Case Report

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Maxillary Ridge Vertical Augmentation Based on a Mixture of Xenogenic and Allogenic Bone Supported by Resorbable Membrane: One Case Report

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Abstract

One 60 years-old patient was scheduled for left posterior maxillary ridge augmentation due to failure of standard implants, followed by successful maxillary dental implants. We used an unproved technique consisting in a mixture of 50% allogenic and 50% xenogenic bone supported by reabsorbable membrane. No complications were found at the different stages of the treatment, and at the ending the patient showed a good level of satisfactory outcomes. Radiological evaluation demonstrated ridge augmentation able to support implant, and within the graft islands of connective and bone-like tissue was found. Within these tissues osteoclasts and osteoblast putative cells were found. Results demonstrate that the used method in addition to support implants has osteogenic and bone remodeling activity.

Keywords: Ridge augmentation, Implants, Mixed bone substitutes, Osteoclasts, Osteoblast

Introduction

The endosseous implants have revolutionized modern dentistry, with a constantly increasing number of patients seeking replacement of lost teeth with this modality of treatment [1]. To ensure the successful of dental implants placement, the volume (both vertical and horizontal) and quality of the alveolar bone are critical. However, an insufficient amount or poor quality of alveolar ridge is usually found in edentulous patients; in these cases, the alveolar ridge must be augmented [2]. Autologous bone grafting is regarded as the gold standard for bone regeneration [3]. However, because the biological and clinical limits of it use and due to safety problems [4,5] the bone-regenerative potential of grafts of different materials has been explored each with its own set of advantages

and disadvantages [6,7]. As a result, bone graft substitutes with predictable regenerative outcomes and minimal complications have been developed [6,8]. At present, guided bone regeneration with particulate graft materials and resorbable collagen membranes is an effective technique for alveolar horizontal ridge augmentation and dental implants [9,10].

Here we present one case of patient in which maxillary vertical ridge augmentation, followed by successful maxillary dental implants, was achieved through an unproved technique consisting in a mixture of allogenic and xenogenic bone supported by reabsorbable membrane and no tent screws. The mixture of allogenic and xenogenic bone substitutes has the advantage of different granule-

sizes that presumably enhances in growth of the new formed bone into the implant, as well as the removal of the implanted material.

Materials and Methods

Case report

One patient 60 years old was scheduled for maxillary vertical ridge augmentation in the atrophic posterior left maxilla due to failure of fixed implants. After reviewing medical history and clinical examination both digital panoramic radiography (Figure 1a) and CT scan were performed (Figure 2a). She showed subsinus residual bone height, ranging from 2.2 to 2.6 mm and bone ridge thickness of 2.2-5.1 mm height x 0.8-0.9 mm (Figure 2a). The clinical procedures were performed in accordance with the Declaration of Helsinki and the Good Clinical Practice Guidelines, and the patient signed a written informed consent form. The study of this case was approved by the Ethical Committee of Centro Estomatológico González-Tuñón, Oviedo, Spain

Figure 1: Two-dimensional radiographic image previous to surgery (a), immediately after surgery (b), at 12 month after graft (c) and at the ending of the treatment.

Surgical Procedure

Surgery was performed under sterile conditions and local anesthesia with articaine plus epinephrine (Artinibsa®, 40mg/ml + 0.01 mg/ml). A two-stage procedure was carried out for maxillary vertical ridge augmentation. On the day of surgery, the patient received Augmentine® (Amoxicillin/clavulanic acid, 875 mg/125mg respectively) 1 day prior to surgery, and this antibiotic treatment was maintained 7 days.

Vertical ridge augmentation was performed as follows: after exposure of the alveolar ridge a resorbable expanded membrane (Xenoprotect, Nobel Biocare, Zürich-Flughafen, Switzerland) was fixed to the bone using minitac (Figure 3a). Then, 8 g of the graft was placed and carefully packed and compacted into the area between the membrane and the bone ridge with pressure as far as membrane elasticity permits (Figure 3b and 3c) using the Urban's technique [11]. The graft consisted of 50% human lyophilized bone with tetracycline antibiotic (OSTEOMycin T™, Wels, Austria) and 50% heterologous bovine bone (Geistlich Bio-OSS®, Geistlich Pharma, Wolhusen, Switzerland). The membrane was sutured with Goretex and Glycolon® (Resoba, Nuremberg, Germany). For the postoperative phase, prophylactic antibiotics (Augmentine®, 2 times daily for 7 days) and analgesic-anti inflammatory medication (Ibuprofeno, 600 mg; 3 times daily) was prescribed. The right position of the graft was controlled radiologically (Figure 1b).

Six months later surgery was performed under local anesthesia (Artinibsa®) to create a 2-3 mm pediculated flap of vestibularly inserted gum that was fixed with miniscrew. The exposed surface was covered with Geistlich Mucrogaf® membrane, then sutured with glycolon. After a healing period of 6 months, clinical and radiographic examination (see below) was performed and the patient was reappointed for biopsy and dental implant placement. Under local anesthesia (Artinibsa®) a gum flap was performed and the fixture implants 4.3 x 11.5 mm (Nobel Parallel®, Nobel Biocare) were placed into the grafted bone substitute at 31.3 Newton of torque (Figures 4a-4d). The correct placement of the implants within the graft was controlled radiologically (Figure 1c). In the same surgical session a graft core biopsy was taken one bone sample (1 cm long, approximately), was retrieved with a trephine bur (6 mm of external diameter and 5 mm of internal diameter; Nobel Biocare) under sterile saline solution irrigation. This sample contained exclusively material from the grafted area and not from the native bone, and it was fixed in 4% formaldehyde solution in 0.1 M phosphate buffer saline (PBS), pH 7.3, and stored at 4° C.

After a period of 6 months a palatine incision was performed to increase the volume of the inserted gum in the vestibular side of the implants a two multi-unit abutment (Nobel Biocare) covered with healing cup, and after healing the zirconia crowns were implanted (Figures 4e-4h), and a new radiological control was performed (Figure 1d).

Figure 2: Two-dimensional radiographic image generated from the tertiary cone-beam CT acquired at the moment of surgery (top) and immediately after graft surgery (bottom).

Radiological Examination

Panoramic radiographs and cone-beam computed tomography (CBCT; Planmeca ProMax 3D Max, Planmeca USA Inc., Roselle IL, USA) was used for 3D assessment of bone volume preoperatively (Figure 2a), immediately after surgery (Figure 2b), and 9 months after dental implant (Figure 5). CBCT of the maxilla was acquired with a voxel size of 0.25 mm and an exposure time of 26 s. The preoperative CBCT (Figure 2a) was obtained to evaluate the bone volume of the alveolar ridge, to exclude pathological changes of the maxillary sinuses and for treatment planning. The postoperative CBCT documented the outcome of the grafting procedure (Figure 2b), and that at 9 months evaluated the volume of the grafted material (Figure 6). Another CBCT was obtained directly after implant placement and biopsy sampling at 12 months of grafting to document the outcome of the implant placement surgery, and a final one was obtained at the ending of the procedure. DICOM (Digital Imaging and Communications in Medicine) data sets of the three CBCT were imported into a 3D image processing software (VoXim®, IVS Technology GmbH, Chemnitz, Germany) to determine the measurements of the grafted area manually selected. Consequently, the CBCT-data allowed following the changes in volume of the grafted area occurring during the all the dental implant procedure.

Figure 3: Surgical procedure of grafting. b: bone, m: membrane. Thin arrow indicates minitac, and large arrow indicates the membrane bag before packing the bone substitutes.
* indicates the final aspect of the graft.

Figure 4: Surgical procedure for implant placement (12 months after grafting; a-d) and for placement of the crowns (18 months after grafting; e-h).

Histology and immunohistochemistry

After fixation of 12 h in the above mentioned solution, the biopsy sample was placed in a solution containing 10% formalin, 15.4 M nitric acid and distilled water (10:5:85 v/v) until decalcification was completed (5 days). After decalcification, the piece washed in tap water for 12 h and included routinely in paraffin, cut 10 µm thick, and the sections mounted on gelatine-coated microscope slides. Deparaffinized and rehydrated sections were stained with hematoxylin and Masson's trichrome, or processed for immunohistochemistry.

Figure 5: Tomographic view (coronal plane) and two-dimensional radiographic image 9 months after grafting.

Figure 6: Structure of the grafted material at 12 months. a,c,d: hematoxylin & eosin; b: Masson's trichome staining.

The immunohistochemical study was performed using the EnVision antibody complex detection kit (Dako, Copenhagen, Denmark), following supplier's Instructions was used. Briefly, in rehydrated sections the non-specific binding was blocked with 1% bovine serum albumin for 20 min. Sections were then incubated overnight at 4°C with primary antibodies whose characteristics are described in table 1. After incubation with the primary antibodies, sections were rinsed in the same buffer and incubated with Dako EnVision System labeled polymer-HR anti-rabbit IgG or anti-mouse IgG (Dako Cytomation, Denmark) for 30 minutes at room temperature. Finally, sections were washed, and the immunoreaction visualized

using 3-3'-diaminobenzidine as a chromogen. For control purposes representative sections were processed in the same way as described above using non-immune rabbit or mouse sera instead of the primary antibodies, or omitting the primary antibodies in the incubation. To ascertain structural details, sections were slightly counterstained with hematoxylin & eosin. For control purposes, representative sections were processed as above but rabbit non-immune serum, or blocking buffer, were used instead of the primary antibody. Under these conditions no specific immunostaining was observed.

Figure 7: Immunohistochemical detection of RANK (a-f) and Runx (g-i) in sections of the grafted material at 12 months.

Histomorphometry and quantitative analysis

Histomorphometric analysis was performed on 5 randomly selected fields per section, in 10 sections 100 µm apart. The sections were measured semiautomatically using a light microscope Olympus BX-61 using a U Plan SApo 0.64 x objectives connected to a Olympus DP-70 camera at a resolution of 4080 x 3072 pixels, (12 Mega pixels). The surface occupied by bone (neoformed bone) was manually delimited and the percent of the measured area was automatically calculated using the software CAST version 2, Copyright Visiopharm 2004 Servicios Científico-Técnicos, Universidad de Oviedo, Spain). The results are expressed as percent of neoformed bone. To evaluate the density of cells positive for the assessed antigens, cells displaying positive immunostaining were counted in 10 randomly selected fields per section, in 2 sections 100 µm apart, using a 20x objective directly under microscope. Results are expressed as number of cells/mm² in the areas containing new formed bone.

Figure 8: Immunohistochemical detection of vimentin (a-c), MMP-9 (d-e) and MMP-2 (f) in sections of the grafted material at 12 months.

Table 1: Primary antibodies used in the study.

Antigen (clone)	Origin	Dilution	Supplier
MMP-2	Rabbit	0.111	Santa Cruz Biotechnology ¹
MMP-9	Rabbit	0.111	Santa Cruz Biotechnology ¹
RANK	Rabbit	0.111	Abcamb ²
Runx2	Rabbit	0.111	Santa Cruz Biotechnology ¹
Vimentin (V-9)	Mouse	0.181	BioGenex ³

MMP-2 and MMP-9: matrix metalloproteinases 2 and 9; RANK: Receptor Activator of Nuclear Factor kappa B; RUNX2: Run-related transcription factor 2.

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Results and Discussion

The maxillary posterior area is the most challenging site for the dental implant and, in addition, vertical augmentation of the alveolar ridge is very difficult. There are numerous procedures to vertical ridge augmentation and all of them have demonstrated similar satisfactory outcomes. Here we present the case of one 60 years-old female that was treated for left posterior maxillary ridge augmentation and subsequent implants after failure of standard implant failure. The graft consisted of a 50% human lyophilized bone with tetracycline antibiotic and 50% heterologous bovine bone supported by a reabsorbable

collagen membrane and was implanted following the above described procedure. As far as we know this method to augmentation maxillary ridge is completely new and improved and we used this technique because the quantity of bone necessary and the patient have not a donor site. Moreover this technique has the additional advantage of no further surgical procedures to remove membrane. No complications were found in our patient at the different stages of the treatment, and at the ending the patient showed a good level of satisfactory outcomes, as habitually reported in studies using osteoinductive materials [7]. Recently used allogenic block bone graft to produce vertical augmentation of maxillary posterior alveolar ridge with good results [12]. Our method offer similar results for simultaneous alveolar bone augmentation together with dental implant placement.

Table 2: Ridge augmentation at different times after grafting.

	Height (mm)	Widness (mm)
Preoperatory	2.2-5.1	0.8-0.9
Immediately after graft	8.8-18.22	11.42-11.83
9 months	11.72-17.45	12.6-13.52
18 months	10.54-15.85	11.94-12.85

The evolution of ridge height and wideness augmentation, as evaluated in CBT images is summarized in table 2. On the basis of the results of the radiological studies the graft was successfully fixed reaching 25 mm in length, 8.60-18.22 mm in heights, and around 11.6 mm in widness (Figure 2). Throughout the time of evolution and until 9 months, the graft was stabilized at 12 mm height and 13 mm in widness. These dimensions remained stable at the ending of the treatment. The graft values obtained with the present technique are in good agreement those reported by Rocchietta et al. (2008) regarding vertical ridge augmentation using different methods [13]. Maintenance of the augmented bone volume over time is important in pre-prosthetic ridge augmentation to control the three-dimensional alveolar bone morphology [14]. It is generally accepted that materials significantly affect the outcome of bone reconstruction procedures in terms of bone volume, quality, and amount of vital bone. Nevertheless, final augmented bone volume or the implant success seems to be independent of the bone augmentation materials [15].

The currently available bone substitutes, independently of their origin, are only osteoconductors and not osteoinductors, and favor the ingrowth of osteoprogenitor

cells and blood enabling new bone formation [7]. On the other hand, the final bone formation was similar when using autologous bone grafts the mixture of autogenous bone with bone substitutes [3]. Moreover, a meta-analysis did not detect superiority of autogenous bone over bone substitutes in the clinical outcomes of alveolar ridge augmentation [6]. In the present study we have observed using image analysis techniques that about $42.4 \pm 11.3\%$ of the graft, valuated in histological sections, was occupied by areolar connective tissue, fibrous connective tissue and bone-like material ($18.5 \pm 6.2\%$) (Figure 6). Since the sample studied was taken from the graft and not native bone, these results clearly suggest bone neofromation in the grafted material, and presumably the ingrowth of osteoprogenitor cells come from the periostium and the residual bone to the graft. On the other hand, the results from the quantitative study are in good agreement with previous reports [16,17] but the heterogeneity of the methods used difficult to compare the results.

To identify bony cells within the graft and better understand the biological process occurring within the graft immunohistochemistry for RANK and Runx was performed. Intense immunostaining for both RANK and Runx was detected in morphologically irregular cells placed at the periphery and inside the connective tissue, in contact with the bone like tissue and the mineral particles of the graft (Figure 7). The density of RANK+ cells was $16.3 \pm 6.8 \text{ mm}^2$ of connective tissue and that of Runx+ cells of $2.7 \pm 1.3 \text{ mm}^2$. Furthermore, based on the expression of vimentin (Figure 8a-8c) most, if not all, of these cells can be identified as superficial osteoclasts. Other markers of osteolytic activity in the graft analyzed were MMPs 2 and 9 cells. MMP-9 was primarily detected in morphologically irregular cells placed at the periphery of the connective tissue and with the islands of connective tissue itself (Figure 8d and 8e). The morphology of these MMP-9 positive cells resembled that of the RANK+ positive ones, thus presumably osteoclasts, but their density was lower ($10.6 \pm 5.1 \text{ mm}^2$). MMP-2 was regularly absent (Figure 8f). Therefore, the implant contains osteoclasts (vimentin+, RANK+, MMP9+) involved in the removal of the grafted material preferably small particles [18,19], and Runx+ cells that participates in the bone formation [20]. In addition of the osteoclasts bioabsorption of remnant bone substitutes and volume maintenance of the augmented tissue over time are inversely related. Since the graft volume is maintained during the time studied here (18 months after grafting) equilibrium between bone re absorption and bone apposition seems to occur.

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