572–7.
10. Ajazuddin, Alexander A, Khichariya A, et al. Recent expansions in an emergent novel drug delivery technology: Emulgel. J Control Release. 2013;171(2):122–32.
11. Panwar AS, Upadhyay N, Bairagi M, et al. Emulgel: A review. Asian J Pharm Life Sci. 2011;1(3):333–43.
12. Mattia CD, Sacchetti G, Mastrocola D, Pittia P. Effect of phenolic antioxidants on the dispersion state and chemical stability of olive oil o/w emulsions. Food Res Int. 2009;42:1163–70.
13. Franco MN, Galeano-Diaz T, Lopez O, et al. Phenolic compounds and antioxidant capacity of virgin olive oil. Food Chem. 2014;163:289–98.
14. Khairnar GA, Sayyad FJ. Development of buccal delivery system based on mucoadhesive polymer. Int J PharmTech Res. 2010;2(1):719–35.
15. Jansen RG, Van Kuppevelt TH, Daamen WF, Kuijpers-Jagtman AM, Von de Hoff JW. Tissue reactions to collagen scaffolds in the oral mucosa and skin of rats: Environmental and mechanical factors. Arch Oral Biol. 2008; 53(4): 376–87
16. Benford HL, McGowan NW, Helfrich MH, Nuttall ME, Rogers MJ. Visualization of bisphosphonate-induced caspase-3 activity in apoptotic osteoclasts in vitro. Bone. 2001;28(5):465–73.
17. Berkovitz BKB, Holland GR, Moxham BJ. Oral Anatomy, Histology and Embryology. 4th ed. St Louis: Mosby Elsevier. 2009:205–52.