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HISTOLOGIC AND HISTOMORPHOMETRIC ANALYSIS OF MAXILLARY SINUS AUGMENTATION WITH DIFFERENT BIOMATERIALS. A PILOT SPLIT-MOUTH HUMAN STUDY

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SUMMARY

Objectives. The aim of this pilot split-mouth controlled human study is to evaluate histologic and histomorphometric results of a highly purified xenogenic grafting material in maxillary sinus floor augmentation after six months of follow-up.
Methods. This pilot split-mouth study was conducted on 11 patients, 7 females and 4 males (mean age 53 +/- 7.9 years; range 41-68 years), who underwent maxillary sinus floor augmentation. The following biomaterials were used: Laddec: a highly purified bovine xenograft, that was the test material; and Bio-oss a natural bone mineral, that was the control material.
Six patients (4 females, 2 males) were treated with unilateral major maxillary sinus floor augmentation with Bio-oss, and five patients (3 females, 2 males) were subjected to bilateral major maxillary sinus floor augmentation, with Bio-oss in one side and Laddec in the other side. Consequently, the test group (Laddec) included 5 samples, while the control group (Bio-oss) included 11 samples.
Results. For the Laddec, the newly formed bone was 36% ± 2.3; the intertrabecular spaces were 34% ± 1.6, and the residual material was 30% ± 1.4. For the Bio-Oss, the newly formed bone was 38% ± 1.6; the intertrabecular spaces were 26% ± 1.6, whereas the residual material was 36% ± 3.1.
Conclusions. Both the xenoimplants obtained a good bone regeneration with a satisfying quantity of newly formed bone and reduced quantity of fibrous bone. The Laddec showed a better absorbability compared with Bio-Oss, whose residual percentage is greater for the same elapsed time.

Key words: sinus floor augmentation, bone grafting, biomaterials, bone regeneration, maxillary sinus, histology.

Introduction

The success of dental implants in sites defined as "favorable" is now well documented and the quantity and quality of the bone seem the determining factors (1-3). Among the anatomical regions, the posterior maxilla (4, 5) is often considered an "unfavorable" site because of the bone quality and resorption (6). To rehabilitate this area, it is often indicated a maxillary sinus floor augmentation, a procedure to surgically elevate the sinus membrane, creating a space for inserting a graft material (6-8). If the height of the residual alveolar bone is less than 5 mm, the implant rehabilitation procedure is performed in two surgical stages: the first one is represented by the sinus lift, followed by a waiting period of about 6 months for the maturation of the grafted biomaterial and bone, the second one consists in

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the placement of the fixture/es (9-11). In maxillofacial surgery and in dentistry, the subject of controversy that still exists is the choice of the most appropriate graft material for the sinus floor augmentation. In this field, the autogenous bone seems to show the best performance, in a early phase (12). But the collection of autogenous bone requires an extra donor site surgery, and is associated with extra risks for morbidity and complaints of the patient. For these reasons, other graft materials are suggested (13). Sinus grafting materials may produce bone formation by osteogenesis, osteoinduction, and osteoconduction. Whereas osteogenesis is obtained by providing osteogenic cells and matrix directly in the graft (e.g. autogenous bone), osteoinduction postulates that the grafted material is chemotactic to undifferentiated progenitor cells inducing them to differentiate into osteoblasts, while osteoconduction permits the outgrowth of osteogenic cells from existing bone surfaces into the graft material. Among the osteoconductive materials, there is a highly purified, xenogeneic graft biomaterial, derived by deproteinized, sterilized bovine bone (Laddec®) which has been introduced to clinicians for its innovative features (the collagen type I matrix is preserved in association with crystals of hydroxyapatite), and recently evaluated in literature (14). Consequently, the organic phase of this material (the collagen type I matrix) is not removed. This material is obtained from bovine bone after a wash with distilled water and a phosphate buffer (0.4 M, pH 7.4), followed by defatting at a temperature <50 °C with ethanol/dichloromethane, and after a proteoglycan removal by urea and mercaptoethanol (International patent: PCT/WO/91/07194). This process of production seems able to preserve the collagen type I fibres in the matrix of this xenograft (14).

In addition, for this material are reported physical characteristics very similar to human cancellous bone (i.e.: an average thickness of trabeculae of about 160 µm, an intertrabecular space of about 340 µm, and the presence of about 2 trabeculae per mm of tissue) (14).

This material seems to stimulate osteoblastic activity in preclinical study: it seems able to facilitate the formation of multiple cell layers, and to increase the expression of alkaline phosphatase in mesenchymal cell cultures (15).

Subsequent clinical and histological studies, also conducted in humans, indicate this material as a reliable option in other oral and maxillofacial surgery procedures (as cyst enucleation or horizontal ridge augmentation) (15, 16).

Data about its use for sinus floor augmentation were also recently reported, but data are not controlled in a split-mouth design (14).

The aim of this pilot split-mouth controlled human study is to evaluate histologic and histomorphometric results of this highly purified xenogenic grafting material in maxillary sinus floor augmentation after six months of follow-up.

Methods

Subjects

This pilot split-mouth controlled study was conducted on 11 patients (7 females and 4 males; mean age 53 +/- 7.9 years; age range 41-68 years), who underwent maxillary sinus floor augmentation.

The subjects were selected according to the following inclusion criteria: the presence of unilateral/bilateral edentulous upper jaw, residual maxillary sinus floor <5 mm. Particular attention paid to the bacterial load of the oral cavity due to the multiple possible interactions, assessed by salivary tests (17-20). Exclusion criteria were: smoking, periodontal disease, maxillary sinus pathologies, systemic contraindications for surgical rehabilitation. All subjects signed an informed consent form before being enrolled in the study. The study protocol was in agreement with the Helsinki Declaration for studies conducted on humans and was approved by the Ethical Committee of the University of L'Aquila.

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Materials

The following biomaterials for sinus floor augmentation were used:

- *Laddec* (xenogeneic biomaterial, Ost Development, Clermont-Ferrand, France; typology granular 600µm): highly purified bovine xenograft. This was the test material.
- *Bio-oss* (xenogeneic biomaterial, Geistlich, Tiani, Italy; granular types: small 0.25-1 mm; and large 1-2 mm): natural bone mineral. This was the control material.

Six patients (4 females, 2 males) were subjected to unilateral major maxillary sinus floor augmentation, and were treated with Bio-oss.
Five patients (3 females, 2 males) were subjected to bilateral major maxillary sinus floor augmentation, by using Bio-oss in one side and Laddec in the other side.
Consequently, the test group (Laddec) included 5 samples, while the control group (Bio-oss) included 11 samples.

Procedures

The following surgical protocol was applied:

1) a first surgical phase of major maxillary sinus floor augmentation (at t0)
2) a second surgical phase after six months, at the time of implants insertion, during which were obtained the bone samples (at t1).

First surgical stage (t0)

The initial clinical situation was documented through preoperative, intra- and extra-oral photos of the patient, and a panoramic X-ray examination. An X-ray dental CT scan examination was also prescribed. Before surgery, the patients were subjected to antibiotic prophylaxis with penicillin, per os, 1 g every 12 hours, 3 days before the surgery, and for 7 days after the surgery.
The surgical procedures were all conducted by the same operator.
After a local anaesthesia with Articaine 40 mg/ ml with adrenaline 1:100.000 vasoconstrictor (Ubistesin 3M ESPE), the maxillary sinus floor augmentation was performed using the lateral window technique (with sinus lift surgical technique) (21).
A full-thickness flap was separated with a manual periosteal elevator, and the opening of a bone window was performed on the lateral side of the sinus, using a 2 mm tungsten carbide surgical bur, with cooling by saline solution. The next step was the detachment of the Schneiderian membrane with manual periosteal elevators. The bone window was then reversed, so that it formed the new sinus floor, under which the tested material (Laddec or Bio-oss) was grafted, following the preparation procedures indicated by the manufacturers. The result was an increased bone thickness, useful for the subsequent implant restoration. No protective membrane was applied on the bone window. The soft tissues were thereafter repositioned above the bone window, with the application of non-absorbable sutures in polimid 4/ with a 16 gauge, 3/8 circle needle. Removal of the sutures was performed after 14 days.

Second surgical stage (t1)

At the time of the implants insertion, six months after the sinus floor augmentation, a sample of newly formed tissue was taken from the graft sites. The blocks were carefully obtained using a trephine bur with copious saline irrigation.

Preparation of the samples

Then, the samples were left in a fixative for 9 days - formaldehyde at 4% dilution - and then, after dehydration with uncatalyzed and hypocatalyzed solutions, included in methacrylate blocks. Histologic sections of 5 microns (µm) were obtained using a microtome (22).

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Some sections were stained with *methylene blue/azure II*, to individuate the structural parameters, whereas the remaining sections were stained with tartrate-resistant acidic phosphatase (TRAP) to verify the presence of osteoclasts (23).
TRAP-positive cells were individuated on light microscope images of the region of interest.

Histological and histomorphometric analysis

The histomorphometric outcomes were:
- the percentage of bone volume on total tissue volume, which expresses the quantity of newly formed bone
- the percentage of intertrabecular spaces on total tissue volume
- the percentage of residual material on total tissue volume.

The measurements were performed using an interactive software for image analysis (IAS 2000, Delta Sistemi, Rome, Italy), which automatically calculates the values (24).
The differences between the two materials were evaluated with the Chi-square test, the significance was set at 0.05.

Results

Descriptive initial data about the included subjects are reported in Table 1.

Table 1 - Sample data.

			Right side		Left side	
Patient	Age	Gender	Type of material	Residual marginal bone	Type of material	Residual marginal bone
1	54	F	Bio-Oss	≤4	/	/
2	44	M	Bio-Oss	≤4	/	/
3	51	M	/	/	Bio-Oss	≤4
4	41	F	Bio-Oss	≥4	/	/
5	58	F	/	/	Bio-Oss	≥4
6	53	F	/	/	Bio-Oss	≥4
7	48	F	Laddec	≥3	Bio-Oss	≥4
8	59	M	Bio-Oss	≥4	Laddec	≥4
9	68	F	Laddec	≥4	Bio-Oss	≤4
10	47	F	Laddec	≤3	Bio-Oss	≤3
11	60	M	Bio-Oss	≤3	Laddec	≤3

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Table 2 - Results of the hystomorphometric analysis.

	Laddec (test group) (n=5)	Bio-Oss (control group) (n=11)
Newly formed bone (bone volume / total tissue volume, %)	36% ± 2.3	38% ± 1.6
Intertrabecular spaces (intertrabecular space / total tissue volume, %)	34% ± 1.6	26% ± 1.6
Residual particles (intertrabecular space / total tissue volume, %)	30% ± 1.4	36% ± 3.1

At t1, from a clinical point of view, no complications were observed in any of the patients. The results of the histomorphometric analysis are reported in Table 2.
In general, the microscopic examination of all the processed specimens confirmed the presence newly formed bone. No acute inflammatory infiltrate was evident.

Bio-Oss

At the histological evaluations, some sections showed the presence of osteoblastic activities, with newly formed bone directly attached to the surface of the particles of graft material, most of which appear surrounded by mature and compact bone, without bone gaps along the interface. The bone always results in close contact with the particles of the graft material. Furthermore, no inflammatory infiltrate of any kind was detected.

Laddec

At the histological evaluation, the newly formed bone tissue, which shows the characteristics of lamellar type, is not detected in the immediate bone-graft material interface. On the contrary, the presence of small capillaries, fibroblasts, and macrophages is detected, even if at a more distant site. Nonetheless, in the inner part of most of the particles of the grafting material, a tissue with a typical colour of the newly formed bone was detected, whereas on the surface of the same particles, numerous osseous gaps were identified. There is, however, no necrotic reaction, no inflammatory infiltrate or foreign body reaction.

Discussion

The purpose of this histologic and histomorphometric evaluation is to evaluate the interactions that occur between the bone and the graft of a test and control osteoconductive xenogeneic graft materials. The clinical findings confirmed that both the xenogeneic bone substitutes, when associated with the sinus lift surgical technique occurred in recent years, allow the placement of implants in atrophic maxillary bone, regenerated with xenografts. In addition, the present findings also showed that Laddec is a suitable material for sinus floor augmentation. Despite the clinical success of xenogeneic graft materials, only few histomorphometric data are reported in the published literature about Laddec (14).
To the best of our knowledge this is the first split-mouth controlled study about it. No evidence of acute inflammatory infiltrate was found in any specimen in the present investigation. This also confirms that Laddec seems to not induce adverse immunologic response.
Specimen from the Laddec group showed newly formed bone of 36% ± 2.3, intertrabecular space of 34% ± 1.6, and residual particles of 30% ± 1.4. Bio-Oss showed 36% ± 3.1 of residual particles. The Laddec showed a better absorbability compared with Bio-Oss, whose residual percentage

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was greater for the same elapsed time. The residual percentage of Bio-Oss observed in the present investigation is in accordance with literature, for the same elapsed time of 6 months (25, 26).
For the Laddec, the present findings appear less encouraging respect to the pre-existing literature, that reports newly formed bone of 64.72% ± 3.44, after 6 months in human specimen (in a study with fifteen subjects, not controlled) (14). The more encouraging results obtained in that sample could be associated to the use of a membrane that was positioned against the packed sinus window, while no membrane was used in the present investigation.
The percentage of residual material over the total tissue was 16.93%, while the present sample shows 30%. The membrane could have influenced the absorbability of the graft particles.
In the present study, the Bio-Oss material is used as a control material, because it was considerably previously analyzed in literature, while lower evidence is reported for the Laddec.
In light of this, from our comparative study we may say that all the biomaterials used gave good results with individual characteristics that allow preferring one to another.
For the Bio-Oss, in the present investigation, the newly formed bone resulted 38% ± 1.6; the intertrabecular space was 26% ± 1.6, whereas the residual material was equal to 36% ± 3.1. These data are comparable with previous literature.
In a study conducted on 20 subjects analyzed after 6 months from sinus augmentation, anorganic bovine bone showed a newly formed bone of about 25% (25.12% ± 7.25%), and residual biomaterial of about 29% (28.65% ± 9.70%) (27).
In other studies on xenografts were reported data of 24.63% and 29.13% of newly formed bone after 6-8 months from the sinus floor augmentation (28, 29).
It was also reported a newly formed bone of 21.1% after six months in a single clinical case (30).
Thus, the results obtained in the present investigation (38% ± 1.6 of newly formed bone) seem slightly more encouraging than the percentages of data reported by previous literature with Bio-Oss. This may depend on the different gravity of the initial cases, and on the individual susceptibility.
Concerning the anatomy, we can assert that there are anatomies characterized by a greater blood supply that facilitate the incorporation of the graft, in which the new formation of bone can be found to be greater (21).
Another hypothesis could be the size of granules (in the present study was used a xenogeneic biomaterial with granular type small 0.25-1 mm and large 1-2 mm), according to a recent finding that the newly bone formation appears more extensive in the large particle grafts compared with the small particle grafts (26.77% ± 9.63% *vs* 18.77% ± 4.74%, respectively) after six months in human specimen (25).

Conclusions

The xenoimplants (Laddec and Bio-Oss) both obtained a good bone regeneration with a satisfying quantity of newly formed bone. The Laddec showed a better absorbability compared with Bio-Oss, whose residual percentage is greater for the same elapsed time. Further studies with Laddec are encouraged on larger samples in order to confirm its optimal outcome for sinus augmentation.

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