PARTICULATE BONE MATRIX USAGE FOR ALVEOLAR BONE CONSERVATION.
A HISTOMORPHOMETRIC STUDY.

Sebastián Fontana [1], Luis Plavnik [1,2], Miguel Filippetti [3], Alicia Inés Malberti [1]

Abstract

Different filling materials have been used in an attempt to repair bone loss situations.
Objective: The present study aimed to examine the effect of a bone matrix in post-extraction remodelling of the alveolar bone, and to perform a histomorphometric analysis of the residual alveolar ridges in Wistar rats.
Material and Methods: Both rat first lower molars were extracted and the right alveoli were filled with particles of a bone matrix with mineral components (MO-UNC) (experimental group, EG). The left alveoli were used as a control group (CG). The animals were sacrificed at 0 hrs, 15, 30 and 60 days after extraction, and the samples were processed. Histological sections were made at the level of the mesial alveolus of the first lower molar. Repair of the alveoli was histologically evaluated and a histomorphometric study of total alveolar volume (TAV), height of the buccal plate (Bh), height of the lingual plate (Lh) and percentage of osseointegration (OI) of the particles was performed to compare the residual ridges of CG with those of the EG. Statistical analysis of the data was performed.
Results: In the cases of the experimental group, newly-formed bone tissue was identified around the MO-UNC particles (osseointegration). Histomorphometric data indicate that, at 60 days post-extraction, TAV was significantly greater for EG when compared with CG ($p<0.05$) and the percentage of osseointegration of the particles increased as a function of time (57.6 %, 90.5% y 95.5%, for EG at 15, 30 y 60 days respectively).
Conclusions: The bone matrix (MO-UNC) evaluated in this study is an osteoconductive material that prevents the collapse of post-extraction alveolar bone.

Key words: Bone transplantation. Alveolar ridges preservation. Post-extraction alveoli. Osteoconductive materials.

1 - Chair of Histology, Dentistry Faculty, National University of Córdoba, Argentina.
2 - Science and Technology Area, CREO Foundation, Córdoba, Argentina.
3 - Human Tissue Processing Plant, UNC-Biotecnia, National University of Córdoba, Argentina.
Correspondence:
Dra Alicia Malberti
Cátedra de Histología, Departamento de Biología Oral, Facultad de Odontología.
Universidad Nacional de Córdoba (UNC). Haya de la Torre s/n, Ciudad Universitaria CP: 5000. Córdoba, Argentina. e-mail: inesmalberti@gmail.com
This project was funded by a subsidy from the Secretariat of Science and Technology at Córdoba National University (SeCyT, UNC. Res 162/12).

Introduction

In dental practice, knowledge of bone tissue is of fundamental importance because the practitioner must deal on a daily basis with injuries and treatments that directly or indirectly affect the maxillary bones. Such dental processes as abscesses, cysts, tumours, trauma or simply the inevitable post-extraction atrophy can cause severe bone loss, with great decrease of the vertical dimension.

After tooth extraction, the healing of the alveolar bone includes clot formation and maturation, and, at the final repair, alternated phenomena of bone apposition and resorption take place [1-3]. In general, it is suggested that the ridges of the alveoli collapse and bone volume decreases in the late stages of alveolar healing [4-5].

In an attempt to preserve the height and volume of alveolar ridges, recent research has focused on different filling materials and the reactions that they promote inside tissues. Biomaterial-induced bone repair starts with the proliferation of capillaries (angiogenesis) and mesenchymal cell proliferation, which run between interparticle spaces. Differentiated cells of the osteoblastic lineage invade the area and usually attach to the particle surface for osteoid matrix secretion that later mineralizes [3,4].

Some of the options for bone fillings [6-9] are: a) autologous or autogenous grafts, (e.g. autologous bone); b) homologous or allogeneic grafts or allografts, (e.g. freeze dried bone allograft (FDBA) and demineralized freeze-dried bone allograft (DFDBA), also called demineralized bone matrix); c) heterologous grafts or xenografts, (e.g. deproteinized bovine bone mineral - DBBM, Bio-Oss®, Giestlich Pharma); and d) alloplastic or synthetic grafts, e.g. bioactive glass and tricalcium phosphate. All these materials, can mediate new bone formation by one/some of these processes: osteogenesis, osteoconduction and/or osteoinduction [6, 7,10,11].

In the references, the autologous bone graft is generally considered as the gold standard [12-14] because it retains both cell vitality and bioactive molecules, such as bone morphogenetic protein (BMP). Also, auto-grafts revascularize easily and do not transmit diseases. However, obtaining it requires a second surgical procedure at a donor site, with the consequent risk of postoperative complications [15,16]. For this reason, the use of substitute materials from human bone banks has increased, and those most often used in the clinic are FDBA and DFDBA [8, 16-18]. In this sense, our work group recently conducted a study on the repair of alveoli at 30 days post-extraction 19 using allograft particles, the bone matrix developed at Córdoba National University (MO-UNC). We concluded that the presence of these particles did not interfere with the habitual repair of the post-extraction alveolus. During the study period, the MO-UNC integrated compatibly with the newly formed bone. It was also established that particle shapes and dimensions (535.42 µm) were appropriate and within the parameters described in other studies, which suggest that the best osteogenic effects occur with specific sizes ranging from 125 to 1000 µm [17, 20].

Having demonstrated that MO-UNC acts as a biocompatible and osteoconductive material, we think that this material could accelerate bone formation and favour the preservation of the volume of post-extraction alveolar bone.

In this sense, the aim of the present study was to evaluate morphologically and histomorphometrically the effect of the bone matrix (MO-UNC) in the process of post-extraction alveolar repair at different experimental time points.

Material and Methods

Filling material features

The bone matrix developed at Córdoba National University, is human bone tissue for therapeutic use from the Bone Bank

at Córdoba Hospital, authorized by the Unique National Coordinating Central Institute of Ablation and Implant (Instituto Nacional Central Único Coordinador de Ablación e Implante, INCUCAI) from Argentina. In the Human Tissue Processing Plant, cortical portion of the long bones from cadaveric donors is selected. The bone is processed in aseptic areas, lyophilized, ground and sterilized by gamma radiation. The final bone particles (Figure 1) has been authorized by Argentina's National Medication, Food and Medical Technology Administration (Administración Nacional de Medicamentos Alimentos y Tecnología Médica, ANMAT) and registered as medical product (number 1007/1-2)

Surgical Procedure

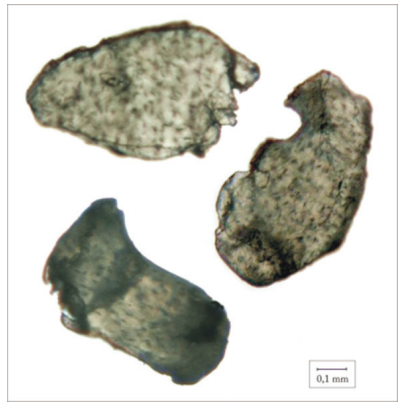

Figure 1

Forty male Wistar rats weighing 80 g (± 10, body weight) were used and kept in biotery. Rats received a balanced diet and water ad-libitum. In all cases, strict controls were carried out to reduce any pain or discomfort in the laboratory animals, complying with the standards of the National Institute of Health (NIH Publication No. 8523 rev 1985). The experimental work protocol was approved by the Committee of Research Bioethics of the Faculty of Medical Science at Córdoba National University.

The animals were anesthetized with Ketamine solution (8 mg/100 g body weight; Ketamine Zoovet®, Lab. Zoovet, Argentina) and Xylazine (1.28 mg/100 g body weight; Sedomín®, Lab König SA, Argentina).

Molar extraction procedures in the rat were carried out following the methodology described by Guglielmotti and Cabrini 1. A special examination table was designed to immobilize the animals and both first molars were extracted (Figure 2). After extraction, the right alveoli were filled with 0.2 cm3 of MO-UNC particles (Experimental Group, EG), while the left alveoli were left unfilled (Control Group, CG). The animals were separated into four (4) groups (n=10 each group) and, sacrificed at 0 h, 15, 30 and 60 days post-extraction respectively. The hemi-maxillaries were resected and fixed in a 10%, PH 7 formalin fixing solution for 24 hours. All the samples were X-rayed, demineralized and embedded in paraffin. Vestibulo-lingual cross sections were made at the level of the mesial alveolus of the first lower molar for microscopic study with hematoxylin-eosin stain.

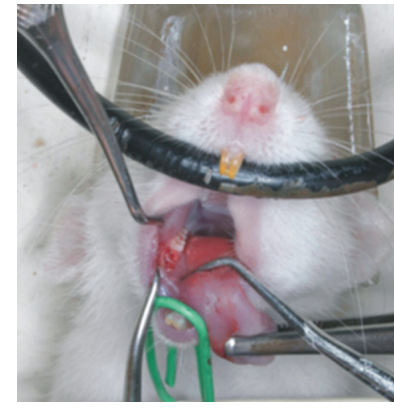

Figure 2

Analysis

A descriptive analysis was made of the presence of MO-UNC particles in the alveoli and the neo-formation of bone tissue in direct relation to them (osseointegra-

tion), at the different time points studied. Histomorphometric analyses were performed using image analysis software (Image Pro-Plus 4.5). A comparison of the total alveolar volume (TAV), height of the buccal plate (Bh), height of the lingual plate (Lh) and percentage of osseointegration (OI) of particles, was made between EG and CG at each experimental time point. For the measurements (Figure 3), a tangent line was drawn to the most salient point of the buccal plate (line t) and another line perpendicular to t, passing through the upper edge of the lower dental nerve canal (line a). The whole bone tissue with its bone marrow spaces, located above line a, was considered as TAV. To define Bh and Lh, the highest points in the buccal and lingual plates respectively were marked. Lines were drawn from these points to the intersection with line a, leaving lines B and L demarcated. For the assessment of OI the perimeter of each particle was marked and the amount of new bone in close contact to its surface was measured. Data were statistically analyzed by nonparametric ANOVA (Kruskall Wallis test), setting p≤ 0.05 for statistically significant differences.

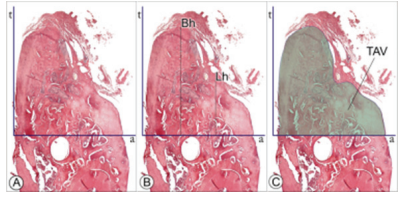

Figure 3

Results

Histological analysis of EG and CG, at each experimental time point.

0 hours: in the CG, the preparations showed the alveoli fully occupied by clot and remnants of periodontal ligament. In the EG, bone particles are arranged within the alveolus leaving wide spaces between them and the alveolar bone (Figure 4).

15 days: in the controls, newly-formed bone of the reticular type was observed in the apical third of the alveoli. In the EG there was also formation of reticular bone tissue and recently synthesized bone was seen around the MO-UNC grafted particles.

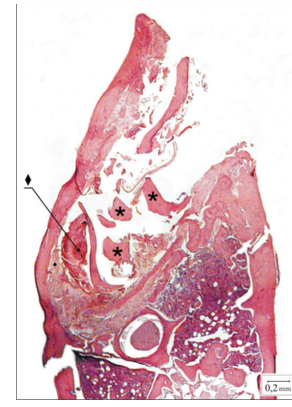

Figure 4

30 days: in the CG, the alveoli were completely occupied by lamellar, homogenous bone, similar to the adjacent cortical bone. In the EG, reparative bone tissue of the lamellar type was observed, as well as bone matrix particles filling the alveolus. The newly-formed bone had closely attached to the surface of the particles: osseointegration (Figures 5 and 6).

60 days: in the CG, the margins of the alveolar ridge had definitively remodelled. In the EG, the alveoli were occupied by particles completely surrounded by newly-formed (osseointegrated) bone, of the lamellar type (Figure 7). In some cases, the surface of the particles showed lacunae, indicative of bone resorption.

Histomorphometric analysis of alveolar ridges

The most significant histomorphometric values are presented in Tables 1 and 2.

Bh and Lh: when comparing the heights of the vestibular and lingual plates between the control and experimental groups, no

Figure 5

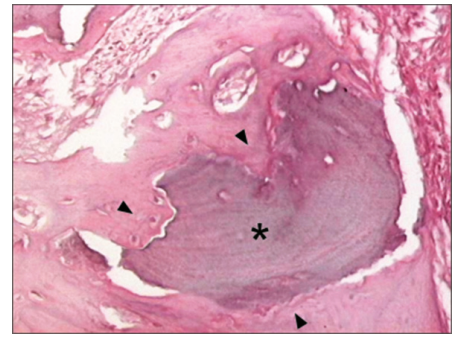

Figure 6

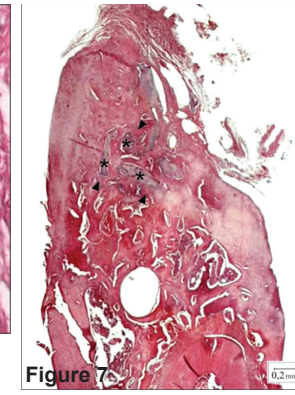

Figure 7

statistically significant differences were found at any of the time points studied.

TAV: At [15, 30] and at 60 days, the TAV values were greater for EG than for the respective CG. However the differences were statistically significant only at 60 days post-surgery (Table 1).

Percentage of ossointegration (OI): When analyzing the cases treated with MO-UNC (EG), at the different times of the study (15, 30 and 60 days), statistical analysis showed that OI of the particles increased as a function of time (Table 2).

Discussion:

This experimental study of post-extraction alveolar repair aims to clarify some aspects about the biological effect of a new allograft based on freeze-dried human bone, the bone matrix developed at Córdoba National University, (MO-UNC).

The first alternative to auto-grafts are the substitutes from human tissue banks (allografts) which have mineral content (FDBA) or are demineralised (DFDBA). Another alternative are xenografts and, among them, the one which has been widely reported in the scientific literature, is the bone of bovine origin (DBBM) [7-11]. A recent literature review concluded that a lack of available scientific data hampers to make clear recommendations on the choice of a specific material for bone regeneration [21]. This is probably due to controversies over experimental methodologies and the interpretation of the results [22].

Time	Control Group (CG)	Experimental Group (EG)	P
15 days	2.51±0.37	2.82±0.6	p>0.05
30 days	2.57±0.57	2.76±0.46	p>0.05
60 days	2.45 ± 0.43	3.40±0.38	p<0.05 *

Table 1. TAV values between CG and EG (expressed in mm2) at the different experimental time points. Note the statistically significant differences at 60 days post-extraction.

Time	Control Group (CG)	Experimental Group (EG)	P
15 days	2.51±0.37	2.82±0.6	p>0.05
30 days	2.57±0.57	2.76±0.46	p>0.05
60 days	2.45 ± 0.43	3.40±0.38	p<0.05 *

Table 2. Percentage of OI of the particles at the different EG study time points. The increase in this parameter is significant as a function of time.

A comparative controlled study suggests that some filling procedures may limit, but not eliminate, the resorption of the alveolar ridge [23].

Our experimental work clearly shows that the material used osseointegrated to the cicatricial bone of the post-extraction alveolus at all the time points studied (0h, 15, 30 and 60 days). In no case did the particles implanted interfere with the usual repair of the alveolus and, moreover, the MO-UNC served as an appropriate physical matrix for the apposition of new bone (osteoconduction). These results match those of Urist [10] and Glowacki [24], who argue that filling non-critical bone defects with biomaterials demonstrates the osteocompatibility and osteoconductivity of bone substitute materials. Histomorphometric data of the present study show no significant statistical differences in the heights of the buccal and lingual plates when comparing the experimental group with controls. At the 15, 30 and 60 days studied, the total alveolar volume (TAV) was higher in the alveoli treated with MO-UNC, compared with controls, but the differences were statistically significant only at 60 days post-extraction. This seems to indicate that the filling used may promote bone modeling of the alveolus and prevent the long-term contraction of the marginal ridge, which is consistent with other studies in which bone fillings were used alone or combined with osseointegrated implants [2,5,12,14,21,24]. We also obtained histomorphometric data of the percentage of osseointegration of the particles. This was seen to increase as a function of the times studied and, at 60 days (EG), reached more than 95% coverage of the particles with newly-formed bone, values comparatively higher than the ones reported by Carmagnola et al [18].

At 60 days post-surgery, we microscopically observed lacunae and irregular areas on the surface of the particles of MO-UNC in the experimental group. We infer that this may be related to a phenomenon of resorption of the material at this advanced stage of repair of the alveolus. Thus, we suggest that the grafted material is biocompatible because, as described previously, biomaterials must meet the requirement of being degraded progressively within the tissues [14].

Other studies [25, 26] reported that placing certain type of filling particles produces a delay in bone repair. This delay is attributed to a foreign body reaction after implantation of the particles, which were surrounded by multinucleated cells and inflammatory tissue. In our study, there were neither inflammatory phenomena nor delay in bone remodelling at any of the time points studied.

The histomorphometric data of total alveolar volume, comparing CG and EG, suggest that MO-UNC promotes conservation of the alveolar ridge post-extraction. The results obtained using this animal model might provide data of interest about the behaviour of biomaterials in bone tissue that can be applied in clinical practice. We believe that more studies should be made to determine the course of the particles over longer time periods.

Conclusións

In view of the results obtained in this study and considering the limitations of the model used, we suggest that:

Bone matrix-UNC (MO-UNC) does not interfere with the process of repair of the post-extraction alveolus and the particles osseointegrate to the newly-formed bone.

This type of filling prevents the collapse of the post-extraction bone ridge.

The percentage of osseointegration of MO-UNC particles in post-extraction alveoli increases as a function of the time.

Acknoeledgments:
The authors wish to thank Dr. Mabel Brunotto for her valuable contribution in the statistical analysis of data.

References

1-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; 43:359-64.

2-Araújo MG, Lindhe J.Dimensional ridge alterations following tooth extraction. An experimental study in the dog. J Clin Periodontol. 2005; 32:212-18.

3-Cardaropoli G, Araújo M, Lindhe J. Dynamics of bone tissue formation in tooth extraction sites. An experimental study in dogs. J Clin Periodontol. 2003; 30:809-18.

4-Araújo M, Linder E, Wennström J, Lindhe J. The influence of Bio-Oss Collagen on healing of an extraction socket: an experimental study in the dog. Int J Periodontics Restorative Dent. 2008;28:123-35.

5-Heberer S, Al-Chawaf B, Hildebrand D, Nelson JJ, Nelson K. Histomorphometric analysis of extraction sockets augmented with Bio-Oss Collagen after a 6-week healing period: a prospective study. Clin Oral Implants Res. 2008; 19:1219-25.

6-Cooper L. Biologic Determinants of bone formation for osseointegration: Clues for future clinical improvements. J Prosthet Dent. 1998; 80:439-49.

7-Marx RE, Carlson ER, Eichstaedt RM, et al. Platelet-rich plasma. Growth factor enhancement for bone graft. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998; 85:638-46.

8-Misch CE, Dietsch F. Bone grafting materials in implant dentistry. Implant Dent. 2003; 2:158-67.

9-Caneva M, Botticelli D, Morelli F, Cesaretti G, Beolchini M, Lang NP. Alveolar process preservation at implants installed immediately into extraction sockets using deproteinized bovine bone mineral. An experimental study in dogs. Clin Oral Implants Res. 2012; 23:789-96.

10-Urist MR. Bone: formation by autoinduction. Clin Orthop Relat Res. 2002; 395:4-10.

11-Fontana S, Olmedo D, Linares J, Guglielmotti MB, Crosa ME. Effect of Platelet Rich Plasma on the Peri-implant Bone Response. An Experimental Study. Implant Dent. 2004; 13:73-8.

12-Becker W, Becker BE, Caffesse R. A comparison of demineralized freeze-dried bone and autologous bone to induce bone formation in human extraction sockets. J Periodontol. 1994; 65:1128-33.

13-Schwartz Z, Mellonig JT, Carnes DL y col. Ability of commercial demineralized freeze-dried bone allograft to induce new bone formation. J Periodontol. 1996; 67: 918-926.

14-Chiapasco M, Casentini P, Zaniboni M. Bone augmentation procedures in implant dentistry. Int J Oral Maxillofac Implants. 2009; 24:237-59.

15-von Arx T, Häfliger J, Chappuis V.Neurosensory disturbances following bone harvesting in the symphysis: a prospective clinical study. Clin Oral Implants Res. 2005 Aug; 16(4):432-9.

16-Zárate-Kalfópulos B, Reyes Sánchez A. Injertos óseos en cirugía ortopédica. Cir Ciruj. 2006; 74:217-22.

17-Guglielmotti MB, Alonso ME, Itoiz ME, Cabrini RL. Increased osteogenesis in alveolar wound healing elicited by demineralised bone powder. J Oral Maxillofac Surg. 1990; 48:487-90.

18-Carmagnola D, Adriaens P, Berglundh T. Healing of human extraction sockets filled with Bio-Oss. Clin Oral Implants Res. 2003;14:137-43.

19-Fontana S, Plavnik LM, Renou SJ, González de Crosa ME. Bone substitute in the repair of the post-extraction alveolus. Acta Odontol Latinoam. 2010;23:42-6.

20-Shapoff CA, Bowers GM, Levy B, Mellonig JT, Yukna RA. The effect of particle size on the osteogenic activity of composite grafts of allogeneic freeze-dried bone and autogenous marrow. J Periodontol. 1980;5 1:625-30.

21-Piattelli A, Scarano A, Corigliano M, Piatelli M. Comparison of regeneration with the use of mineralized and demineralised freeze dried bone allografts: a histological and histochemical study in man. Biomaterials. 1996; 17:1127-1131.

22-Chiapasco M, Zaniboni M, Boisco M. Augmentation procedures for the rehabilitation of deficient edentulous ridges with oral implants. Clin Oral Implants Res. 2006;17:136-59.

23-Horváth A, Mardas N, Mezzomo LA, Needleman IG, Donos N. Alveolar ridge preser-

vation. A systematic review. Clin Oral Investig. 2013; 17(2):341-63.
24-Glowacki J. A review of osteoconductive testing method and sterilization processes for demineralised bone. Cell and Tissue Banking 2005; 63-12.
25-Araújo M, Linder E, Lindhe J. Effect of a xenograft on early bone formation in extraction sockets: an experimental study in dog. Clin Oral Implants Res. 2009; 20:1-6.
26-Calixto RF, Teófilo JM, Brentegani LG, Lamano-Carvalho TL. Grafting of tooth extraction socket with inorganic bovine bone or bioactive glass particles: comparative histometric study in rats. Implant Dent. 2007; 16(3):260-9.