The Effect of Xenogenic Bone Graft Particle Size on the Quality of Newly Formed Tissue in Alveolar Ridge Preservation (Clinical and Histomorphometric Study)

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Abstract

Aim of the study: To investigate alveolar socket preservation with two different particle sizes of demineralized bovine bone mineral (DBBM) small-sized particles (0.25–1 mm) and large-sized particles (1–2 mm) by means of histological, clinical and radiographic analysis.

Materials and methods: Clinical parameters included; vertical bone height and bone width that were recorded at baseline and at three months after extraction. Moreover, histomorphometric parameters included; area fraction of newly formed bone three months after extraction by histomorphometric analysis. Radiographic parameters included superimposition of baseline CBCT and another one done three months after extraction with subsequent measurement of bone height, bone width and bone density. A core biopsy was taken three months after tooth extraction which has undergone histomorphometric analysis; implant placement was also done at the same time.

Results: The histomorphometric analysis showed a significant difference between different groups. The highest value was found in the large particles group, followed by the small particles group while the lowest value was found in the control group.

Conclusion: Xenogenic bone graft Particle size has no effect on the quality of newly formed tissue after 3 months of alveolar ridge preservation.

Keywords: Xenogenic Bone Graft, Particle Size, Newly Formed Tissue, Alveolar Ridge Preservation, Socket preservation.

Introduction

Attaining satisfying esthetics in the anterior maxilla involves many clinical parameters but is mainly related to tissues around the dental implant. An adequate three-dimensional (3D) osseous volume of the alveolar ridge, comprising an intact facial bone wall of appropriate thickness and height, together with the proper restoration-driven implant placement, are required to support the peri-implant mucosa. Inadequate facial bone architecture has a detrimental influence on aesthetics and is a major cause of difficulties and failures after implant placement. However, the integrity of the hard and soft tissue dimensions is jeopardized by physiological and structural...
changes following tooth loss (Chappuis et al., 2017).

Teeth extraction causes alveolar bone resorption that starts and continues for years. Different studies showed that alveolar bone loss within first 12 months after tooth extraction was 11 - 22% of alveolar bone height and 29 - 63% of width while two-thirds of ridge is lost during first 3 months after tooth extraction. Major alterations in the alveolar ridge's dimensions are brought on by a thin buccal bone plate, mainly in the aesthetic and premolar regions. In these conditions, fibrous tissue ingrowths into the socket following extraction prevents alveolar bone regeneration and promotes alveolar ridge resorption (Stumbras et al., 2019).

Recent studies have concentrated on a number of materials and methods in order to decrease or prevent alveolar ridge alterations. The use of soft tissue grafts, the use of hard tissue graft materials, or the use of a mix of soft tissue and hard tissue biomaterials are the three alternatives available for maintaining the alveolar ridge, according to recent systematic reviews. The primary objectives include: preventing, or at least limiting, post-extraction ridge alterations; promoting the healing of soft and hard tissue within the former extraction socket; and making it easier to place dental implants in an optimal position for prosthetics without the need for additional augmentation procedures. (Jung et al., 2018).

Atraumatic tooth extraction accompanied by bone grafting of the extraction socket with particulate bone graft or substitutes is called alveolar ridge preservation (ARP) (Horváth et al., 2013).

The large majority of studies that evaluated the dimensions of soft and hard tissues following tooth extraction concluded that the ideal bone graft material should not only have osteoconductive characteristics but also induce osteoinduction and osteogenesis. These three features are specific to autologous bone, which is still regarded as the gold standard for bone augmentation techniques (Scarano et al., 2011).

However, additional surgical procedures and longer recovery times, donor-side morbidity, lack of autologous bone, and postoperative discomfort necessitate the adoption of alternate bone substitutes for bone regeneration. Materials for bone grafts are selected based on their capacity to act as scaffolds, maintain space for the ingrowth of new bone, and have only osteoconductive activity.

The osteoinductive characteristics are specific to DFDBA. In addition to acting as a scaffold for new bone formation, it encourages osteoblast differentiation in mesenchymal cells. Multiple publications on DFDBA claim that demineralization of alloegenic bone exposes the inner structure of the bone graft, which contains growth hormones and osteoinductive bone morphogenetic proteins (Stumbras et al., 2019).

Bone from equine, porcine, or bovine origin that has undergone additional processing and deproteinization is known as xenograft material. The organic components of these substances are removed to minimize immunological response or disease transmission. The minerals that are still present act as a framework for the development of natural bone. They could be mixed with allografts or growth factors to mimic autogenous bone. The most typical process for generating bone is osteoconduction. The crystal structure that results resembles that of human cancellous bone (Chavda & Levin, 2018).

Today, there is a growing industry for improved bone tissue regeneration. Bone tissue has the capacity to fully recover after injury if the necessary conditions are met. The size of the defect, underlying medical disorders, and/or poor vascularization, on the other hand, may restrict healing, leading to a non-healing defect that is unable to finish self-repair processes. Improved osteoconductive and osteoinductive characteristics for bone tissue regeneration are therefore becoming more and more useful (Kurien et al., 2013).

Both the osteoconductivity and resorption characteristics of xenograft bone can be impacted
by the processing method. The graft should provide even healing and osteoconductivity for bone growth. The processed graft should be anorganic, which is a common characteristic. All remnants of the donor animal must be removed to reduce antigenic reactions. Healing and bone formation are influenced by the pore size between the xenograft particles, grain size, surface shape, and crystallinity (Block, 2019).

There are few studies comparing these different materials using the same patient model. There are different processing methods available, and these methods can affect how the material behaves in its natural environment. The surface morphology of these materials is not well described in the literature. The grain size and actual pore size of these materials, as well as evidence of their impact on bone conductivity (Block, 2019).

This study was performed to investigate alveolar socket preservation with two different particle sizes of demineralized bovine bone mineral (DBBM) small-sized particles (0.25–1 mm) and large-sized particles (1–2 mm) by means of clinical, histological and radiographic analysis.

Aim of the Study

A randomised controlled trial to compare histologically and radiographically the quality of newly formed tissue after using xenogenic bone grafts having two different particle sizes in alveolar ridge preservation. The primary objective was histomorphometric analysis of bone quality at the time of implant placement after 3 months of alveolar ridge preservation. The secondary objective was clinical measurements of dimensional changes that occur after 3 months of extraction, cone beam computed tomography (CBCT) to access changes in alveolar ridge dimensions and alveolar bone density that occur after 3 months of extraction.

Subjects and Methods

A power analysis was designed to have adequate power to apply a statistical test of the null hypothesis that there is no difference would be found between different tested groups. By adopting an alpha level of (0.05) a beta of (0.2) i.e. power=80% and an effect size (f) of (0.599) calculated based on the results of a previous study; the predicted sample size (n) was a total of (30) cases (i.e. 10 cases per group). Sample size calculation was performed using G*Power version 3.1.9.7 (NB 1): The calculated number is a minimum estimation of the required sample size. Sample size can be safely increased to any number of choice as long as it’s higher than the estimation). NB (2): References for the paper and the program used for the calculations are in footnotes at the bottom of the page if not visible on mobile try opining on a computer.

Thirty patients were selected from the outpatient clinic of the department of Oral medicine, Periodontology, and Oral diagnosis, Faculty of Dentistry, Ain Shams University. Patients who met the eligibility criteria were randomized using a computer-generated randomization list and were allocated to three treatment modalities groups:

* Group 1: (test group) ten extraction sockets where alveolar ridge preservation was performed using xenogenic bone graft with a particle size ranges (0.25-1 mm),
* Group 2: (test group) ten extraction sockets where alveolar ridge preservation was performed using xenogenic bone graft with a particle size ranges (1-2 mm),
* Group 3: (control group) ten extraction sockets underwent natural socket healing. All patients will receive the implants after 3 months of alveolar ridge preservation (Jambhekar et al., 2015).

A-Inclusion criteria:

1- Healthy adult patients as evidenced by health questionnaire using modified Cornell Medical Index.
2- Age from 20 – 50 years old.

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3- Hopeless tooth indicated for extraction (a non-restorable or remaining root that filling, fixed restoration placement and crown lengthening can't be done located in the maxillary arch including premolar and anterior tooth).

4- Sockets type I or II.

Based on the hard and soft tissue topography, extraction sockets can be classified as follows: **Type I**: The facial soft tissue and buccal plate of bone are at normal levels in relation to the cementoenamel junction of that pre-existed tooth and remain intact postextraction, **type II**: Facial soft tissue is present but the buccal plate is partially missing following extraction of the tooth, **type III**: The facial soft tissue and the buccal plate of bone are both markedly reduced after tooth extraction.

**B- Exclusion criteria:**

1- Smokers (Marenzi et al., 2015).
2- Patients with poor oral hygiene.
3- Pregnant and breast-feeding females.
4- Medically compromised patients.
5- Prisoners and handicapped patients.

**Surgical procedure:**

Extraction and Socket Augmentation Procedure: Baseline CBCT was taken at day of extraction to evaluate type of socket, patients had local anesthesia**, atrumatic extraction was performed using periotome** for severing the periodontal ligament with minimal damage to the surrounding alveolar ridge to facilitate removal of the involved tooth and to preserve bone and soft tissue, then using extraction forceps*** for tooth extraction (figures 4-5-6), Socket curettage was done using bone curettes****.

**Bone height**

The clinical parameters were assessed using a periodontal probe****** till reaching bone. A stent of thickness one mm was fabricated before the extraction using a cast. The tooth to be extracted was removed from the cast. Two holes were made in the resin plate in the following positions: mid buccal and mid palatal. Measurements were taken after tooth extraction from base of the stent to the crest of alveolar ridge (figures 7-8) and 3 months after extraction at time of implant placement (Madhan and Singh, 2017).

**Bone width**

The width of the alveolar ridge was measured immediately after extraction and after 3 months using a caliper clamp*. The measurement was done perpendicular to the tangent of the dental arch at the mid-point of the extraction site approximately 4mm apical to the level of the marginal gingiva of the adjacent teeth (figure 9).

5- For group (I) socket was filled with small particles bovine xenogenic bone graft.
6- For group (II) socket was filled with with large particles bovine xenogenic bone graft (Figure 10).
7- For group (III) socket was allowed to heal spontaneously.
8- Medications were prescribed (Augmentin*** 1g t.d.s, Metronidazole*** 500 mg twice/day and antiseptic**** mouth wash for 1 week).
9- Post-operative instructions were given to the patients; all patients were instructed not to wear any prosthetic restoration.

**Implant placement and core biopsy procedure:**

After 3 months, another cone beam CT was done. Changes in the width and height at the center of the extraction socket were evaluated in merged axial and sagittal views using On-demand superimposition system. Clinical measurements were repeated for the alveolar ridge bone height and width before implant placement by the same clinician (figures 11:14). An open flap reflection was done for core biopsy using trephine bur* and placement of submerged implant**, and then flaps

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* Artinbisa 40mg/0.01 mg/ml- solution injectable-inisha-Spain
** Periotome straight 2.5 mm / Periotome angled 2.5 mm TRINOVO - Germany
*** Martin-Nelson-Germany
**** Reicodent-Germany
***** Hu-Friedy UNC 15 Co.,LLC-USA

(MCT), GAU-04, South Korea
4-0 polypropylene Non-resorbable monofilament, Assut sutures-Switzerland
Augmentin®-GlaxoSmithKline, medical union pharmaceuticals, Egypt.
Flagyl, Sanofi aventis, Egypt
Orovex, MACRO Group Pharmaceuticals, Egypt
Bio-Oss (0.25-1 mm), Geistlich, Switzerland.
were closed (figures 15:17). Three months later patients received the prosthetic part of implant restoration.

Histological Examination and Histomorphometric measurements:

After 3 months from extraction, bone biopsy was taken by a trephine bur*, specimens were fixed in 10% buffered formaldehyde for five days [the formaldehyde is buffered in PH 7.2 phosphate buffer saline (PBS)]. The specimens were decalcified using a solution containing 12% Ethylene diamine tetra-acetic acid (EDTA) buffered in pH7.2 PBS for three weeks at 4°C (Bancroft and Gamble, 2008).

After complete decalcification, specimens were assigned for the following:

1- Histological staining:

A- Hematoxylin and Eosin stain:

Specimens were decalcified, properly washed under running water, dehydrated by being transported through progressive alcohol concentrations (50%, 60%, 80%, 90%, 96%, and absolute alcohol), and then transferred to xylol to remove the alcohol from the specimen. The specimens were then inserted in the middle of blocks of paraffin wax after being coated in the substance. The embedded samples were cut into sections using a microtome (3 microns thick), which were then transferred to descending alcohol concentrations (96%, 70%, and finally distilled water), before being stained with hematoxylin and eosin (H&E) stain for routine histological examination under a light microscope (Bancroft and Gamble, 2002).

B- Masson trichrome special stain:

Areas with new collagen synthesis were marked by a blue colour using the Masson trichrome (MT) special stain, while those without new collagen formation were marked by a reddish tint (Bancroft and Gamble, 2002). For each MTC-stained section, four microscopic fields were selected and photomicrographs were captured at original magnification of X20. All images were captured using digital camera (EOS 650D, Canon, Japan) which was mounted on a light microscope (BX60, Olympus, Japan). Images were then transferred to the computer system for analysis. This was performed in the Precision Measurement Unit, Oral Pathology Department, Faculty of Dentistry, Ain Shams University. All the steps of immunohistochemical assessment were carried out using Image J, 1.41a, (NIH, USA) image analysis software. The steps are shown in fig. (35). Images were first corrected for brightness and contrast. Corrected images were then converted into 8-bit type grayscale. Color thresholding was, then, adjusted. The area fraction (AF) of the red MTC-stained newly formed calcified bone was measured automatically. The area fraction represented the percentage of the new bone to the total area of the microscopic field. The mean area fraction (MAF) for each case was calculated. The collected data were tabulated in a Microsoft Excel sheet.

Statistical analysis:

Values were presented as mean and standard deviation (SD) values. Data were explored for normality using Kolmogorov-Smirnov test of normality. The results of Kolmogorov-Smirnov test indicated that most of data were normally distributed (parametric data), therefore, unpaired t test was used to compare both groups. Paired t test was used to compare the baseline and after 3 months values. The significance level was set at p < 0.05. Statistical analysis was performed with SPSS 18.0 (Statistical Package for Scientific Studies, SPSS, Inc., Chicago, IL, USA) for Windows. Numerical data were presented as mean and standard deviation (SD) values. They were explored for normality by checking the data distribution, and using Shapiro-Wilk test. Data showed parametric distribution so they were analyzed using one-way ANOVA followed by Tukey’s post hoc test for intergroup comparisons and paired t-test for intragroup comparisons. Statistical analysis was performed with R statistical analysis software version 4.1.2 for Windows3.

Results

A-Clinical:

Table 1: Mean, Standard deviation (SD) values of alveolar bone height (buccal) (mm) for different groups

<table>
<thead>
<tr>
<th>Interval</th>
<th>Alveolar bone height (buccal) (mm) (mean±SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Small particles</td>
<td>Large particles</td>
</tr>
<tr>
<td>Before</td>
<td>4.56±0.53^A</td>
<td>4.35±0.67^A</td>
</tr>
<tr>
<td>After</td>
<td>5.72±0.51^AB</td>
<td>5.40±0.77^B</td>
</tr>
<tr>
<td>Percentage change (%)</td>
<td>26.06±6.97^B</td>
<td>24.48±6.51^B</td>
</tr>
</tbody>
</table>

Means with different superscript letters within the same horizontal row are significantly different *; significant (p ≤ 0.05) ns; non-significant (p>0.05)

Table 2: Mean, Standard deviation (SD) values of alveolar bone height (palatal) (mm) for different groups

<table>
<thead>
<tr>
<th>Interval</th>
<th>Alveolar bone height (palatal) (mm) (mean±SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Small particles</td>
<td>Large particles</td>
</tr>
<tr>
<td>Before</td>
<td>4.78±0.67^A</td>
<td>5.35±0.53^A</td>
</tr>
<tr>
<td>After 3 months</td>
<td>5.50±0.61^B</td>
<td>5.90±0.61^AB</td>
</tr>
<tr>
<td>Percentage change (%)</td>
<td>15.61±6.63^B</td>
<td>10.26±2.41^B</td>
</tr>
</tbody>
</table>

Means with different superscript letters within the same horizontal row are significantly different *; significant (p ≤ 0.05) ns; non-significant (p>0.05)

Table 3: Mean, Standard deviation (SD) values of alveolar bone width (mm) for different groups

<table>
<thead>
<tr>
<th>Interval</th>
<th>Alveolar bone width (mm) (mean±SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Small particles</td>
<td>Large particles</td>
</tr>
<tr>
<td>Before</td>
<td>8.00±0.71^A</td>
<td>7.70±0.79^A</td>
</tr>
<tr>
<td>After 3 months</td>
<td>6.50±0.71^AB</td>
<td>6.70±0.79^A</td>
</tr>
<tr>
<td>Percentage change (%)</td>
<td>-18.88±1.64^B</td>
<td>-13.11±1.34^A</td>
</tr>
</tbody>
</table>

Means with different superscript letters within the same horizontal row are significantly different *; significant (p ≤ 0.05) ns; non-significant (p>0.05)

A-Radiographic:

Table 4: Mean, Standard deviation (SD) values of alveolar bone height (radiographic) (mm) for different groups

<table>
<thead>
<tr>
<th>Interval</th>
<th>Alveolar bone height (radiographic) (mm) (mean±SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Small particles</td>
<td>Large particles</td>
</tr>
<tr>
<td>Before</td>
<td>13.87±3.07^A</td>
<td>14.18±3.39^A</td>
</tr>
<tr>
<td>After 3 months</td>
<td>12.92±0.09^AB</td>
<td>13.10±0.40^A</td>
</tr>
<tr>
<td>Percentage change (%)</td>
<td>-11.17±1.78^AB</td>
<td>-8.07±2.67^A</td>
</tr>
</tbody>
</table>

Means with different superscript letters within the same horizontal row are significantly different *; significant (p ≤ 0.05) ns; non-significant (p>0.05)
Table 5: Mean, Standard deviation (SD) values of alveolar bone width (radiographic) (mm) for different groups

<table>
<thead>
<tr>
<th>Interval</th>
<th>Alveolar bone width (mm) (mean±SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Small particles</td>
<td>Large particles</td>
</tr>
<tr>
<td>Before</td>
<td>7.47±0.63\textsuperscript{A}</td>
<td>7.30±0.79\textsuperscript{A}</td>
</tr>
<tr>
<td>After 3 months</td>
<td>6.35±0.16\textsuperscript{A}</td>
<td>6.49±0.15\textsuperscript{A}</td>
</tr>
<tr>
<td>Percentage change</td>
<td>-12.93±2.85\textsuperscript{A}</td>
<td>-11.14±2.29\textsuperscript{A}</td>
</tr>
</tbody>
</table>

Means with different superscript letters within the same horizontal row are significantly different *; significant (p ≤ 0.05) \textit{ns}; non-significant (p>0.05)

Table 6: Mean, Standard deviation (SD) values of alveolar bone density (HIU) for different groups

<table>
<thead>
<tr>
<th>Interval</th>
<th>Alveolar bone density (HIU) (mean±SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Small particles</td>
<td>Large particles</td>
</tr>
<tr>
<td>Before</td>
<td>550.63±124.40\textsuperscript{A}</td>
<td>578.78±163.57\textsuperscript{A}</td>
</tr>
<tr>
<td>After 3 months</td>
<td>733.61±116.71\textsuperscript{A}</td>
<td>822.81±163.69\textsuperscript{A}</td>
</tr>
<tr>
<td>Percentage change</td>
<td>35.57±16.62\textsuperscript{A}</td>
<td>47.36±22.91\textsuperscript{A}</td>
</tr>
</tbody>
</table>

Means with different superscript letters within the same horizontal row are significantly different *; significant (p ≤ 0.05) \textit{ns}; non-significant (p>0.05)

III-Histological evaluation

Table 7: Mean, Standard deviation (SD) values of Area fraction of new bone formation (%) for different groups

<table>
<thead>
<tr>
<th>Area fraction of new bone formation (mean±SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small particles</td>
<td>Large particles</td>
</tr>
<tr>
<td>40.57±5.26\textsuperscript{A}</td>
<td>45.41±5.44\textsuperscript{A}</td>
</tr>
</tbody>
</table>

Means with different superscript letters within the same horizontal row are significantly different *; significant (p ≤ 0.05) \textit{ns}; non-significant (p>0.05)

III- Histological Assessment

A- Group (1) Small particles

Figure 1: A photomicrograph of a G1 case stained with MTC showing a generalized pattern of maturation of the newly developed bone trabeculae, as exhibited by the uniform red staining (green arrows). The blue stain is confined to the delicate stromal collagen fibers (black arrows), while some dense collagen bundles demonstrate an evidence of heavy mineralization as confirmed by the red staining (yellow arrows)(Original magnification X20).
B- Group (2) Large particles

Figure 2: A photomicrograph of a G2 case stained with MTC showing an extensive maturation of the newly formed bone trabeculae, as denoted by the exclusive red staining (blue arrows). The dense collagenous surrounding stroma also expresses areas of mineralization (green arrow), while other areas retain the unmineralized blue stained collagen fibers (yellow arrows) (Original magnification X20).

C- Group (3) Natural healing

Figure 3: A photomicrograph of a G3 case stained with MTC showing a limited formation of a mixture of small strands of blue-stained osteoid (black arrows), thin short red-stained bone (green arrows), surrounded by both blue-stained delicate collagen fibers (white arrows) and calcified red-stained scar-like tissue (yellow arrows) (Original magnification X20).

Case Presentation

Figure 4: Badly decayed upper left first premolar indicated for extraction.  

Figure 5: Atraumatic extraction using periotome.
Figure 6: Empty socket after atraumatic extraction.

Figure 7: Showing periodontal probe measuring mid buccal bone height (8 mm).

Figure 8: Showing periodontal probe measuring mid palatal bone height (7 mm).

Figure 9: Showing measuring bone width using bone caliper after extraction (7 mm).

Figure 10: Bone graft and interrupted suture.

Figure 11: Healing site after three months.
Figure 12: Showing periodontal probe measuring mid buccal bone height after three months (10 mm).

Figure 13: Showing periodontal probe measuring mid palatal bone height after three months (7.5 mm).

Figure 14: Showing measuring bone width using bone caliper three months after extraction (6 mm).

Figure 15: Osteotomy site preparation.

Figure 16: Implant in place and covered by cover screw.

Figure 17: Showing interrupted sutures.
Discussion

Xenograft is regarded as one of the best available grafting materials for alveolar ridge preservation after tooth extraction, according to a systematic review that assessed the efficacy of various bone-material substitutes for alveolar preservation after tooth extraction (Canellas et al., 2021).

The bovine xenogeneic cancellous bone with a similar architecture to natural bone could help more patients. For developing the extracellular matrix required to restore damaged or injured tissue, the cells engaged in the healing process should be able to migrate, differentiate, and proliferate in bone substitute materials. Thus, the physicochemical criteria of these synthetic materials, such as ions composition, crystallinity, particle size, surface properties, and porosity architecture, are directly connected to their biological activities (Gehrke et al., 2019).

The limited number of trials comparing ARP with and without biomaterials to seal the socket led Del Fabbro to conclude that no specific sealing method or biomaterial can be suggested over another in ARP (Del Fabbro et al., 2022).

The alveolus can be sealed using a variety of methods during (ARP). One of these is leaving the biomaterial exposed to the oral cavity with no barriers (OHNB), which greatly reduces the complexity and length of the surgical procedure. Some studies that evaluated this strategy concluded that it was successful and caused only minor ridge resorption (Martins et al., 2022).

Upper and lower impressions were obtained in accordance with the preoperative preparation to create a working model. As a fixed reference guide for the vertical measurements taken with a standardised periodontal probe, the base of the acrylic stent was used to create acrylic stents on the working model that included at least one tooth next to the tooth that would be extracted. The measurements were performed both immediately following extraction and three months later (Madhan and Singh, 2017).

It has been observed that a socket usually heals in three months. Moreover, for years, clinicians have applied a minimum three-month as a socket healing duration prior to reentry for implant surgery (Jambhekar et al., 2015).

The effectiveness and accuracy of various radiographic techniques used for clinical applications in dentistry have been examined by several trials. Bite-wing and periapical intraoral films offer a two-dimensional picture, whereas computed tomography creates a three-dimensional image. Instead of layering numerous slices together like a traditional CT scanner does, the CBCT scans the head in two dimensions to get this image. This enables a picture that is more effective, affordable, and low energy output. Along with these advantages, the CBCT does not generate large radiation doses (Sukovic, 2003).

Because it can distinguish between mineralized and osteoid tissue, Masson trichrome stain was utilised for the histomorphometric examination of bone quality and quantity (Suvik et al., 2012).

The histo-morphometric analysis of bone samples is the most biologically accurate technique for evaluating bone structure, but it is not frequently used in clinical practice (Ivanova et al., 2021).

The bone alterations assessed three months following tooth extraction were the aim of our study. Our findings indicated that some degree of bone loss might be predicted and that no grafting material can entirely stop post-extraction resorption. Similar findings have been published in earlier systematic reviews (Canellas et al., 2021).

The current study's findings showed that all groups had decreased bone height and bone width. The fact that ridge preservation techniques decrease bone dimensional alterations when compared to extraction without ridge preservation operations has been supported by literature. These findings are in agreement with a systematic review that found no material to be clearly superior for maintaining both horizontal and vertical dimensions in post-extraction sockets. However,
all of the treatments were more effective than spontaneous healing (Canullo et al., 2021).

Large particles group and small particles group of xenografts clearly demonstrated a better clinical behavior regarding vertical and horizontal bone preservation compared to spontaneous healing. This can be explained by the fact that xenografts have been demonstrated to be resistant to resorption due to de-antigenation that occurs during processing (Canullo et al., 2021).

Additionally, 25 bone-substitute materials that were applied to preserve sockets were evaluated in a network meta-analysis. Two xenograft materials demonstrated the highest number of statistically significant variations for width and height preservation when bone alterations were assessed 3-6 months following tooth extraction (Canellas et al., 2021).

Regarding bone width, large particles group showed a slight favourable result compared to both control and small particles groups. This result might be due to slow particles resorption. As, if the particles are smaller in size, they may get resorbed at a faster rate (Anil et al., 2020).

These findings agree with the hypothesis that anorganic xenogenous graft could slow the resorption of autogenous bone, increasing the volume to the grafted area (de Azambuja Carvalho et al., 2019).

In a study by Kheur et al., the large particle size graft (1 to 2 mm) resulted in a greater ridge width gain compared with the small particle size graft (0.25 to 1 mm) when used for staged ridge split procedures in the posterior mandible (Kheur et al., 2018).

The inflammatory response in the surrounding tissue and particularly the induction of MNGCs were examined in relation to the effects of the granule size of three bone substitutes that were made in an identical manner (as a parameter of the material degradation). While the medium-sized and large granules both lead to a fibrous encapsulation without an ongoing cellular breakdown, the small granules appear to be entirely degradable (Abels et al., 2021).

Unlike a study that examined the impact of granule size on the induction of multinucleated giant cells (MNGCs) and implant bed vascularization in a subcutaneous implantation model in rats using 2 biphasic bone substitutes (400-700 mm and 500-1000 mm), the findings demonstrated that mononuclear cell phagocytosis was unaffected by granule size in the studied range (Barbeck et al., 2015).

Radiological analysis of different views of CBCT showed that the maximum amount of resorption have occurred within the control group (group III) after 3 months. The volume of resorption was significant compared to both group I and II that showed less vertical and horizontal bone resorption.

Similar results were concluded by Al Qabbani et al., when he found that radiological evaluations showed that the use of bovine bone granules to fill in the socket alveolar bone defects showed better soft and hard tissue healing and it seems essential in preserving the alveolar bone dimension, specifically the thin buccal plate (Al Qabbani et al., 2018).

Alveolar ridge preservation in groups I and II increased bone density compared to natural healing in group III. This finding is not different from Lorenz et al., who found that the bone density increased within two groups grafted with xenogeneic bone substitute in maxillary sinus augmentation. The presented increase in bone density in both bone substitute material groups seems to represent the replacement of the graft material with newly formed bone (Lorenz et al., 2018).

In 2018, measurements of bone density were made after socket preservation with xenograft and averages of bone densities showed the mean of the experimental group to be higher than that of the control group; resulting in a similar result of our study (Ferreira Júnior et al., 2018).

By means of histological and histomorphometric analysis, there was no statistically significant difference in the percentage of biomaterial, newly formed bone or connective tissue between the small and large-sized particle
groups. These results are in agreement with de Molon et al. who investigated sinus floor augmentation with two different particle sizes of demineralized bovine bone (de Molon et al., 2019, de Azambuja Carvalho et al., 2021).

Histologically, both group I and II showed more new bone formation compared to group III. This result was also found by Barone et al., when he analyzed and compared histologic and histomorphometric aspects of the extraction-alone sites to the grafted sites. Also Crespi et al., had the same result when he examined the use of xenograft in fresh sockets via histomorphometric and in vivo gene expression profiling (Canullo et al., 2021).

Different results were obtained by Lim et al., when he compared histologically the difference between using deproteinized bovine bone mineral (DBBM) in ARP and natural healing; more percentage of new bone was observed in the control group (25.16±18.45%) compared to (16.92±14.86%) in test group (Lim et al., 2019).

This result may be due to the use of ARP without primary flap closure. Also, he used molar areas (wider defects) for his study rather than anterior or premolar areas.

Another different result was concluded by Ben Amara et al., The histomorphometric analysis demonstrated that alveolar ridge preservation hindered bone formation in extraction sockets and resulted in less amount of new bone formation (Ben Amara et al., 2021).

But, The result of his study may be affected by using extraction sockets of periodontally compromised teeth as test groups.

The method of sectioning the biopsy specimen and the method of measuring the section played important roles in the result. In our study we believe that a longitudinal section can fully show the gradual change in the ratio of new bone from crown to root. By observing the entire longitudinal layer of slices, a more objective judgment regarding the overall histological result can be made. Therefore, the difference in histological methods among studies might have affected the results and led to bias.

The possible reason for the differences between studies is that the position of the natural healing tissue section was biased toward the root, resulting in a significantly higher proportion of new bone than in other articles. However, the bias of the tissue slices in the test group toward the crown side may have increased the residual rate in the DBBM group (Zhao et al., 2020).

Our results suggested that no grafting material can totally prevent post-extraction resorption, and some quantity of bone loss might be expected. Similar results have been reported in previous systematic reviews (Canellas et al., 2021).

Scaffold properties could modulate cell behaviors, mainly for bone regeneration applications. Some of the physicochemical properties are intertwined, thus making it difficult to separately study the effect of individual properties. Discrepancies between studies may be due to the difference in particle size, scaffold composition, specific surface area, pore size range and bone regeneration assessment method. Another challenge is the limitation in materials fabrication. Traditional manufacturing techniques render it challenging to fabricate scaffolds with different pore sizes while maintaining similar mechanical properties. Hence, the effect of pore size can be challenging to be studied alone, and the concept of a universal optimal pore size remains disputable (Zhu et al., 2021).

Conclusion

Based on the results of the present study, it can be concluded that:

Xenogenic bone graft Particle size has no effect on the quality of newly formed tissue at the time of implant placement after 3 months of alveolar ridge preservation. The spontaneous healing group (control group) showed more immature bone and granulation tissue formation after three months of socket preservation. Xenogenic bone grafts can lead to formation of mature bone in augmented sockets after three months. Xenogenic bone graft Particle size has no effect on dimensional reduction occurs in the.
alveolar ridge following dental extraction. Extraction sockets grafted with xenogenic bone grafts showed better bone quality compared with spontaneous healing both clinically and radiographically. Socket grafting appeared to improve bone density compared to non-grafted ones. A complete prevention of remodeling is not achievable, irrespective of the technique used.

**Recommendations**

Further long-term studies need to be carried out, soft tissue augmentation could be done to improve esthetic outcome of final restoration, porosity between bone particles may be a much important factor to be investigated.

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**References**


Shokry et al.


