Original article

Clinical and histomorphometric comparison of autologous dentin graft versus a deproteinized bovine bone graft for Socket Preservation

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Abstract

Dentin has been an important topic of study for its potential use as a bone substitute because it has a higher mineral content than any material derived from bone. Furthermore, dentin is similar to autologous bone in two ways: it is both osteocompatible and osteoconductive, thus providing a physical matrix for the sediment of new bone.
In this comparative study, we evaluated the osteoinductive and osteoconductive possibilities of various materials normally used in "socket preservation" or alveolar ridge preservation.
From the results obtained, it can be seen that autologous dentin matrix and bovine-derived xenografts (Bio-Oss) achieved better bone regeneration with a greater amount of newly formed bone (expressed by the BV/TV parameter) and less fibrous bone, which has unfavorable characteristics for implant biomechanics.

Key words: dentin, socket preservation, osteoconductive

Introduction

The human body has intrinsic mechanisms that allow self-healing, but 'restitutio ad integrum' is not recurring, particularly in the oral cavity and at the level of the alveolar bone. Biomaterials can promote and enhance the natural capacity for healing and can be successfully used to restore certain structures in the human body 1-2. Dental implants represent a method with predictable results for rehabilitating chewing function in edentulous patients as long as there is an adequate residual bone thickness to allow their insertion, primary stability, and osseointegration. If such thickness is not present, implant treatment may be possible only through regenerative surgery techniques, the success of which is linked to an accurate diagnosis involving the correct choice of graft material, which is crucial for adequate bone formation.
In current times, when implant placement is the favored option for replacing a missing tooth, preservation of the alveolar ridge is vital. After tooth extraction, dimensional changes in the residual alveolar ridge are inevitable, which pre-existing pathologies such as periodontal disease and periapical lesions accelerate3.
When a tooth is lost, the lack of stimulation of residual bone causes a decrease in trabeculae and bone density in the edentulous socket, along with a loss of width in the buccal bone and a subsequent loss of height in the volume of the alveolar process. These risks are particularly significant during the first 8 weeks4.
Within the first 6 months after tooth extraction, the alveolar ridge loses height and width ≥50%5-6. Therefore, to maintain the bone volume useful for optimal functional and esthetic outcomes of a dental implant, it is necessary to intervene conteporary or immediately after extraction. Placement of various grafting materials within the postextraction socket is supported by numerous studies as a "ridge preservation technique." 5- 6-7-8-9
Techniques for alveolar ridge preservation using autogenous, allogeneic, and xenograft graft materials have been mentioned in the literature.
For most favorable results, an ideal bone graft should have the properties of osteoconduction, osteoinduction, and osteoproliferation10-11. Among all available options, allografts and xenografts demonstrate only osteoconduction (except allograft with demineralized freeze-dried bone, which is osteoinductive). Considering that autologous bone possesses all three ideal properties, it is still considered the gold standard. However, infection hazard from the donor site, limited availability, and marked resorption are some of the shortcomings of autologous bone grafting.
Advances in tissue engineering and stem cell science are leading to the development of new techniques for bone regeneration in the maxillofacial area constituting an additional therapeutic possibility.
Dentin has been an important topic of study for its potential use as a bone substitute because it has a higher mineral content than any material derived from bone. Furthermore, dentin is similar to autologous bone in two ways: it is both osteocompatible and osteoconductive, thus providing a physical matrix for the sediment of new bone. For the above reasons, dentin is considered an ideal bioac-

tive material for hard tissue regeneration 12-13-14.
The literature agrees that proteins with a weight similar to bone morphogenetic proteins (BMPs) are abundant in dental substance. BMPs help promote the differentiation of mesenchymal cells into odontoblasts and ameloblasts 15-16-17-18-19.
These proteins can improve the osteoinductive properties of bone substitutes if they can be successfully retained during the processing of the graft material. The idea of being able to use the tooth as a bone substitute dates back to studies on the identification of growth factors known as bone morphogenetic proteins (Bmps). In 1967, U.S. orthopedic surgeon Marshall Urist, discoverer of Bmps, first demonstrated their presence in dentin as well. Subsequently, Bmps in the dentin matrix were isolated and characterized 17-18-19.
Autogenous dentin grafting has been developed and clinically applied in Korea from 2008 onward.
The patient's extracted tooth represents the ideal biomaterial because it is autologous, does not require a second harvest site, has high osteoconductive and osteoinductive properties, and is remodeled and fully replaced by new bone.
The osteoinductive properties are due to the presence of significant amounts of Bmp within the dentin structure. These proteins are preserved even long after extraction and are unaffected by storage conditions: consider that intact Bmps have been identified even in fossil human teeth. The complete or partial demineralization treatment that is carried out by some of the current methods increases the bioavailability of dentin Bmps, which are otherwise constrained by the high degree of crystallinity of hydroxyapatite. This makes dentin the most biological and osteoinductive material available, with the sole exception of autologous bone. In this regard, it should be kept in mind that in theory enamel also has good osteoconductivity characteristics but, having a higher inorganic component (96% compared to 60-70% of dentin), it is less easily resorbed even after demineralization 19.
Many studies on the use of autogenous dentin as a graft have shown good clinical and histological results 20-21-22-23-24. However, there is a paucity of data supporting the use of autogenous tooth graft (ATG) in clinical applications.
Avoiding autogenous bone harvesting by opening another surgical site is advantageous in all cases where the need to increase bone volumes is accompanied by the extraction of dental elements. It also helps with the cost savings associated with the use of an autologous material compared with those of animal or synthetic origin. 25-26-27-28
The primary objective of the present study is to evaluate clinically and radiographically the efficacy of an autologous dentin graft versus a deproteinized bovine bone graft in the technique of alveolar ridge preservation in post-extraction sites. The secondary purpose was to determine histologically the bone formation potential of ATG (Autogenous Tooth Graft).
The data obtained were evaluated by histomorphometry, or quantitative histology, which allows the acquisition of the most important bone parameters, including the bone remodeling index. Indeed, suffice it to say that it is used for the diagnosis of metabolic diseases of the skeleton (on bicortical biopsies from the iliac crest).
As a quantitative technique, histomorphometry may also find wider application in "measuring" the response of bone to biomaterials implanted during surgery. 29

Materials and methods

In this comparative study, we evaluated the osteoinductive and osteoconductive possibilities of various materials normally used in "socket preservation" or alveolar ridge preservation. The study was carried out on a single patient to get rid of all the variables that inevitably may arise when evaluating a larger audience of subjects. The patient, a volunteer, of the present study, is a candidate for treatment and rehabilitation with implant-prosthetic therapy at the Dental Clinic of the University of L'Aquila. In addition, the candidate patient required dental extractions for periodontal reasons. The patient included in the study met the following exclusion criteria:

- History of systemic disease that would make surgery contraindicated
- Long-term therapy with nonsteroidal anti-inflammatory drugs
- Lack of antagonistic elements in the area being extracted and thus of implant placement
- Oral therapy with bisphosphonates
- Lack of elements adjacent to the site undergoing therapy
- Inability to present at subsequent follow-ups
- Subject smoker of more than 10 cigarettes per day.

Before participating in the study, the patient received clear explanations and signed the informed consent. In addition, he or she was carefully evaluated through analysis of diagnostic patterns and panoramic/periapical radiographs, and data such as age, sex, smoking habits, indication for dental extraction based on both clinical and radiographic data, location of the tooth element, and presence/absence of adjacent teeth were acquired. Once informed consent was performed, it was possible to proceed with the surgical procedure.
The clinical situation of the patient examined presented the dental arches with edentulousness of numerous elements.
To study the effectiveness of autogenous dentin in preserving the alveolar ridge, the following experimental protocol was established.
The patient had extracted eight elements in the two arches.
The post-extraction sites were divided into 3 groups:
GROUP 1: in which the alveolus was filled with freshly prepared autologous dentin matrix;
GROUP 2: in which deproteinized bovine bone (Geistlich Bio-Oss) was used;
GROUP 3: control, in which the alveolar bone defect was not filled, reproducing natural post-extraction conditions.

Surgical protocol

- Antibiotic prophylaxis.

Following the latest guidelines about antibiotic prophylaxis in oral surgery, the patient received prophylactic antibiotic therapy with 2g amoxicillin 1 hour before extraction and continued therapy in the postoperative period with 1g amoxicillin twice daily for 4 days.

Figure 1. Condition of the dental arches immediately following multiple extractions.

- Anesthesia

Lidocaine-based local-regional and plexic anesthesia was performed, with adrenaline 1:50000 where possible.

- Dental extractions

Elements 1.4, 1.2, 2.2, 2.4, 3.4, 3.2, 4.2 and 4.4 were extracted with manual syndesmotomes or extraction forceps.
The atraumatic extraction and subsequent grafting for the alveolar ridge preservation technique were performed without dislodging a full-thickness flap using the flapless technique.
Great care was taken to minimize trauma to the buccal bone surface and to maintain the integrity of bone morphology.

- Curettage of the alveolus

For proper performance of this study, it was necessary to ensure the removal of all root fragments, fibers, and soft tissues from the alveolus prior to the insertion of graft material.
Curettes were used to remove these tissues in the post-extraction socket.

- Preparation of autologous dentin matrix

Parallel to curettage, according to the manufacturer's recommendations, treatment of the extracted teeth for the creation of the autologous dentin matrix graft was performed.
The Smart Dentin Grinder manufactured by KometaBio was chosen for the preparation of the autologous material.

Figure 2. Grinding and sorting process completed.

The first step was to select teeth for treatment. Endodontically treated elements were excluded.
A high-speed handpiece and an ultrasonic scaler were used to remove all cavities, artificial materials (crowns or fillings of any kind, amalgam or composites), and debris until completely clean elements remained. It was not necessary to remove the crown or enamel.
The prepared teeth were dried and introduced into the grinding chamber, they were then ground and sorted through the machine functions obtaining particles between 300 and 1200 microns in size. Particles smaller than 300 microns were discarded.

Figure 3. KometBio's Smart Dentin Grinder with the extracted and cleaned teeth inserted into the grinding chamber.

Drawer with particle sizes between 300 and 1200 microns following the shredding and sorting process.
Next, the sterilization step was carried out with substances provided by the manufacturer (NaOH with ethanol 20%), respecting the timing and procedures.
This process resulted in the organic residues dissolution of, bacteria, and toxins in the dentin, leaving the dentin particles ready for use.

- Grafting of biomaterials

In group 1, which includes the post-extraction sites of elements 1.2, 2.2, 3.2, and 4.2, the freshly prepared dentin matrix was placed as an autogenous graft.
In group 2 comprising sites 3.4 and 4.4, a deproteinized bovine bone graft (Bio-Oss. Geistlich) was placed, and in group 3, comprising sites 1.4 and 2.4, no graft was placed and the postextraction socket was allowed to heal naturally. The graft material was condensed into the socket by gently pressing it while the patient's blood was used as the preferred medium to blend it.
The use of collagen membrane is mandatory to ensure the protection of the site from gingival proliferation (tenting effect) to allow graft rooting and osteogenesis, as well as to allow optimal retention of the material. For this purpose, the membrane was appropriately shaped according to the dimensions of the socket and then adapted with the ends gently pushed underneath the adjacent soft tissues previously unglued (envelope technique).

- Sutures

Mucosal margins were fixed in situ, using sutures without achieving complete soft tissue closure.
Collagen membranes remained exposed to the oral cavity with healing by the second intention. Removal of the sutures was scheduled after 10 days.
The patient was recommended to continue antibiotic prophylaxis and take, as an anti-inflammatory, naproxen sodium in 550 mg tablets twice daily as long as needed, in addition to the use of a chlorhexidine 0.2% mouthwash twice daily.

- Sample

After about 6 months, the implants were placed by taking the bone to be analyzed from the alveolar site by full-thickness flap and the use of core drills (hollow toothed, internally cooled, handpiece-mounted drills) in the area where the biomaterial graft had been placed.
Subsequently, the implant was placed.
The patient received the same drug therapy at the time of the initial surgery. The retrieval was left in a tube with a fixative for 9 days to preserve and stabilize its constituents.
The purpose of fixation is to prevent the postmortal degenerative processes that occur in tissues while preserving their morphology, structure, and reactivity as best as possible by obtaining as extensive and truthful information as feasible about the "in vivo" condition of the tissue specimen under examination81.
Indeed, the fixative allows instant blocking of enzyme activity, and preservation of all tissue components and does not alter the structure or allow tissue dislocation.
In the present study, formaldehyde (or formalin) used in 4% dilution pH7 was chosen as the fixative.
This was followed by the inclusion step, which consists of allowing a substance to permeate the tissue under examination, which, as it solidifies, allows it to be cut with a microtome into thin sections a few microns (μm) thick.
The inclusion material chosen was methacrylate.
The resulting piece was then placed for longitudinal cutting using the microtome.
Histological sections, on the order of 5 microns (μm), were thus made for staining and analysis.
The sections, arranged on object slides, were immersed in xylol, the inclusion solvent, to allow rehydration and subsequent staining.
Once the slide was stained, depending on the need for investigation, the section was sealed using Canada balsam and coverslip.
Several sections were made of each sampling, of which, some were stained with methylene blue/blue II, for anal-

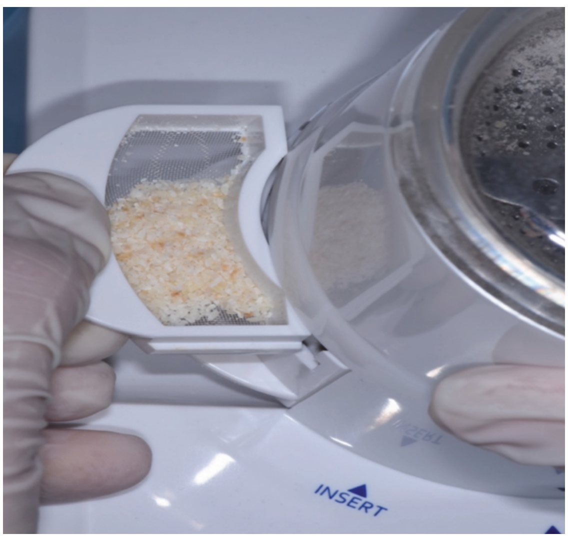

Figure 4. Drawer with particle sizes between 300 and 1200 microns following the shredding and sorting process.

Figure 5. Placement of Group 1 grafting material (dentin) in the post-extraction socket.

ysis of structural parameters, and the remaining with TRAcP staining for analysis of bone cell parameters.

- Histomorphometric analysis

Histomorphometric analysis involved the entirety of the sectioned specimen, and the following were measured:

- osteoclast number/bone surface area (number/mm2),
- osteoclast surface area/bone surface area (percent),
- osteoblast surface area/bone surface area (percent),
- bone volume/total volume (percent).

The nomenclature, symbols, and measure units of the histomorphometric indices were expressed as recommended by the Histomorphometry Nomenclature Committee of the American Society for Bone and Mineral Research.

Cells were fixed in 3% paraformaldehyde in cacodylate buffer

0.1 M for 15 min, then washed with the same buffer. TRAcP activity was detected histochemically using Sigma- Aldrich kit #386, following the manufacturer's suggested instructions.

Results

From the results obtained, it can be seen that autologous dentin matrix and bovine-derived xenografts (Bio-Oss) achieved better bone regeneration with a greater amount of newly formed bone (expressed by the BV/TV parameter) and less fibrous bone, which has unfavorable characteristics for implant biomechanics.

This assessment resulted not only from histomorphometric examination but also from histological sections analyzed by light microscopy.

The percentage of residual material, examined by histomorphometry, was also found to be higher for the first two groups than for the third, an important finding to allow its replacement with newly formed bone tissue (osteoconduction). For group I, with autogenous dentin grafting, the following parameters were examined:

- BV/TV, expressing the percentage of newly formed bone,
- OC.N/micron, expressing the number of osteoclasts per micron,
- OCS/BS, expressing the number of total osteoclasts observed on bone volume.

The results obtained were as follows:

- **BV/TV (Bone volume/Tissue Volume) %.**

Site	Section 1	Section 2	Section 3	Average ± SEM
1	27%	30%	28%	28.3 ± 0.88
2	55%	61%	58%	58.0 ± 1.73
3	21%	17%	23%	20.4 ± 1.76
4	80%	83%	88%	83.7 ± 1.30

- ***OC.N/micron***

Site	Section 1	Section 2	Section 3	Average ± SEM
1	8µm	11µm	5µm	8µm
2	21µm	22µm	20µm	21µm
3	5µm	8µm	6µm	6µm
4	1µm	0µm	2µm	1µm

- ***OCS/BS***

Site	Section 1	Section 2	Section 3	Average ± SEM
1	15%	20%	18%	17.6%
2	35%	32%	42%	36.3%
3	13%	16%	21%	16.6%
4	3%	0%	2%	1.6%

Regarding on group 2, which included xenograft with deproteinized bovine bone (Bio-Oss), and group 3, including sites left to heal naturally, the following were examined:
- BV/TV, expressing the amount of newly formed bone,
- The percentage of intertrabecular spaces,
- The percentage of residual material.

The results obtained were as follows:

	No fillers	Bio-Oss
New bone	37%±3.2%	38%±1.6%
Inter-trabecular spaces	44%±1.3%	32%±1.6%
Residual material	0%	30%±1.4%

As for Bio-Oss, sections of some samples showed osteoblastic activities with affixation of bone directly on the surface of the particles, most of which appeared to be surrounded by newly formed, mature, and compact bone tissue, with no bone gaps along with the interface.
The bone was always found to be in close contact with the particles themselves. In addition, no inflammatory infiltrate was evident. Histomorphometry showed that the newly formed bone was 38%± 1.6%, the intertrabecular spaces 33%±1.6%, and the residual material was 30%± 1.4%.
Regarding the sites without any graft, where there was natural healing, there was evidence of neoformation of bone tissue with wide gaps.
No inflammatory infiltrate was found.
The neoformed bone was 37%± 3.2%, the intertrabecular spaces 44% ±1.3%, and the residual material was 0%.

Discussion

The presence of neoformed bone was observed in all samples examined.
Therefore, it can be stated that the biomaterials considered in this study resulted in bone neoformation.
However, the phenomena of bone neoformation are not sufficient to clarify the greater usefulness of one biomaterial over another. Therefore, the amount of mineralized bone, the amount of intertrabecular spaces, and the amount of residual biomaterial were also considered in our study.
Based on the parameters analyzed, there are differences between the various biomaterials, particularly in the amount of residual biomaterial.
Autologous dentin matrix turns out to be the most stable “biomaterial” in comparison with Bio-Oss and traditional healing whose residual percentage is higher, given the same elapsed time (in agreement with the international literature and concerning Bio-Oss identified as the “gold standard” among grafting biomaterials).
Regarding the type of material replacement, in bone tissue or fibrous tissue, we can argue that traditional healing was the process that gave the highest percentage of fibrous tissue formation.

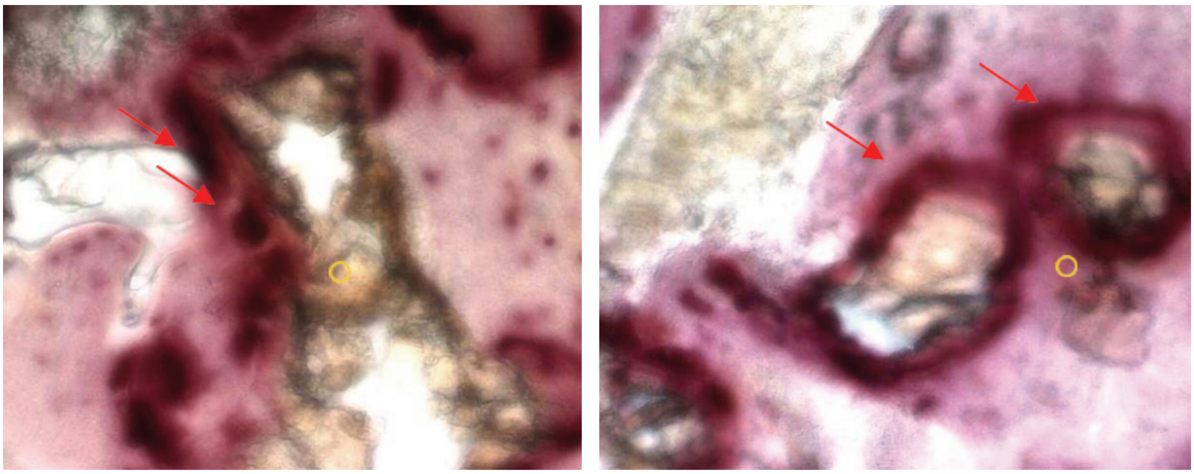

Figure 6. Transverse section of patient’s jaw bone.
TRAcP histochemical staining highlighting cells expressing the enzyme tartrate-resistant acid phosphatase enzyme.
40X magnification.
Red arrows = osteoclasts \ o = bone tissue.

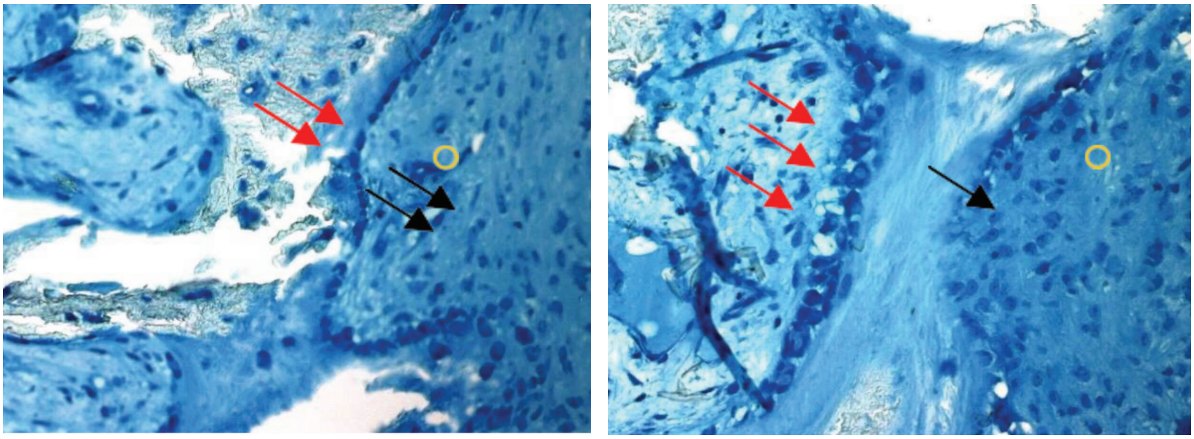

Figure 7. Longitudinal section of patient's maxillary bone.
2.5X magnification. Methylene blue II staining.
Images are representative of at least 3 sections analyzed at different depths.
O = bone tissue \ red arrows = osteoblasts \ black arrows = osteocytes

Nevertheless, differences, even considerable ones, were still found among the various retrievals, even with the same material used, in the type of tissue regenerated. These disparities are related to individual patient factors that play an equally important role such as:
- general health conditions of the same,
- anatomical conditions,
- amount of residual bone.

It is inferred, in fact, through the radiological study performed before and after surgery, how the anatomical shape of the post-extraction site and the thickness of residual bone are determining factors for the surgery to be succesfull.
As far as anatomy is concerned, we can state that there are types of anatomy that facilitate graft rooting as they are characterized by greater irrigation, in which the neoformation of bone was greater.
The results obtained confirm the data from the literature review.
The study demonstrated and confirmed the necessity of the alveolar ridge preservation technique in maintaining the height and width of residual alveolar bone following dental extraction.
Grafted sites show variable alveolar ridge preservation not only depending on the type of graft, but also the morphological condition of the post-extraction alveolus. Indeed, if greater difficulty could be expected in areas where previous periodontal lesions had caused extensive bone destruction, equally predictable was bone resorption in alveoli not treated at all and left to heal spontaneously. Between the two types of grafts, on the other hand, the differences in results appeared to be non-deterministic, allowing it to be said that in any case, both materials produced good results.
Therefore, rather than disposing of extracted teeth as biomedical waste, they can and should be used as autogenous grafting material, which may be considered the best alternative to most conventional grafting materials.
Regarding the limitations of the present study, it should be mentioned that since its nature is purely experimental, further studies are needed to confirm these results using a larger sample size.
Further randomized clinical trials are needed to establish its regenerative potential in various periodontal weaknesses.

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