

# Clinical and histopathological comparative study of two equine-derived bone graft: a human study

Selen Adiloglu<sup>1</sup>, Calal Bahadır Giray<sup>2</sup>, Ibrahim Kulac<sup>3</sup>, Alp Usubutun<sup>4</sup>, Alper Aktas<sup>5</sup>

## Abstract

**Objective:** To compare the performance of collagenated bone graft substances with different collagen ratios after sinus floor augmentation.

**Methods:** The cross-sectional study was conducted at Hacettepe University, Ankara, Turkey, from September 2011 to September 2013. Sinus floor augmentation was done with two different equine-derived xenografts in patients before dental implant application. Of the two randomised groups, one was treated with 100% collagenated bone mix (Group A), and the other half with 90% collagenated bone mix + 10% collagen gel (Group B). Six months after sinus augmentation, prior to dental implant surgery, a specimen was taken from the implant socket with trephine drill for histopathological evaluation of new bone, connective tissue and residual graft material at each augmented site. SPSS 19 was used for data analysis.

**Results:** Of the 19 patients, 12(63%) were females and 7(37%) were males. The overall mean age was 51.68±11.96 years (range: 24-69 years). A total of 30 sinus floor augmentations were done. New bone formation was significantly better in Group A(15 sinus floor augmentation) than in Group B (the other 15 sinus floor augmentation) ( $p<0.05$ ), but there was no significant difference in connective tissue formation and residual graft materials between the groups ( $p>0.05$ ).

**Conclusion:** Collagenated bone mix was found to be a suitable graft material for sinus floor augmentation, but increased collagen ratio did not improve new bone formation over the 6-month healing process.

**Keywords:** Equine-derived graft materials, Sinus floor augmentation, New bone formation, Connective tissue, Human study. (JPMA 69: 1617; 2019). doi: 10.5455/JPMA.296194.

## Introduction

The limited crest height can be a contraindication for implant surgery because the bone volume is not adequate for dental implant stabilisation.<sup>1</sup> Maxillary sinus floor augmentation is a preferable approach for the rehabilitation of severely resorbed posterior edentulous maxilla with implant-supported prosthesis. Although surgical techniques of sinus floor augmentation in order to apply dental implant to severely resorbed edentulous posterior maxilla are well described,<sup>2</sup> the preferred material for bone graft remains unclear.<sup>1</sup>

Intraoral and extraoral autologous bone grafts (ABGs) are known as gold standard because of their high

capacity to promote osteogenesis and optimal ability to incorporate into normal tissue without immunological sequelae.<sup>1,3</sup> Nonetheless, ABGs have some disadvantages, including secondary surgical zones,<sup>4</sup> undesirable postoperative symptoms,<sup>5</sup> prolonged surgical time,<sup>6</sup> limited yield of graft materials in quantity, and severe resorption.<sup>2</sup> These disadvantages have led to new research studies on novel graft materials that can reduce surgical time,<sup>7</sup> increase application easiness, and have a higher capacity of new bone formation.<sup>8</sup> Consequently, bone grafts alternative to ABGs were investigated, including allogenic-, xenogenic-, and alloplastic-derived materials.<sup>1,3</sup>

Xenografts, obtained from various sources, like bovine, porcine and equine, have osteoconductive properties,<sup>9</sup> and numerous studies have been conducted on the positive effect of xenograft on the healing of bone

<sup>1,5</sup>Department of Oral and Maxillofacial Surgery, Hacettepe University, Ankara, Turkey; <sup>2</sup>Private Practice, Ankara, Turkey; <sup>3,4</sup>Department of Pathology, Hacettepe University, Ankara, Turkey.

**Correspondence:** Alper Aktas. e-mail: alperaktas@gmail.com

defects.<sup>10,11</sup> Because of this osteoconductive property, the xenografts can be a scaffold for osteogenic cells, such as osteoblasts.<sup>6,8</sup> Many clinical and histological studies have reported the use of xenograft for sinus floor augmentation, but most of the studied xenografts were from bovine or porcine sources, and were used in combination with allogenic, alloplastic, or autogenous grafts.<sup>1-3,5</sup> The current study was planned to investigate two different xenografts of equine origin that differed in physical form and collagen composition.

## Materials and Methods

The cross-sectional study was conducted at the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Hacettepe University, Ankara, Turkey, from September 2011 to September 2013. Sinus floor augmentation procedures were performed after approval was obtained from the institutional ethics committee and informed consent from all the subjects. Patients with 4 mm or less alveolar bone height in the posterior maxilla were included, while those with uncontrolled and severe systematic diseases and contraindications for a sinus floor augmentation and dental implant application were excluded. Bone heights at implant sites were measured on orthopantomographs and the average bone height was 3mm.

Two types of graft materials, namely 100% collagenated bone mix (100CBM) (Gen-Os®250-1000 µm OsteoBiol, TecnoSS Dental, Torino, Italy) and 90%CBM + 10% collagen gel (CG) (Mp3® 600-1000 µm OsteoBiol, TecnoSS Dental, Torino, Italy), were used, and both the materials were of equine origin. Their granule size were approximately similar in the same chemical composition, but the collagen concentrations (Mp3 has additional 10% collagen gel) and physical form (Mp3® is injectable, Gen-Os® is granular form) were different. Both the materials consist of heterogeneous, osteoconductive, biocompatible and bioavailable collagenated bone matrix.

Surgical procedures were performed by the same surgical team. All patients underwent maxillary sinus floor elevation surgery through the lateral window approach under local anaesthesia with articaine hydrochloride (HCl) with epinephrine (1:200,000) (Maxicaine VEM ilaç, Istanbul, Turkey). The two equal groups of patients that differed only in the type of the graft material were formed randomly. After membrane elevation, the space was

filled with 2 different equine graft materials (1.5-2 cc); half of the sinuses were filled 100CBM and the other half with 90CBM/10CG. After the graft material was placed, it was delicately condensed. In both groups, the lateral bony windows were covered with a resorbable collagen membrane (Evolution Membrane 20x20 mm, OsteoBiol, TecnoSS Dental, Torino, Italy). The operation sites were closed primarily with number 3/0 vicryl sutures, which were removed 10 days postoperatively.

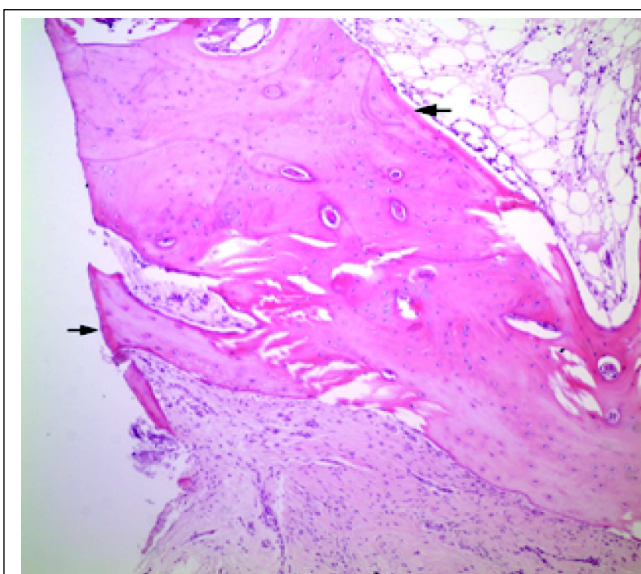
All the patients were prescribed 1g of amoxicillin and clavulanate twice a day, 550mg naproxen sodium three times a day, and chlorhexidine gluconate and benzydamine HCl three times a day, starting prior to surgery.

In all patients, implant insertions were planned at 6 months after sinus floor augmentation. During this period, orthopantomographs were taken at 1, 3 and 6 months to evaluate any complications. At 6 months, all patients underwent local anaesthesia for dental implant insertion. An incision was then performed, and a mucoperiosteal flap was elevated. A biopsy was taken from the implant site by a trephine drill (2.9mm inner diameter, 4.0mm outer diameter, Komet, GEBR, BRASSELER). At 100CBM (Group A) and 90CBM/10CG (Group B) augmented sites, dental implants were applied. All retrieved grafted bone specimens were fixed by immersion in 10% neutral formaldehyde solution for at least 48 hours for histopathological analysis.

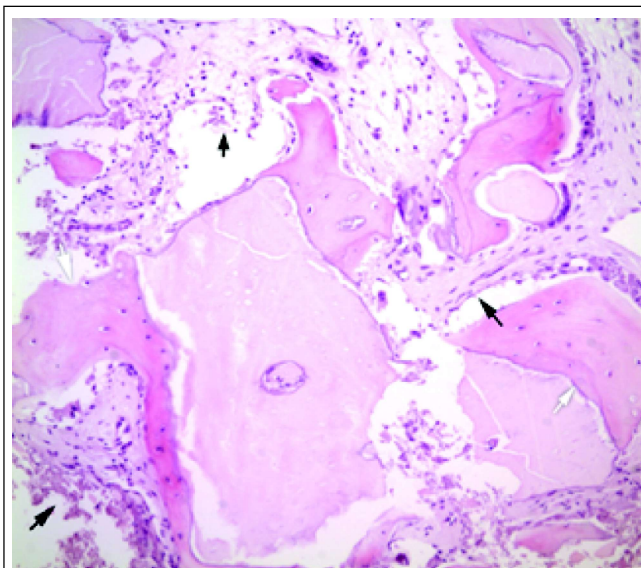
Bone specimens were decalcified by 10% formic acid for 24 hours. Following decalcification, the specimens were dehydrated by placing them in alcohol and xylol. The samples then were embedded in paraffin wax. These blocks were cut and ground to approximately 4µm thickness, and then stained with haematoxylin and eosin (H&E).

The stained sections were evaluated under light microscope by a histopathologist who was blinded to the type of graft material. The percentage of each tissue, newly formed bone, connective tissue, residual graft materials in the sections were calculated (Figures 1-2).

SPSS 19 was used for data analysis. Mean values and standard deviation (SD) and the difference in the histopathological parameters between the two graft materials were analysed by Mann-Whitney U test, and Fisher exact tests was preferred for the evaluation of the implant success.



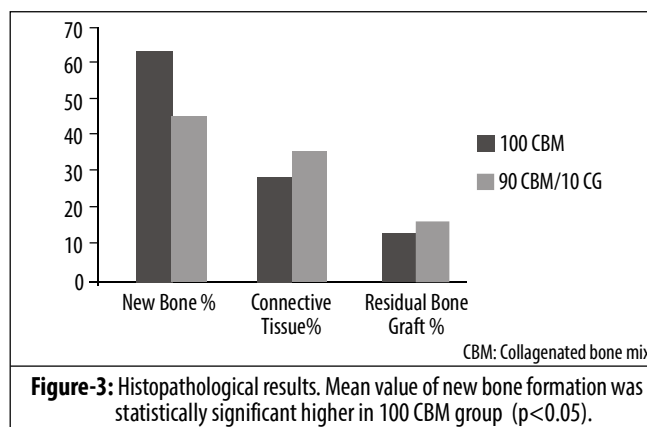
**Figure-1:** Histopathological photograph of the bone sample taken from sinus augmented with 100 CBM. Large amount of new bone formation can be seen over connective tissue. Black arrows: Large amount of new bone formation. (CBM: Collagenated bone mix.)



**Figure-2:** Histopathological photograph of the bone sample taken from sinus augmented with 90CBM/10CG. New bone formation can be seen beneath host bone. Large amount of connective tissue can be observed all around the sample. Black arrows: Large amount of connective tissue formation. White arrow: New bone formation. (CBM: Collagenated bone mix; CG: Collagen gel.)

## Results

Of the 19 patients, 12(633%) were females and 7(37%) were males. The overall mean age was  $51.68 \pm 11.96$  years (range: 24-69 years). Sinus floor augmentations were



done bilaterally in 11 patients (57.9%) and unilaterally in 8 patients (42.1%) in total 30 sinus floor augmentations were performed. 90CBM/10CG<sup>15</sup> - 100CBM<sup>15</sup> were applied for 30 sinus floor augmentation. Perforation of the Schneiderian membrane ( $\leq 2$  mm) was seen in 3(10%) augmentations, and collagen membrane was applied. Postoperative complications, such as sinusitis, infection, bleeding, wound dehiscence and pain, were not observed at the follow-up period till the second surgery.

In Group A, the mean values of residual graft materials, new bone formation and connective tissue formation were  $8.6 \pm 6.3\%$ ,  $63.6 \pm 19.5\%$  and  $28.6 \pm 18.9\%$  respectively. In Group B, the corresponding values were  $19 \pm 19.4\%$ ,  $45.3 \pm 23\%$ , and  $35.7 \pm 14.9\%$  respectively (Figure 3).

Histopathologically, there was no statistically significant difference between the two graft materials in terms of residual graft materials ( $p > 0.05$ ). A significant difference was observed for new bone formation ( $p = 0.030$ ). Group A showed more new bone formation than Group B ( $p = 0.269$ ) for connective tissue formation between the two groups.

Implant failures were observed in 3(7.5%) of the dental implants during prosthetic abutment fixture. All the failures were observed in Group A. The rest of the dental implants in Group A were successfully osteointegrated in the augmented sites and the same was the case with all(100%) the implants in Group B ( $p > 0.05$ ). After the mean loading period of  $6 \pm 2$  years, the survival rate was 100%.

## Discussion

Many bone substitutes have been studied, particularly with regard to the host immune response and new bone forming capability. The aim of these studies was to

identify the best material for use in augmentation. The present study aimed at increasing the knowledge on equine bone graft material.

Maxillary sinus floor augmentation is generally mandatory for dental implant treatment because of severe resorption. Thus far, several graft materials have been investigated by different researchers. Autogenous grafts have been approved as gold standard in bone augmentation because of their osteogenetic and osteoinductive characteristics.<sup>12</sup> Many studies have reported successful results for autogen, allogeneic and xenogeneic graft materials when used alone or in combination for maxillary sinus floor augmentation. These studies have discussed the advantages and disadvantages of different graft materials.<sup>5,13-15</sup>

Studies have investigated xenogeneic grafts alone and in combination with autogenous grafts.<sup>5,16</sup> A study examined deproteinised bovine bone (DBB) and polylactic-co-glycolic acid / hydroxyapatite (PLGA/HA) augmentation after sinus floor elevation. Six months after grafting, it found that DBB had  $27.51\% \pm 9.39$  and PLGA/HA had  $44.45\% \pm 17.62$  new bone tissue.<sup>16</sup> Another study used equine-derived bone grafts for sinus floor augmentation, and at 6 months after augmentation, it found  $39.84 \pm 2.96\%$  new bone formation.<sup>5</sup> In the current study, new bone formation was 63.6% for 100CBM and 45.3% for 90CBM/10CG. The difference between the two graft materials seems to be related to the collagen ratio. Similar to earlier findings,<sup>5,16</sup> graft materials used in the current study showed higher ability for new bone formation. This result may be related to increased collagen rate in the used graft materials.

A study found residual graft material  $0 \pm 0\%$  for PLGA/HA grafts and  $16.6 \pm 8.62\%$  for DBB grafts.<sup>16</sup> The other study found that equine-derived bone graft yielded 33% residual bone graft.<sup>5</sup> In the present study, residual bone graft of 8.6% for 100CBM and 19% for 90CBM/10CG seems better than those reported earlier.<sup>5,16</sup> In a study, the PLGA/HA group had the least per cent of residual bone grafts, but the amount of newly formed bone was lower than that for the equine-derived graft materials. It linked the result to the limited number of specimens it had obtained, and commented that newly-formed bones seem to be the most important data for comparing graft healing, and insisted on the importance of newly-formed bone percentage.<sup>15</sup> These findings confirmed that the graft used in the present study can be a good

candidate for routine clinical usage.

One study evaluated radiologically and histomorphologically two different xenografts (OsteoBioL Mp3®- porcine and Endobone® bovine) on a tibia defect model of rabbit. It concluded that both graft materials had osteointegrative capacity and fibrosis was not observed at the augmented sites.<sup>6</sup> In another study, sinus floor augmentation was performed using equine-derived graft materials (Bio-Gen Mix, Bioteck, Vercelli, Italy), and these materials were compared in terms of new bone tissue, existing fibrous tissue, and residual graft materials. There was no foreign body reaction and immunological response.<sup>5</sup> The present study showed that augmentation with these graft materials did not cause fibrosis.

There are many opinions on the osteoclastic activity at augmented sites with the application of xenografts. In some studies, osteoclastic activity was related to bone apposition.<sup>17</sup> A study dwelled on the penetration of new bone between pores of the graft particles as a sign of graft degradation and reported a mean bone graft density at 7.1 months of 29.6%.<sup>18</sup> In another bovine-derived graft material study, a study explained the mechanism of conversion of the graft material to bone by observing that woven bone was formed first between graft particles, then tissue mineralisation occurred, and, finally, mature lamellar bone remodelling was completed.<sup>19</sup> In the present study, new bone formation, mineralisation, and maturation were compared with the ratio of bone formation between two equine-derived graft materials. For histopathological analysis, samples of bone tissue, consisting of mature and woven bone between graft particles, as described in a study, were collected at 6 months after the surgery.<sup>19</sup>

When autogenous graft and bovine-derived graft materials were used together for sinus floor augmentation in a histopathological study, new bone formation was found to occur at the sides closer to the sinus floor, but bone apposition was not recognised near the sinus membrane.<sup>19</sup> Studies have showed that a high incidence of new bone formation could be achieved near the bone borders of the augmented sinus, although graft particles enclosed with fibrous cover could be seen at distant sides from the bone.<sup>20,21</sup> Bone formation was observed evenly at each section for the 90CBM/10CG group in our study, but in the 100CBM group, bone formation was seen on the outer sides and a high number of graft particles was observed closer to the sinus membrane.

The shape, size and morphogenic properties of graft materials play a role in the activity of graft material. Surface area and the size of pores in addition to chemical composition affects osteogenic cell activation at the surface of the biomaterials.<sup>22,23</sup> Particle size increases the contact area and also affects the condensation properties of graft materials.<sup>22</sup> In the present study, new bone formation of the 100CBM material was similar to that of human bone. Because the graft materials used for the present study were similar, the effect of porosity or density of graft materials could not be evaluated. Even though one of the graft materials was in granular form, an assessment of the particle size could not be performed because there was no standard size of graft particles. The manufacturing company specifies that 100CBM has 250 to 1000- $\mu$ m particle size and 90CBM/10CG has 600 to 1000- $\mu$ m particle size.<sup>11</sup>

A study showed that the application of injectable allograft materials was 11% faster (7.75 min) than that for the control group.<sup>24</sup> Another study indicated that easy application of injectable graft material to an irregular bone cavity reduced operation time and minimised surgical trauma.<sup>25</sup> A study claimed that injectable graft materials transmit lower strength to sinus membrane.<sup>24</sup> Our study showed that the application of injectable 90CBM/10CG graft material was faster and easier than 100CBM application. Further studies can be done to record surgery time for manipulation easiness of the injectable graft materials.

The present study showed that equine-derived bone graft material could be used as an alternative material for sinus floor augmentation because new formation was histopathologically and clinically successful. The density of the new bone was quite similar to human bone, and the surface area was sufficient for successful grafting. In addition, the injectable form of graft material can be an easy and fast alternative for application.

To analyse the effect of increased collagen amount on bone formation, further studies need to be done with short sample collection periods.

## Conclusion

Equine-derived bone graft material could be used for sinus floor augmentation for adequate new bone formation and successful dental implant rehabilitation.

**Disclaimer:** The study is part of a PhD thesis.

**Conflict of Interest:** None.

**Source of Funding:** None.

## References

1. Scarano A, Degidi M, Iezzi G, Pecora G, Piattelli M, Orsini G, et al. Maxillary sinus augmentation with different biomaterials: a comparative histologic and histomorphometric study in man. *Implant Dent* 2006; 15: 197-207.
2. Lutz R, Berger-Fink S, Stockmann P, Neukam FW, Schlegel KA. Sinus floor augmentation with autogenous bone vs. a bovine-derived xenograft - a 5-year retrospective study. *Clin Oral Implants Res* 2015; 26: 644-8.
3. Zerbo IR, Zijderveld SA, de Boer A, Bronckers AL, de Lange G, ten Bruggenkate CM, et al. Histomorphometry of human sinus floor augmentation using a porous beta-tricalcium phosphate: a prospective study. *Clin Oral Implants Res* 2004; 15: 724-32.
4. Nkenke E, Radespiel-Troger M, Wiltfang J, Schultze-Mosgau S, Winkler G, Neukam FW. Morbidity of harvesting of retromolar bone grafts: a prospective study. *Clin Oral Implants Res* 2002; 13: 514-21.
5. Rivara F, Negri M, Lumetti S, Parisi L, Toffoli A, Calciolari E, et al. Maxillary Sinus Floor Augmentation Using an Equine-Derived Graft Material: Preliminary Results in 17 Patients. *BioMed Res Int* 2017; 2017: 9164156.
6. Ramirez-Fernandez M, Calvo-Guirado JL, Delgado-Ruiz RA, Mate-Sanchez del Val JE, Vicente-Ortega V, Meseguer-Olmos L. Bone response to hydroxyapatites with open porosity of animal origin (porcine [OsteoBiol mp3] and bovine [Endobon]): a radiological and histomorphometric study. *Clin Oral Implants Res* 2011; 22: 767-73.
7. Arcuri C, Cecchetti F, Germano F, Motta A, Santacroce C. Clinical and histological study of a xenogenic bone substitute used as a filler in postextractive alveolus. *Minerva Stomatologica* 2005; 54: 351-62.
8. Pikkdoken L, Gurbuzer B, Kucukodaci Z, Urhan M, Baris E, Tezulas E. Scintigraphic, histologic, and histomorphometric analyses of bovine bone mineral and autogenous bone mixture in sinus floor augmentation: a randomized controlled trial--results after 4 months of healing. *J Oral Maxillofac Surg* 2011; 69: 160-9.
9. Santos FA, Pochaspski MT, Martins MC, Zenobio EG, Spolidoro LC, Marcantonio E. Comparison of biomaterial implants in the dental socket: histological analysis in dogs. *Clin Implant Dent Relat Res* 2010; 12: 18-25.
10. Orsini G, Scarano A, Piattelli M, Piccirilli M, Caputi S, Piattelli A. Histologic and ultrastructural analysis of regenerated bone in maxillary sinus augmentation using a porcine bone-derived biomaterial. *J Periodontol* 2006; 77: 1984-90.
11. Nannmark U, Sennerby L. The bone tissue responses to prehydrated and collagenated cortico-cancellous porcine bone grafts: a study in rabbit maxillary defects. *Clin Implant Dent Relat Res* 2008; 10: 264-70.
12. Canullo L, Dellavia C. Sinus lift using a nanocrystalline hydroxyapatite silica gel in severely resorbed maxillae: histological preliminary study. *Clin Implant Dent Relat Res* 2009; 11 Suppl 1: e7-13.
13. Furst G, Gruber R, Tangl S, Zechner W, Haas R, Mailath G, et al. Sinus grafting with autogenous platelet-rich plasma and bovine hydroxyapatite. A histomorphometric study in minipigs. *Clin Oral Implants Res* 2003; 14: 500-8.
14. Velich N, Nemeth Z, Toth C, Szabo G. Long-term results with different bone substitutes used for sinus floor elevation. *J Craniofac Surg* 2004; 15: 38-41.
15. Peker E, Karaca IR, Yildirim B. Experimental evaluation of the effectiveness of demineralized bone matrix and collagenated heterologous bone graft used alone or in combination with platelet-rich fibrin on bone healing in sinus floor augmentation. *Int J Oral Maxillofac Implants* 2016; 31: e 24-31.

16. Marco P, Marco C, Floriana L, Gabriele C, Maurizio M, Enrico G, et al. Histomorphometric Evaluation of Two Different Bone Substitutes in Sinus Floor Augmentation Procedures. *J Craniofac Surg*, 2017. Published Ahead-of-Print
  17. Cabbar F, Guler N, Kurkcu M, Iseri U, Sencift K. The effect of bovine bone graft with or without platelet-rich plasma on maxillary sinus floor augmentation. *J Oral Maxillofac Surg* 2011; 69: 2537-47.
  18. Yildirim M, Spiekermann H, Handt S, Edelhoff D. Maxillary sinus augmentation with the xenograft Bio-Oss and autogenous intraoral bone for qualitative improvement of the implant site: a histologic and histomorphometric clinical study in humans. *Int J Oral Maxillofac Implants* 2001; 16: 23-33.
  19. Tadjoedin ES, de Lange GL, Bronckers AL, Lyaruu DM, Burger EH. Deproteinized cancellous bovine bone (Bio-Oss) as bone substitute for sinus floor elevation. A retrospective, histomorphometrical study of five cases. *J Clin Periodontol* 2003; 30: 261-70.
  20. Stavropoulos A, Karring T, Kostopoulos L. Fully vs. partially rough implants in maxillary sinus floor augmentation: a randomized-controlled clinical trial. *Clin Oral Implants Res* 2007; 18: 95-102.
  21. Stavropoulos A, Kostopoulos L, Nyengaard JR, Karring T. Deproteinized bovine bone (Bio-Oss) and bioactive glass (Biogran) arrest bone formation when used as an adjunct to guided tissue regeneration (GTR): an experimental study in the rat. *J Clin Periodontol* 2003; 30: 636-43.
  22. Figueiredo M, Henriques J, Martins G, Guerra F, Judas F, Figueiredo H. Physicochemical characterization of biomaterials commonly used in dentistry as bone substitutes—comparison with human bone. *J Biomed Mater Res B Appl Biomater* 2010; 92: 409-19.
  23. Li X, van Blitterswijk CA, Feng Q, Cui F, Watari F. The effect of calcium phosphate microstructure on bone-related cells in vitro. *Biomaterials* 2008; 29: 3306-16.
  24. Irinakis T. Efficacy of injectable demineralized bone matrix as graft material during sinus elevation surgery with simultaneous implant placement in the posterior maxilla: clinical evaluation of 49 sinuses. *J Oral Maxillofac Surg* 2011; 69: 134-41.
  25. Zhang W, Wang X, Wang S, Zhao J, Xu L, Zhu C, et al. The use of injectable sonication-induced silk hydrogel for VEGF(165) and BMP-2 delivery for elevation of the maxillary sinus floor. *Biomaterials* 2011; 32: 9415-24.
-