ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

The Effects of Local Alendronate with Biphasic Calcium Phosphate on Augmented Rabbit Sinus

Zainab M.Hameed[1*], Mazin J. Mousa[2]

1. *Department of Periodontics, Faculty of Dentistry, University of Babylon, Iraq.*

2. *Department of Clinical Laboratory Sciences, Faculty of Pharmacy, University of Babylon, Iraq.*

***Corresponding Author: Zainab M. Hameed**

Abstract

Background: Biphasic calcium phosphate considered a good synthetic bone graft substitutes, and its effective role in sinus floor lifting. Alendronate (ALN) is a common formula of bisphosphonates that have potential effects to decrease the osteoclast differentiation. Aim of study is to evaluate histologic and histomorphometric effect of local alendronate with BCP with ratio(30: 70)when used with a rabbit sinus. Materials and Methods: 8 male adult (New Zeeland) rabbits were selected. Sixsteen sinus floor elevation procedures, were prepared, 2 on each animal and were distributed into two groups (n=4): the BCP group, where the sinus was grafted with B CP with HA :ß-TCP (30 :70).And BCP/ALN group, where the sinus grafted with (OSTEON II), mixed with Alendronate solution, the solution was prepared by dissolving of 20 mg of ALN with 1 ml of distal water. The rabbits killed at 7, 14, 30 and 60 days after surgery and the histological analysis were achieved. The histologic evaluation was performed using a light microscope. The new bone area represents the proportion of dark red area to the total augmented area, while the (FCT) represents the proportion of light red area to the total augmented area. Results: Although there were significant differences of new bone formation between two study groups after 7,14 and 30 days periods ,but there's non-significant differences occurred after 60 days . In addition, the FCT area was decreased in these two groups with the progressing healing periods, at the same time the FCT area was less in BCP/AL group; and there were statistically nonsignificant differences between two groups after 60 days periods. Conclusions: Local application of ALN with BCP with the (HA: ß-TCP (30:70)) ratio, did not have beneficial effects when augmented sinus rabbit model to increase bone formation.

Keywords: *Biphasic calcium phosphate; Alendronate; Rabbit maxillary sinus.*

Introduction

Reduce amount of bone and density that presents in maxillary posterior region. Therefore; dental implant construction in this region; is considered one of most interesting procedure. Sinus floor lifting procedures with bone graft are common surgical technique, to reconstruct the highly atrophied maxillary posterior region [1,3].

For many years autologous bone grafts considered as the best standard; because of both osteo-inductive and osteo-conductive properties. At the same time harvested of this material from the donor region is limited, and required for second surgical traumatic and morbidity of this donor area. For these reasons; those have improved the demand for natural graft, and synthetic biomaterials.

However, allograft and xenografts may provoke an immunological response [4]. Therefore, synthetic biomaterials being established .These synthetic biomaterials are osteo-conductive, and donot prompt immune-genicity [5]. Biphasic calcium phosphate (BCP) considered a good synthetic bone graft substitutes, and its active role in sinus floor

lifting has been proved [6, 10] .Bi-CP contains a slow-resorbing hydroxyllapatite (HA) and rapid resorbed of ßTricalcium phosphate.

Mixing bone graft substitute with other materials in order to accelerate recovery and inhibit resorption of graft material has been confirmed in some applications. Bisphosphonates (BPs) are chemically unchanging, and are commonly used for treating illnesses that caused of increasing of resorption of bone; as an inhibitor of bone resorption, such as, osteoporosis, hypercalcemia [11, 14].

Lowering of resorption of bone occurred by inducing apoptosis of osteoclastscells, through the formation of intracellular metabolic action in the osteoclast cells [15, 16]. In addition, in vitro studies had demonstrated that some Bisphosphonates that improved osteoblast cells differentiation [17, 20].

At the same time, the effect of BP on bone would depend to its capability; to reduce resorption of bone and its possible capability to stimulate bone formation [21]. Alendronat (ALN) is one of common BP, with a great antiresorptive action, by decreasing osteoclast cell differentiation during the maturation procedure [22].

It is known that ALN reduced the action of inflammation; by, inhibiting of some of proteinasis [23]. System administration of BP treatment, may be helpful in the inhibition of bone damage that accompanying period onto disease in beigle dogs[24],in addition to reduction of bone resorption after flap procedure in rat model [25,26].De Almeida et al found that the topical use of ALN as a companied to mechanical debridement, is effective for the treatment of induced periodontitis in rat [27].

A yranci et al, shown that adding of Alendronate, improved new bone construction, and reduced fibrous connective tissue production, with rabbits; when treated by two different materials (either xenograft, or auto- iliac crest bone graft) for maxillary sinus base augmenting [28].

Recent study by Özer et al reported that there's enhancement in osteo-conductive properties of the autogenous bone graft in conjunction with local sodium alendronate in mandibular bone defects created in rabbits [29]. In addition, Sung .Kim et al, found in their vitro study that alendronate and BCP scaffolds have the latent to enhance calcium deposition by osteoblast cells and markedly stimulate osteoblast differentiation [30].

However, synergic effect of the adding of local alendronate in maxillary sinus floor augmentation is unknown.BCP with local alendronate when used with a rabbitsinus is UN available. Therefore, our study was conducted in order to estimate the results through a histological and histomrphometric evaluation.

Materials and Methods

Scientific committee in periodontal department /Faculty of Dentistry / University of Babylon approved this study, and the "guidance for care of animals," as written by the Animal House at the College of Medicine at Babylon University.

Eight male adult rabbits; were selected. Sixteen sinus floor elevation operations were accomplished, two on each animal and were distributed into two groups(n=4) : the BCP group, where the sinus was grafted with (OSTEON II Korea),that the ratio of HA:ß-TCP was 30:70. And BCP/ALN group, where the sinus augmented with[OSTEON II] mixed with Alendronate solution ,the solution was prepared by dissolving of 20 mg of ALN with 1 ml of distal water [31].There have been many studies of sinus augmentation in rabbit models [32, 35, 28, 10].

Surgical Procedure

Each animal underwent a surgical procedure, for the bilateral sinus lift technique. General anesthesia was given to animal using a mixed of intra -muscle ketamine (50 mg/kg) and xylazine hydrochloride. Lido cane was also injected subcutaneous of the nasol bone region to decrease flow of blood in the surgical area.

An incision was made along the sagittal midline on the nasal bone, to elevate a flap [32]. A trephine bur 5mm in diameter was used for creation a bone opening for the sinus. The bur was used with irrigation with saline solution. The nasal bone disk was detached from both the nasal bone and the sinus membrane; then, the grafting materials

were implanted into the space that was produced after a careful elevation of the sinus membrane (Figure 1). After that, the operation areas were sutured.

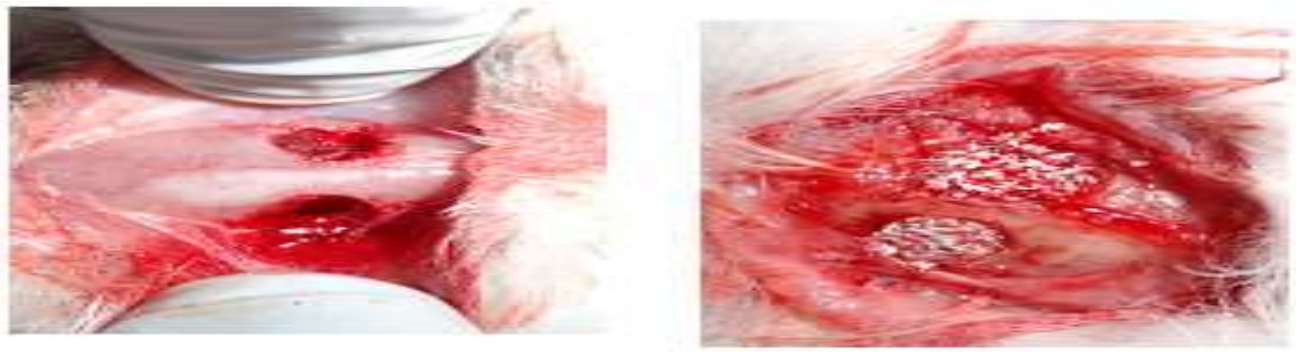

Figure 1: Photo-graphs for surgical procedure area

Tissue Perpetrated

Rabbits were killed, at 7,14 ,30, and 60 days subsequently after surgery (4 for each).The operation area was cutting into small blocks which included both the nasal and maxillary sinus, and then fixed with 4% formaldehyde solution for(48)hours at (4°C),and decalcified . Then samples fixed in paraffin wax and sliced into sections 5µm thick for histological analysis, after having been stained with Hematoxylin Eosin.

Histological, and Histomorphometrical Analysis

The histologicel and histomorphometrical analysis were achieved according with the previously described method [28].Histological evaluation was performed by a light-microscope. The measurements were taken using an image processing software program (Image J.exe). The new bone area [36] represents the proportions of dark red area to the total augmented area, while the fibro connective tissue (FCT) represents the proportion of pale red area to the total augmented area.

Statistical Analysis

All analyses of study data were done by [Statistical Package for Social Sciences] Ver. 22. Mean values of the measurements, were occurred in (95% CI), all data were written as (means ± SD), by using an (ANOVA) test; in order to determine the differences. A Turkey's test was also used and the ($P < 0.05$) values were regarded as statistical significant.

Results

Histologically Analysis

After 7 days, most of the implant areas of the sinuses in both groups were filled by well-maintained by graft materials with all the animals. The grafted materials surround by highly fibrous tissues, and some newly formed bone appeared during this period in groups, the newly bone were seen around graft particles; and more pronounced in the TCP 70 group, than in the TCP 30group.The osteoclast cells were pronounced in both groups (Fig. 2 A & B).

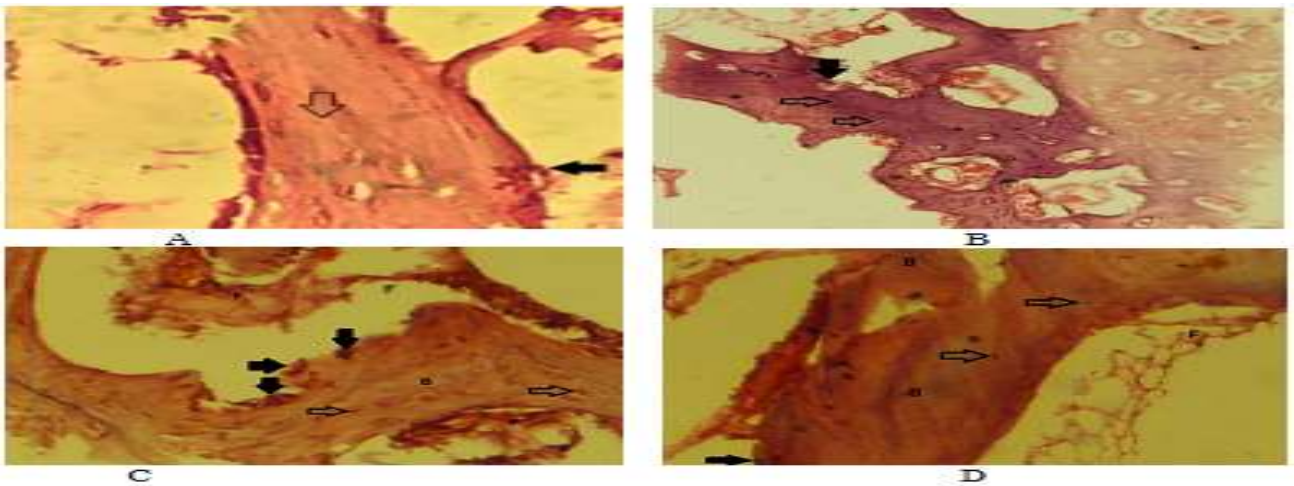

Figure 2: Photo micro graph showed the bone formation : after 7 days of the BCP group (A) and BCP/AL group (B),and after 14days of the BCP group (C) and BCP/AL group(D).B= bone, F = fibrous connective tissue, the empty arrows indicate osteocyte cells, and black arrows indicated osteoclastcell H&E stain. X 400

After 14 days, the graft materials were well maintained beneath the elevated sinus membrane in both groups and surrounded by dense, vascularized tissue and thin trabecular bone .The presence of osteoblasts and osteocytes were also observed in both groups. The osteoclast cells were more pronounced in BCP group more than in BCP / ALN group, and these cells appeared with ruffled borders, while in BCP/ALN group appeared with clear contact with adjacent bone.

The new bone was shown to be in partial connection with graft elements and closed to the Schneiderian membrane in both groups, at the same time this bone appeared to be more dense in BCP/ALN group than in BCP group. The amount of fibrous connective tissue was less in BCP/ALN group (Figure.2.A and B). After 30 days, the amount of the new bone in ugmented sinus area had increased more compared to the new bone after 14 days in both groups.

The new bone was a more pronounced; many osteocyte cells were observed inside the new bone in both groups. Large number of osteoclastcells seemed during this period of time in BCP group when compared with the BCP/AL group, and the amount of fibrous connective tissue was more pronounced in BCP group (Fig.3.Aand B). After 60 days, the amount of the newly bone in the augmented area had increased more compared to the new bone after 30days in both groups.

New bone was thicker and more matured bone; numerous osteocytes were observed in the new bone in both groups. Large number of osteoclastcells appeared during this time in BCP group when compared with the BCP/AL group, and resemble what found after 30 days of healing period; these cells appeared with ruffled bordered in BCP group and clear borders with adjacent bone in BCP/ALN group. The amount of fibrous connective tissue was more pronounced in BCP group (Figure .3 C and D).

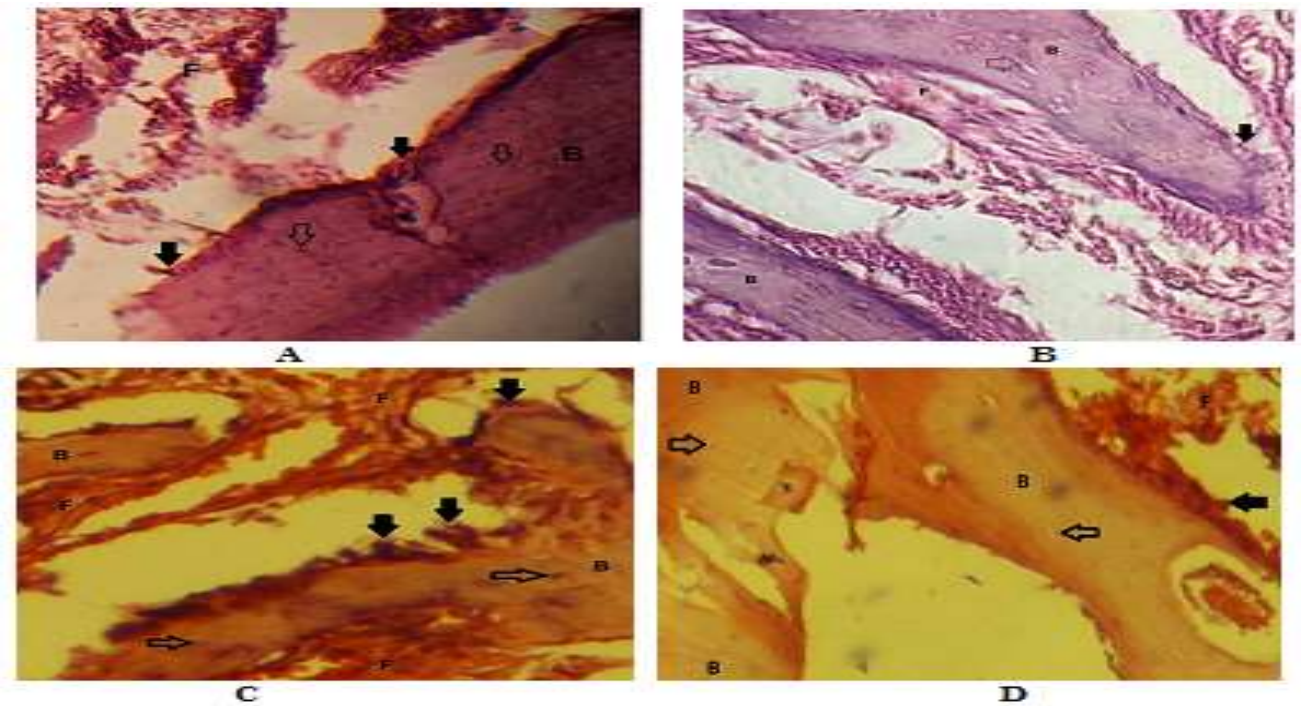

Figure 3: Photo-showing the bone formation after 30 days of the BCP group (A) and BCP/AL group(B) ,and after 60 days of the BCP group (C) and BCP/AL group (D).B= bone, F = fibrous connective tissue, the empty arrows indicate osteocyte cells, and black arrows indicate osteoclast cells. H-E stain X 400

Histo-morphometric Analysis

The histomorphometrical outcomes of the augmented area are shown in Tables1and 2. The NBA was increased in all groups with the progressing healing periods. There were statistically significant differences between two groups after 7, 14 &30 days periods. While there was non-significant difference occurred between them after the last period of healing. In general, there's an increased bone formation in BCP/AL group more than in BCP group. In addition, the FCT was decreased in these two groups with the progressing healing periods ; and there were

same results of differences had been occurred between these two groups that were found with new bone formation ,and also the same findings at last period of healing (60 days) that there was nonsignificant difference occurred between them these two groups.

Table1: The results of histomorphometric evaluation of New Bone Area (Mean % ±SD) for BCP and BCP/AL groups after 7, 14, 30 and 60 days

	BCP	BCP/AL
7 days.	10.05±0.21	14.75±0.92*
14 days.	13.8±0.4	16.4±0.5*
30 days.	47.35±1.62	59.5±1.6*
60 days.	55±1.2	58.5±1.9

* Statistically significant difference ($p < 0.05$)

Table2: The results of histomorphometric evaluation of Fibrous Connective Tissue (Mean % ±SD) for BCP and BCP/AL groups after 7,14,30 and 60 days .

	BCP	BCP/AL
7 days.	89.95±0.21	85.25±0.9*
14 days.	86.2±0.4	83.6±0.2*
30 days.	52.65±1.6	40.5±1.6*
60 days.	40±1.13	41.5±1.8

* Statistically significant difference ($p < 0.05$)

Discussion

Sinus lifting procedure with bone graft material was the commonest procedure to increase the bone in maxillary posterior region, in order to overcome the complications that accompanied placement of dental implant in this area. Different graft materials were used to augment the sinus; and auto-bone graft is stay the best graft, due to its osteoinductive e, osteoconductive, and osteogenic effects [37].

However, the disadvantages of autogenous bone graft material including, limited amount of bone that harvested from intra-oral donor area, and discomfort of this donor area post operatively. All these limitations had tended towered searching for an alternative bone substitute materials. Bi-phasic calcium phosphete BCP is considered to be one of many different bone materials, which has bio-compatible, osteo conductive, and cost-effective properties by providing framework that facilitate cells and vascular infiltration and subsequently bone formation [6, 10, 38, 39].

BCP [OSTEON II] ,which contain ratio of HA:ß - TCP(30:70) The proper ratio of slow resorb (HA) and quick- resorbing of ß-Tri-calcium phosphate (ß-TCP) could improve new bone formation by (ß-TCP) and provide augmented space by (HA) in a grafted sinus. ß TCP was showed similar actions to autogenous bone in a canine model [40]. Thus, our hypothesis based on that the adding of ALN to the grafted area would increase new bone formation that formed by ß-TCP by inhibiting the osteoclast cells activity in order to decreased bone resorption and simultaneously increased bone formation. ß- TCP occurred in high ratio in BCP(OSTEON II) with ratio of (30 : 70). The present study was showed in order to estimate the outcomes of augmented sinus with BCP [OSTEON II] with local ALN when used with a rabbit sinus model .A rabbit sinus is usually good model to evaluate bone formation because to its anatomic similarity to a human sinus [3].

Systemic and topical administrations of alendronate to bone graft have been demonstrated in many previous studies, and that were aimed to control bone resorption by inhibiting of action of osteoclast cells [22, 24, 27, 28, 41]. Reddy et.al, have showed that systemically administration of ALN treatment may be helpful in the management of bone loss that related with moderate and severely period disease in sixteen beagli dogs were studied for a 6-month period [24].

In addition to Juliano De Almeida et al found that the effective treatment of experimental periodontitis in rat by scaling and root planning followed by topical irrigation with 1 ml of sodium alendronate solution [27].

Ayrance eal, have found that, bisphosphonate improved new bone formation accompanied with reducing fibrous connective tissue formation for more than six weeks follow up in sinus floor augmentation of rabbits, that were treated with two types of bone graft [28].

In our study, BCP were mixed with the Alendronat solution, the solution was prepared by, dissolving of 20 mg of ALN, with 1 ml of distal water, and then planted for the surgical areas.

The histological and histo- morphometric analysis, revealed that the formation of newly bone, increased throughout the healing time in both study groups (BCP/ALN group and BCP group), and as expecting of using alendronate with BCP is that the new bone formation will be more, but these results were incorporated with previous studies, because although the new bone formation were increased in BCP/ALN group more than in BCP group, but there were non-significant differences occurred between them at the last period of healing. The results of current study were corporate with Chacon et al study that used systemic therapy of Alendronate for 20 rabbits treated with dental implant in femur and tibia, and torque removal data, showed that there's non-statistical differences occurred among the alendronate and control groups [22].

BCP materials provide advantageous properties due to close similarity to natural bone mineral, with respect to Alendronate treatment. Many studies focusing on adverse effects of systemic used of Alendronate, such as esophagitis, gastric ulcers, and osteonecrosis the jaw, and these all adverse effects increased with increased doses with long periods of using of this drug [42].

On the other hand, low dose of local application of this drug, did not report adverse effects in many studies [24, 43]. And results of present study also confirmed these findings. Thus local application of low dose of Alendronate mixed with BCP may have no beneficial effects for bone formation since this application showed nonsignificant differences when compared with BCP alone.

References

1. Araujo M, Sonohara M, Hayacibara R, Cardaropoli G, Lindhe J (2002) Lateral ridge augmentation by the use of grafts comprised of autologous bone or a biomaterial. An experiment in the dog. Journal of clinical periodontology, 29: 1122-1131.
2. Boyne PJ (1980) Grafting of the maxillary sinus floor with autogenous marrow and bone. J. Oral Surg., 38: 613-616.
3. Tatum JH (1986) Maxillary and sinus implant reconstructions. Dental Clinics of North America, 30: 207-229.
4. Sukumar B, Nagamani K, Raghavan RS (2008) Evaluation of strength at early ages of self-compacting concrete with high volume fly ash. Construction and Building Materials 22: 1394-1401.
5. Bauer TW, Muschler GF (2000) Bone graft materials: an overview of the basic science. Clinical Orthopaedics and Related Research® 371: 10-27.
6. Bae J-H Kim, Y-K, Kim S-G, Yun P-Y, Kim J-S (2010) Sinus bone graft using new alloplastic bone graft material (Osteon)-II: clinical evaluation. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 109: e14-e20.
7. Favato MN, Vidigal BC, Cosso MG, Manzi FR, Shibli JA, Zenóbio EG (2015) Impact of human maxillary sinus volume on grafts dimensional changes used in maxillary sinus augmentation: a multislice tomographic study. Clinical Oral Implants Research 26: 1450-1455.
8. Kim YK, Yun PY, Lim SC, Kim SG, Lee HJ, Ong JL (2008) Clinical evaluations of OSTEON® as a new alloplastic material in sinus bone grafting and its effect on bone healing. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 86: 270-277.
9. Ohayon L (2014) Maxillary sinus floor augmentation using biphasic calcium phosphate: a histologic and histomorphometric study. International Journal of Oral & Maxillofacial Implants, 29.
10. Lim H-C, Hong J-Y, Lee J-S, Jung U-W, Choi S-H (2016) Late-term healing in an augmented sinus with different ratios of biphasic calcium phosphate: a pilot study using a rabbit sinus model. Journal of periodontal & implant science, 46: 57-69.
11. Liberman UA, Weiss SR, Bröll J, Minne HW, Quan H, Bell NH, Rodriguez-Portales J, Downs Jr RW, Dequeker J, Favus M (1995) Effect of oral alendronate on bone mineral density and the incidence of fractures in postmenopausal osteoporosis. New England Journal of Medicine 333: 1437-1444.
12. Adami S, Zamberlan N, Mian M, Dorizzi R, Rossini M, Braga B, Gatti D, Bertoldo F,

Locascio V (1994) Duration of the effects of intravenous alendronate in postmenopausal women and in patients with primary hyperparathyroidism and Paget's disease of bone. Bone and mineral, 25: 75-82.

13. Aapro M, Abrahamsson P-A, Body J-J, Coleman R, Colomer R, Costa L, Crino L, Dirix L, Gnant M, Gralow J (2007) Guidance on the use of bisphosphonates in solid tumours: recommendations of an international expert panel. Annals of oncology, 19: 420-432.
14. Civitelli R, Napoli N, Armamento-Villareal R (2007) Use of intravenous bisphosphonates in osteoporosis. Current osteoporosis reports 5: 8-13.
15. Murakami H, Takahashi N, Sasaki T, Udagawa N, Tanaka S, Nakamura I, Zhang D, Barbier A, Suda T (1995) A possible mechanism of the specific action of bisphosphonates on osteoclasts: tiludronate preferentially affects polarized osteoclasts having ruffled borders. Bone, 17: 137-144.
16. Sato M, Grasser W (1990) Effects of bisphosphonates on isolated rat osteoclasts as examined by reflected light microscopy. Journal of Bone and Mineral Research, 5: 31-40.
17. Gandolfi M, Pugnaloni A, Mattioli-Belmonte M, Muzzarelli R, De Benedittis A, Mengucci P, Zucchini C, Tesei M, Caudarella R, Biagini G (1999) Osteoblast behaviour in the presence of bisphosphonates: ultrastructural and biochemical in vitro studies. Clinical and experimental rheumatology, 17: 327-334.
18. Giuliani N, Pedrazzoni M, Negri G, Passeri G, Impicciatore M, Girasole G (1998) Bisphosphonates stimulate formation of osteoblast precursors and mineralized nodules in murine and human bone marrow cultures in vitro and promote early osteoblastogenesis in young and aged mice in vivo. Bone, 22: 455-461.
19. Garcia-Moreno C, Serrano S, Nacher M, Farre M, Diez A, Marinoso M, Carbonell J, Mellibovsky L, Nogues X, Ballester J (1998) Effect of alendronate on cultured normal human osteoblasts. Bone, 22: 233-239.
20. Itoh F, Aoyagi S, Furihata-Komatsu H, Aoki M, Kusama H, Kojima M, Kogo H (2003) Clodronate stimulates osteoblast differentiation in ST2 and MC3T3-E1 cells and rat organ cultures. European journal of pharmacology, 477: 9-16.
21. Shinoda H, Takeyama S, Suzuki K, Murakami S, Yamada S (2008) Pharmacological topics of bone metabolism: a novel bisphosphonate for the treatment of periodontitis. Journal of pharmacological sciences, 106: 555-558.
22. Chacon GE, Stine EA, Larsen PE, Beck FM, McGlumphy EA (2006) Effect of alendronate on endosseous implant integration: an in vivo study in rabbits. Journal of oral and maxillofacial surgery 64: 1005-1009.
23. Farina AR, Cappabianca L, Di Ianni N, Ruggeri P, Ragone M, Merolle S, Gulino A, Mackay AR (2012) Alendronate promotes plasmin- mediated MMP- 9 inactivation by exposing cryptic plasmin degradation sites within the MMP- 9 catalytic domain. FEBS letters, 586: 2366-2374.
24. Reddy MS, Weatherford TW, Smith CA, West BD, Jeffcoat MK, Jacks TM (1995) Alendronate Treatment of Naturally- Occurring Periodontitis in Beagle Dogs. Journal of periodontology, 66: 211-217.
25. Yaffe A, Golomb G, Breuer E, Binderman I (2000) The effect of topical delivery of novel bisacylphosphonates in reducing alveolar bone loss in the rat model. Journal of periodontology, 71: 1607-1612.
26. Yaffe A, Herman A, Bahar H, Binderman I (2003) Combined local application of tetracycline and bisphosphonate reduces alveolar bone resorption in rats. Journal of periodontology 74: 1038-1042.
27. De Almeida J, Ervolino E, Bonfietti LH, Novaes VCN, Theodoro LH, Fernandes LA, Martins TM, Faleiros PL Garcia VG (2015) Adjuvant therapy with sodium alendronate for the treatment of experimental periodontitis in rats. Journal of periodontology 86: 1166-1175.
28. Ayranci F, Gungormus M, Omezli MM, Gundogdu B (2015) The effect of alendronate on various graft materials used in maxillary sinus augmentation: a rabbit study. Iranian Red Crescent Medical Journal 17.
29. Özer T, Akta A, Barı E, Çelik HH, Vatansever, A. (2017) Effects of local alendronate administration on bone defect healing. Histomorphometric and radiological evaluation in a rabbit model. Acta cirurgica brasileira, 32: 781-795.
30. Kim SE, Yun Y-P, Lee D-W, Kang EY, Jeong WJ, Lee B, Jeong MS, Kim HJ, Park K, Song H-R (2015) Alendronate-eluting biphasic calcium phosphate (BCP) scaffolds stimulate

osteogenic differentiation. Bio Med research international.

31. Kaynak D, Meffert R, Günhan M, Günhan Ö, Özkaya Ö (2000) A histopathological investigation on the effects of the bisphosphonate alendronate on resorptive phase following mucoperiosteal flap surgery in the mandible of rats. Journal of periodontology, 71: 790-796.
32. Xu H, Shimizu Y, Onodera K, Ooya K (2005) Long-term outcome of augmentation of the maxillary sinus using deproteinised bone particles experimental study in rabbits. British Journal of Oral and Maxillofacial Surgery 43: 40-45.
33. Sohn D-S, Kim W-S, An K-M, Song K-J, Lee J-M, Mun Y-S (2010) Comparative histomorphometric analysis of maxillary sinus augmentation with and without bone grafting in rabbit. Implant dentistry 19: 259-270.
34. Moon Y-S, Sohn D-S, Moon J-W, Lee J-H, Park I-S, Lee J-K (2014) Comparative histomorphometric analysis of maxillary sinus augmentation with absorbable collagen membrane and osteoinductive replaceable bony window in rabbits. Implant dentistry, 23: 29-36.
35. Lim H-C, Zhang M-L, Lee J-S, Jung U-W, Choi S-H (2015) Effect of different hydroxyapatite: ß-tricalcium phosphate ratios on the osteoconductivity of biphasic calcium phosphate in the rabbit sinus model. International Journal of Oral & Maxillofacial Implants, 30.
36. Yang C, Unursaikhan O, Lee JS, Jung UW, Kim CS, Choi SH (2014) Osteoconductivity and biodegradation of synthetic bone substitutes with different tricalcium phosphate contents in rabbits. Journal of Biomedical Materials Research Part B: Applied Biomaterials 102: 80-88.
37. Galindo- Moreno P, Moreno- Riestra I, Avila G, Padial- Molina M, Paya JA, Wang HL, O'valle F (2011) Effect of anorganic bovine bone to autogenous cortical bone ratio upon bone remodeling patterns following maxillary sinus augmentation. Clinical Oral Implants Research, 22: 857-864.
38. Yang C, Unursaikhan O, Lee JS, Jung UW, Kim CS, Choi SH (2014) Osteoconductivity and biodegradation of synthetic bone substitutes with different tricalcium phosphate contents in rabbits. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 102: 80-88.
39. Hameed ZM (2018) The osteoconductive influence of biphasic calcium phosphate when augmented in a rabbit sinus with different hydroxyapatite:b-tricalcium phosphate ratios(60:40)and (30:70):a histological and histomorphometric study. Biochem. Cell. Arch., 18 (1): 535-541
40. Artzi Z, Weinreb M, Givol N, Rohrer MD, Nemcovsky CE, Prasad HS, Tal H (2004) Biomaterial Resorption Rate and Healing Site Morphology of Inorganic Bovine Bone and ß-Tricalcium Phosphate in the Canine: A 24-month Longitudinal Histologic Study and Morphometric Analysis. International Journal of Oral & Maxillofacial Implants, 19.
41. de Souza Vieira J, Giovanini A, Görhinger I, Gonzaga CC, Costa-Casagrande TA, Deliberador TM (2017) Use of Low-Dose Alendronate Improves Cranial Bone Repair and Is Associated With an Increase of Osteocalcin: An Experimental Study. Journal of oral and maxillofacial surgery, 75: 1873-1881.
42. Bonnet N, Lesclous P, Saffar JL, Ferrari S (2013) Zoledronate effects on systemic and jaw osteopenias in ovariectomized periostin-deficient mice. PLoS One 8: e58726.
43. Pradeep AR, Kanoriya D, Singhal S, Garg V, Manohar B, Chatterjee A (2017) Comparative evaluation of subgingivally delivered 1% alendronate versus 1.2% atorvastatin gel in treatment of chronic periodontitis: a randomized placebo-controlled clinical trial. Journal of investigative and clinical dentistry 8.

