USE OF ANORGANIC BOVINE BONE MATRIX IN AN EXPERIMENTAL MODEL OF BONE HEALING

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ABSTRACT

The dimensions of the alveolar bone surrounding the tooth are not maintained post tooth-extraction probably as a consequence of the bone remodeling process and the biomechanical demands on bone. The use of biomaterials as bone substitutes in the post-tooth-extraction socket promotes bone repair, regardless of damage to bone structures during the surgical procedures.

The aim of the present study was to evaluate the effectiveness of anorganic bovine bone matrix (ABBM) as a bone substitute, in an experimental model of post-tooth extraction bone healing in the rat. Radiographic follow-up was performed at 7, 14, and 30 days, and showed persistence of the biomaterial inside the experimental alveoli.

At 14 and 30 days post-tooth extraction, particles surrounded by bone tissue were observed in the middle sector of the alveoli. The osteoconductive property of ABBM was demonstrated using the present experimental model of active osteogenesis, thus showing its usefulness as a bone substitute. Persistence of the particles at the studied experimental time points did not affect post-tooth extraction bone healing.

Key words: *Bone substitutes - bone healing - tooth extraction - rat.*

UTILIZACIÓN DE MATRIZ ÓSEA ESPONJOSA ANORGÁNICA BOVINA EN UN MODELO EXPERIMENTAL DE REPARACIÓN ÓSEA

RESUMEN

Las dimensiones del hueso alveolar que rodea a la pieza dentaria, no se mantienen después de la exodoncia. Este hecho sería consecuencia del proceso de remodelado óseo y del requerimiento biomecánico. La utilización de biomateriales como sustitutos óseos en los alvéolos, facilitan o promueven la reparación ósea, independientemente que se haya producido traumatismo de las estructuras óseas durante la maniobra quirúrgica.

El objetivo del presente estudio fue evaluar la efectividad de una matriz ósea esponjosa anorgánica (MOEA) como sustituto óseo, en un modelo experimental de reparación ósea en el alvéolo post-extracción en ratas. Se realizó el estudio radiográfico en los distintos tiempos experimentales: 7, 14 y 30 días, evidenciando la persistencia del biomaterial. A los 14 y 30 días post-exodoncia se evidenciaron las partículas rodeadas de tejido óseo en el sector medio del alvéolo. Es importante destacar que la utilización de (MOEA), como sustituto óseo en el alvéolo post-exodoncia de rata, evidenció su capacidad osteoconductiva. La persistencia de las partículas del biomaterial en los tiempos estudiados no interfirió en la reparación ósea.

Palabras clave: *Sustituto óseo - reparación ósea - exodoncia - ratas.*

INTRODUCTION

Post tooth-extraction socket bone healing requires approximately two months before complete bone repair can be observed, after which bone remodeling takes place[1,4].

Although it holds true that the reported descriptions of the stages of bone repair from the moment the tooth is extracted are based on histologic and histomorphometric studies performed in experimental models in rats and dogs, among other experimental animals[5-9], it is also well documented that observations in animal models apply to humans, rendering post-tooth extraction socket healing in animals and humans comparable[10-12]. It is also well documented that bone tissue metabolism in each of the stages and the mechanisms involved in socket bone healing are species specific, differing between animals and humans. For example, post tooth-extraction socket healing is slower in humans than in dogs[13], and the metabolic activity index is faster in rats than in humans[14].

The dimensions of the alveolar bone surrounding the tooth are not maintained post tooth-extraction[1-4],

probably as a consequence of the bone remodeling process and the biomechanical demands on bone[15].
The use of biomaterials as bone substitutes in the post-tooth extraction socket promotes bone repair, regardless of damage to bone structures associated with the surgical procedures[3,16-17].
There are different types of bone substitutes, each of which exhibits different properties:

1) Osteoinduction: It implies chemotaxis, mitosis, and differentiation of mesenchymal cells to osteoblasts or chondroblasts; eg, demineralized bone powder[18-22].
2) Osteoconduction: The implanted biomaterial serves as a scaffold for osteoblasts; such is the case of bank-bone grafts and hydroxyapatite[23-26].
3) Osteopromotion: The implanted biomaterial stimulates viable osteoblasts; for example: bioactive glass ceramic materials[27-31].

A wide variety of biomaterials has been used in experimental[20,22,25,32-34] and clinical studies[17,27].
Although most grafts are capable of preserving bone tissue volume and contour at the extraction site, there is controversy regarding the quality of the bone that forms around the graft. This issue gains further significance when treatment involves placing an implant, for when bone repair around the filling is not adequate, the physical and biological properties of the newly formed bone tissue are not suitable to meet the biomechanical requirements of the implant. In addition, Irinakis T. emphasized the importance of monitoring peri-implant mucosa, since preserving the bone structures before and after surgical procedures ultimately facilitates reaching the mucosa surrounding the implant-supported prosthesis and maintaining adequate hygiene[35]. Thus, adequate maintenance of peri-implant mucosa has significant clinical implications, given that oral hygiene is an important determinant of long-term treatment success.
The bone graft of choice is human demineralized freeze-dried allografts of cortical and cancellous bone. They are obtained from human bone-banks, and are subjected to a number of treatments, such as fragmentation, saponification, lyophilization, and decalcification, among others. Based on the above, the aim of the present study was to evaluate the effectiveness of Anorganic Bovine Bone Matrix as a bone substitute, in an experimental model of post-tooth extraction bone healing in the rat.

MATERIALS AND METHODS

Thirty male Wistar rats, 70 ± 10 g body weight (b.w.), were used. The animals were anesthetized by intraperitoneal injection of 8 mg of Ketamine (Ketalar®, Parke-Davis, Morris Plains, NJ) and 1.28 mg Xylaxine (Rompum®, Bayer, Leverkusen, Germany) per 100g / b.w. The right and left lower first molars were extracted following the technique described by Guglielmotti et al[5]. Anorganic Bovine Bone Matrix (Osteodens®-Pharmatrix, Argentina) particles ranging in size from 250 to 1000 mm, were placed in the fresh extraction socket of the extracted right mandibular first molar. No filling material was placed in the left post-tooth extraction socket, which served as control.
The guidelines for the care and use of laboratory animals were observed[36]. The experimental protocol was approved by the Ethics Committee of the School of Dentistry of the University of Buenos Aries. The rats were fed regular chow and water ad libitum; no antibiotic therapy was administered.
The animals were euthanized 7, 14, and 30 days post-tooth extraction respectively, and weighed; the mandibles were resected and fixed in 10% formalin solution.
All hemimandibles were radiographed and decalcified in 25% formic acid for 48 hours, and processed for embedding in paraffin. The samples were sectioned in a bucco-lingual orientation at the level of the mesial alveolus of the lower first molar to obtain 5μm to 7μm thick sections. Both experimental and control sections were stained with hematoxylin-eosin.

RESULTS

Radiographic study

A time-dependent increase in radiopacity was observed in control alveoli (Fig. 1a). The presence of the bone substitute, which was more opaque than the newly formed bone tissue, was detected in the experimental group at all experimental time points (Fig. 1b).

Histologic Results

The control group showed the typical features of post-tooth extraction bone repair: granulation tissue filling the alveolus and newly formed woven bone in the apical third of the alveolus at 7days; woven bone filling a large portion of the alveolus at 14 days; and lamellar bone replacing the woven bone at 30 days.

Fig. 1: a: Control group: 30 days post-tooth extraction. Note the presence of radiopaque tissue filling the post-tooth extraction socket of the first molar. b: Experimental group: 30 days post-tooth extraction. Note the presence of bone substitute particles surrounded by the newly formed bone tissue in the post-tooth extraction socket of the first molar.

The histological study of the experimental group showed the presence of bone substitute particles at all the experimental time points. Granulation tissue and woven bone were observed around and close to the particles at 7 days post-tooth extraction, and laminar bone tissue surrounding and aggregating the particles could be seen at 14 and 30 days (Fig.2 a-b, Fig. 3 a-b).

DISCUSSION

Artzi et al[37] studied the influence of porous bovine bone mineral on human extraction socket healing 9 months post-extraction. The authors found newly formed bone characterized by abundance of cellular woven-type bone in the coronal area, while lamellar arrangements could be identified only in the more apical region. They concluded that the biomaterial was "an appropriate biocompatible bone derivative in fresh extraction sockets for ridge preservation".

In agreement with the above, an experimental study in dogs showed the filling biomaterial to be in contact with woven bone first, and with lamellar bone at a later stage[38]. The study also analyzed particle density at different experimental time points until

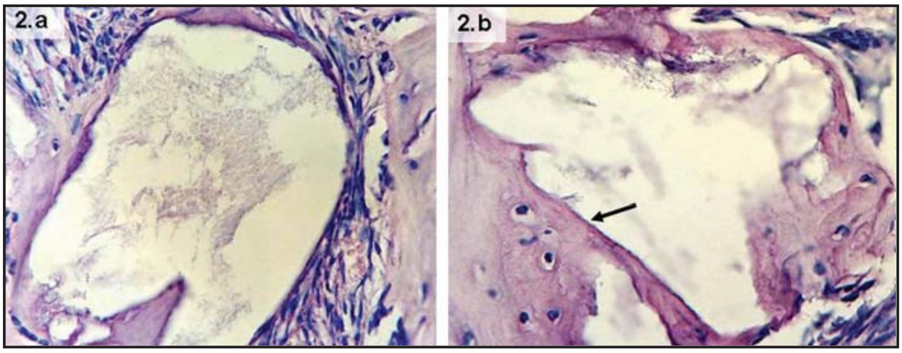

Fig. 2: a: Experimental group: 14 days post-tooth extraction. Note the negative histologic image corresponding to the particles, which were surrounded by bone and granulation tissue. (H-E- Orig. Mag. X40). b: Experimental group: 14 days post-tooth extraction. Note the negative histologic image corresponding to the particles, and the surrounding bone tissue (↑). (H-E – Orig. Mag. X40).

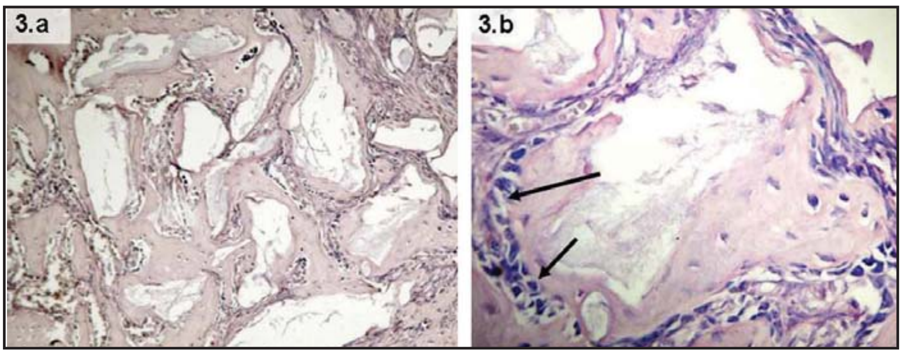

Fig. 3: a:Experimental group: 30 days post-tooth extraction. Note the lamellar bone tissue surrounding the particles, which differ in shape and size. (H-E – Orig. Mag. X10). b: Experimental group: 30 days post-tooth extraction. Higher magnification allows observing the presence of lamellar bone tissue surrounding a particle and covered by osteoblasts (↑). (H-E – Orig. Mag. X40).

particle resorption and replacement by lamellar bone was complete[39].

The aforementioned studies demonstrate the osteoconductive capacity of anorganic bovine bone, which has also been reported in experimental studies in rats and dogs under different experimental conditions, as well as in clinical studies[3, 20-22].

It must be pointed out that the experimental works cited above were performed using the biomaterial alone or in combination with guided tissue regeneration membranes.

The results of the present study demonstrate the osteoconductive capacity of anorganic bovine bone matrix, and show its usefulness as a post-tooth extraction filling biomaterial. The experimental model used herein could serve to further evaluate the effect of systemic and local factors, which previous works by our research group have shown to affect bone healing[40-47].

Other aspects that must be taken into account are the time and mechanisms involved in the resorption of the filling biomaterial. Ideally, this process ends when the material is resorbed or biodegraded and fully replaced by lamellar bone, which is able to withstand biomechanical loads[48]. This process can take from five months to over a year in humans, as shown by Skoglund et al[49] and Avera et al[50], who encountered particles upon surgical reentry 44 months post-placement, and by Paolantonio et al[51], who found that particles persisted 4 years post-placement. The mechanism involved in filling biomaterial resorption is not yet fully understood. Some authors have suggested that osteoclasts are involved, whereas others posit that enzymes play a role in this process[52,53]. Zitzmann et al observed lacunar type resorption, both in areas with bone filling and in those with newly formed bone. It is therefore evident that bone remodeling takes place normally in both areas[53].

Another advantage to this biomaterial is its radiopacity, since it allows performing radiographic follow-up in both animal models and in humans. In fact, Schlegel and Donath[54] performed a 7-year radiographic follow-up of patients receiving a bone substitute.

In the present study, radiographic follow-up was performed at each of the studied time points (7, 14, and 30 days), and confirmed the presence of the filling biomaterial in the experimental alveoli.

CONCLUSION

The experimental model used in the present study demonstrates the osteoconductive properties of locally manufactured anorganic bovine bone matrix, and confirms that it does not affect post-tooth extraction bone healing.

Further studies should be conducted to analyze the bone substitute in combination with a Guided Bone Regeneration membrane.

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REFERENCES

1. Trombelli L, Farina R, Marzola A, Bozzi L, Liljenberg B, Lindhe J. Modeling and remodeling of human extraction sockets. *J Clin Periodontol*. 2008;35:630-639.
2. Tan WL, Wong TL, Wong MC, Lang NP. A systematic review of post-extractional alveolar hard and soft tissue dimensional changes in humans. *Clin Oral Implants Res*. 2012;23:1-21.
3. Zubillaga G, Von Hagen S, Simon BI, Deasy MJ. Changes in alveolar bone height and width following post-extraction ridge augmentation using a fixed bioabsorbible membrane and demineralized freeze-dried bone osteoinductive graft. *J Periodontol* 2003; 74:965-975.
4. Evian CI, Rosenberg ES, Coslet JG, Corn H. The osteogenic activity of bone removed from healing extraction sockets in humans. *J Periodontol*. 1982;53:81-85.
5. Guglielmotti MB, Cabrini RL. Alveolar wound healing and ridge remodeling after tooth extraction in the rat: a histologic, radiographic, and histometric study. *J Oral Maxillofac Surg*. 1985;43:359-364.
6. Araújo MG, Lindhe J. Dimensional ridge alterations following tooth extraction. An experimental study in the dog. *J Clin Periodontol*. 2005;32:212-218.
7. Cardaropoli G, Araujo M, Lindhe J. Dynamics of bone tissue formation in tooth extraction sites. An experimental study in dogs. *J Clin Periodontol*. 2003;30:809-818.

8. Cardaropoli G, Araújo M, Hayacibara R, Sukekava F, Lindhe J. Healing of extraction sockets and surgically produced-augmented and non-augmented-defects in the alveolar ridge. An experimental study in the dog. *J Clin Periodontol*. 2005;32: 435-440.
9. Busenlechner D, Kantor M, Tangl S, Tepper G, Zechner W, Haas R, Watzek G. Alveolar ridge augmentation with a prototype trilayer membrane and various bone grafts: a histomorphometric study in baboons. *Clin Oral Implants Res*. 2005;16:220-227.
10. Lekovic V, Kenney EB, Weinlaender M, Han T, Klokkevold P, Nedic M, Orsini M. A bone regenerative approach to alveolar ridge maintenance following tooth extraction. Report of 10 cases. *J Periodontol*. 1997;68:563-570.
11. Amler MH, Johnson PL, Salman l. Histological and histochemical investigation of human alveolar socket healing in undisturbed extraction wounds. *J Am Dent Assoc*. 1960**;**61: 32-44.
12. Boyne PJ. Osseous repair of the postextraction alveolus in man. *Oral Surg Oral Med Oral Pathol*. 1966;21:805-813.
13. Claflin RS. Healing of disturbed and undisturbed extraction wounds. J Am Dent Assoc. 1936;6:945-959.
14. Amler MH. Age factor in human alveolar bone repair. *J Oral Implantol*. 1993;19:138-142.
15. Pagni G, Pellegrini G, Giannobile WV, Rasperini G. Postextraction alveolar ridge preservation: biological basis and treatments. *Int J Dent*. 2012;2012:151030.
16. Carmagnola D, Adriaens P, Berglundh T. Healing of human extraction sockets filled with Bio-Oss. *Clin Oral Implants Res* 2003;14:137-143.
17. Sbordone L, Bortolaia C, Perrotti V, Pasquantonio G, Petrone G. Clinical and histologic analysis of calcium sulfate in treatment of a post-extraction defect: a case report. *Implant Dent*. 2005;14:82-87.
18. Pinholt EM, Bang G, Haanaes HR, Roervik M. Alveolar ridge augmentation by osteoinduction in rats. *Scand J Dent Res*. 1990;98:434-441.
19. Reddi AH, Weintroub S, Muthukumaran N. Biologic principles of bone induction. *Orthop Clin North Am*. 1987;18:207-212.
20. Guglielmotti MB, Alonso C, Itoiz ME, Cabrini RL. Increased osteogenesis in alveolar wound healing elicited by demineralized bone powder. *J Oral Maxillofac Surg*. 1990;4:487-490.
21. Torricelli P, Fini M, Giavaresi G, Giardino R. In vitro osteoinduction of demineralized bone. *Artif Cells Blood Substit Immobil Biotechnol*. 1998;26:309-315.
22. Hosny M, Sharawy M. Osteoinduction in rhesus monkeys using demineralized bone powder allografts. *J Oral Maxillofac Surg*. 1985;43:837-844.
23. Albrektsson T, Johansson C. Osteoinduction, Osteoconduction and osseointegration. *Eur Spine*. 2001:96-101.
24. Allegrini S Jr, Koening B Jr, Allegrini MR, Yoshimoto M, Gedrange T, Fanghaenel J, Lipski M. Alveolar ridge sockets preservation with bone grafting—review. *Ann Acad Med Stetin*. 2008;54:70-81.
25. Hockers T, Abensur D, Valentini P, Legrand R, Hammerle CH. The combined use of bioresorbable membranes and xenografts or autografts in the treatment of bone defects around implants. A study in beagle dogs. *Clin Oral Implants Res*. 1999;10:487-498.
26. Brandão AC, Brentegani LG, Novaes AB Jr, Grisi MF, Souza SL, Taba Júnior M, Salata LA. Histomorphometric analysis of rat alveolar wound healing with hydroxyapatite alone or associated to BMPs. *Braz Dent J*. 2002;13:147-154.
27. Stvrtecky R, Gorustovich A, Perio C, Guglielmotti MB. A histologic study of bone response to bioactive glass particles used before implant placement: a clinical report. *J Prosthet Dent*. 2003;90:424-428.
28. Gorustovich A, Rosenbusch M, Guglielmotti MB. Characterization of bone around titanium implants and bioactive glass particles: an experimental study in rats. *Int J Oral Maxillofac Implants*. 2002;17:644-650.
29. Gorustovich AA, López JM, Guglielmotti MB, Cabrini RL. Biological performance of boron-modified bioactive glass particles implanted in rat tibia bone marrow. *Biomed Mater*. 2006;1:100-105.
30. Camargo PM, Lekovic V, Weinlaender M, Klokkevold PR, Kenney EB, Dimitrijevic B, Nedic M, Jancovic S, Orsini M. Influence of bioactive glass on changes in alveolar process dimensions after exodontia. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2000;90:581-586.
31. Teófilo JM, Brentegani LG, Lamano-Carvalho TL. Bone healing in osteoporotic female rats following intra-alveolar grafting of bioactive glass. *Arch Oral Biol.* 2004;49:755-762.
32. Artzi Z, Nemcovsky C. The application of deproteinized bovine bone mineral for ridge preservation prior to implantation. Clinical and histological observations in a case report. *J Periodontol*. 1998;69:1062-1067.
33. Nevins M, Mellonig JT. Enhancement of the damaged edentulous ridge to receive dental implants: a combination of allograft and GORE-TEX membrane. *Int J Periodontics Restorative Dent* 1992;12:96-111.
34. Mellonig JT, Nevins M, Sanchez R. Evaluation of a bioabsorbable physical barrier for guided bone regeneration. Part ll. Material and bone replacement graft. *Int J Periodontics Restorative Dent*. 1998;18:129-137.
35. Irinakis T. Rationale for socket preservation after extraction of a single-rooted tooth when planning for future implant placement. *J Can Dent Assoc* 2006;72:917-922.
36. Institute for Laboratory Animal Research 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
37. Artzi Z, Tal H, Dayan D. Porous bovine bone mineral in healing of human extraction sockets. Part 1: histomorphometric evaluations at 9 months. *J Periodontol*. 2000;71: 1015-1023.
38. Berglundh T, Lindhe J. Healing around implants placed in bone defects treated with Bio-Oss. An experimental study in the dog. *Clin Oral Implants Res* 1997**;**8:117-124.
39. Artzi Z, Weinreb M, Givol N, Rohrer MD, Nemcovsky CE, Prasad HS, Tal H. Biomaterial resorption rate and healing site morphology of inorganic bovine bone and beta-tricalcium phosphate in the canine: a 24-month longitudinal histologic study and morphometric analysis. *Int J Oral Maxillofac Implants*. 2004;19:357-368.
40. Guglielmotti MB, Ubios AM, Cabrini RL. Alveolar wound healing after x-irradiation: a histologic, radiographic, and histometric study. *J Oral Maxillofac Surg*. 1986;44:972-976.
41. Ubios AM, Guglielmotti MB, Cabrini RL. Effect of diphosphonate on the prevention of X-radiation-induced

inhibition of bone formation in rats. *J Oral Pathol*. 1986;15: 500-505.
42. Ubios AM, Jares Furno G, Guglielmotti MB. Prevention of post-irradiation alveolar bone resorption by diphosphonate. *Acta Odontol Latinoam*. 1986;3:3-6
43. Gorustovich A, Veinstein F, Costa OR, Guglielmotti MB .Histomorphometric evaluation of the effect of bovine collagen granules on bone healing. An experimental study in rats. Acta Odontol Latinoam. 2004;17:9-13.
44. Giglio MJ, Gorustovich A, Guglielmotti MB. Bone healing under experimental anemia in rats. *Acta Odontol Latinoam*. 2000;13:63-72.
45. Gorustovich A, Guglielmotti MB. Histomorphometric study of the peri-implant bone healing in case of nerve injury: an experimental model in rats. *Implant Dent* 2001; 10: 203-207
46. Mandalunis PM, Guglielmotti MB, Ubios AM. Alveolar wound healing in rachitic animals. *Acta Odontol Latinoam*. 1986;3:75-79.
47. Puia SA, Renou SJ, Rey EA, Guglielmotti MB, Bozzini CE.. Effect of bismuth subgallate (a hemostatic agent) on bone repair; a histologic, radiographic and histomorphometric study in rats. *Int J Oral Maxillofac Surg* 2009;38: 785-789.
48. Araujo MG, Sonohara M, Hayacibara R, Cardaropoli G, Lindhe J. Lateral ridge augmentation by the use of grafts comprised of autologous bone or a biomaterial. An experiment in the dog. *J Clin Periodontol*. 2002;29:1122-1131.
49. Skoglund A, Hising P, Young C. A clinical and histologic examination in humans of the osseous response to implanted natural bone mineral. *Int J Oral Maxillofac Implants*. 1997;12:194-199.
50. Avera SP, Stampley WA, McAllister BS. Histologic and clinical observation of resorbable and nonresorbable barrier membranes used in maxillary sinus graft containment. *Int J Oral Maxillofac Implants*. 1997;12:88-94.
51. Paolantonio M, Scarano A, Di Placido G, Tumini V, D'Archivio D, Piattelli A. Periodontal healing in humans using anorganic bovine bone and bovine peritoneum-derived collagen membrane: a clinical and histologic case report. *Int J Periodontics Restorative Dent*. 2001; 21:505-515.
52. 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;30:261-270.
53. Zitzmann NU, Schärer P, Marinello CP, Schüpbach P, Berglundh T. Alveolar ridge augmentation with Bio-Oss: a histologic study in humans. *Int J Periodontics Restorative Dent*. 2001; 21:288-295.
54. Schlegel AK, Donath K. BIO-OSS a resorbable bone substitute?. *J Long Term Eff Med Implants*. 1998;8:201-209.