Reactive Soft Tissue Preservation in Large Bone Defects After Tooth Extractions: A Cone Beam Study

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Purpose: Reduction of alveolar height and width after tooth extraction may present problems for implant placement, since bone volume is important for biologic and esthetic reasons. The aim of this study was to assess bone healing of large bone defects grafted with collagen sheets and maintenance of reactive soft tissue, evaluating cone beam computed tomography (CBCT) scans and histomorphometric results.

Materials and Methods: Patients presented large bone defects after tooth extractions. Reactive soft tissue was left in the defects filled by collagen sheets. Vertical bone volume was assessed by CBCT examinations before tooth extractions and 3 months later. At 3 months, cylinder bone samples were obtained for histology and histomorphometry analysis. Results: Twenty-six patients were included in the clinical study. Examined defects reported mean bone gain of 12.13 ± 3.91 mm, and mean vertical bone levels showed a statistically significant increase (P < .05) at 3 months after extraction. Histologic examinations revealed bone formation; mean vital bone measurements were 41.59% ± 5.80%, and connective tissue percentages averaged 50.37% ± 7.73%. Conclusion: Reactive soft tissue left in large bone defects after tooth extraction and grafted collagen may support a significant vertical bone gain and vital bone formation.

Keywords: bone defects, collagen, cone beam computed tomography, reactive soft tissue

Several studies reported significant structural changes and bone resorption in fresh sockets following extraction procedures, with important dimensional changes in the surrounding alveolar bone.1–3 Removal of teeth and granulation tissue in large bone defects during surgery procedures was followed by loss in height and width of the alveolar process. It resulted in narrowing and shortening of the residual ridge.4 Bone resorption continues over time, but the most statistically significant loss of tissue contour occurs during the first month after tooth extraction, averaging 3 to 5 mm in width and between 0.4 and 3.9 mm in height at 6 months.5 The alteration of the ridge occurs concomitantly with the healing of bone tissue, but the process of remodeling may also continue after the conclusion of de novo bone formation in the socket.6 Concomitant with these intraalveolar healing events, the bone buccal plate of the socket underwent marked resorption of 2.5 mm on average,1 in comparison to the lingual bone plate.

As reported by ten Heggeler et al7 in a systematic review, socket preservation techniques may aid in reducing the bone dimensional changes following tooth extraction, but they do not prevent bone resorption, so a loss in width up to 3.48 mm and in height up to 2.64 mm may still be expected. With this procedure, key factors to ensure successful grafting of bone into extraction sites include asepsis and complete removal of reactive granulation tissue, ensuring that the blood supply to the graft site is adequate, and ensuring primary soft tissue closure.8Also, bone regeneration may be adversely affected by a lack of primary wound closure during the healing period.9 However, Lindhe and Nyman10 reported that removal of granulation tissue in conjunction with flap surgery is not critical for establishing conductive conditions for the efficient healing of the periodontal tissues. Hence, the implications of the removal of the granulation tissue on the repair process of the alveolar bone have been interpreted in different ways over time. The fact that granulation tissue formed after
tooth extraction can differentiate into bone, filling up the empty defects, indicates that some precursor cells are present.\textsuperscript{11,12} The presence of multipotent progenitor stem cells has been demonstrated in the case of infected granulation tissues from chronic periodontitis lesions,\textsuperscript{13} so the commonly practiced removal of granulation tissue during bone surgery may also result in removal of vital cells with characteristics of multipotent stem cells, which could support tissue healing.

The demanding suggestion of this study was that intrasocket reactive soft tissue containing granulation and epithelial tissue was left in the bone defect, and collagen sheets filled the large bone defects.

The aim of this study was to assess bone healing of large bone defects grafted with collagen sheets and maintenance of reactive soft tissue, using cone beam computed tomography (CBCT) and histomorphometric evaluation.

MATERIALS AND METHODS

Patient Selection

The prospective clinical study was composed of a population of patients presenting to the Department of Dentistry, San Raffaele Hospital, Milan, Italy, between October 2012 and June 2014. The inclusion criteria were the presence of large bone defects after tooth extractions, with the loss of interdental alveolar buccal bone plate and presence of active or chronic infections in the defects. All patients were in good health, with no chronic systemic disease, and did not smoke. Exclusion criteria were: coagulation disorders and alcohol or drug abuse. The local ethical committee approved the study, and all patients signed an informed consent form. The diagnosis was performed both clinically and radiographically by CBCT. The patients included in this clinical study were treated by one oral surgeon in the Department of Dentistry, San Raffaele Hospital, Milan, Italy.

Surgical Protocol

One hour prior to surgery, the patients received 1 g amoxicillin and then 1 g twice a day for a week after the surgical procedure. Surgery was performed under local anesthesia (optocaine 20 mg/mL with adrenaline 1:80,000 [Molteni Dental]).

The following surgical procedure was performed:

1. Teeth were gently separated from the intrasocket reactive soft tissue and bony wall in mandibular and maxillary bones.
2. The periosteum was not detached, avoiding flap raising.
3. Reactive soft tissue was left in the defect (Table 1). A periodontal probe (Hu-Friedy PGF-GFS, Hu-Friedy) was used to verify the defect wall assessment.
4. Collagen sheets, secured by sutures (Condress, Abiogen Pharma), filled bone defects.\textsuperscript{14,15}
5. The gingival edges of the wound were not sutured together; the wound was left “open” to gradually close and heal on its own. The sutures were not tight and only served to achieve soft tissue adaptation without obtaining primary closure of the wound margins\textsuperscript{16,17} (Fig 1).

Three months after surgery, the biopsy specimens of core bone 2 × 7 mm in length were harvested with a trephine bur from 15 patients where titanium implants would be inserted; histology was performed.

Histomorphometry

The samples were fixed in 4\% buffered formaldehyde, dehydrated in a graded series of alcohols from 50\% to 100\%, and embedded in epoxy resin (Epon, Polysciences), according to a previously described procedure.\textsuperscript{18} Thirty undecalcified, 10-mm-thick sections along the axis of the biopsy specimen were obtained with a microtome. The sections were stained with toluidine blue and observed using Normasky differential interference contrast microscope (D.I.C.).

A microscope (Fomi III, Carl Zeiss) connected to a computer and a digital camera (Leica DC 280, Leica Microsystems) was used for histomorphometric measurements. Histomorphometry was performed with software (Alexasoft, Microcontrol). The percentage of mineralized tissue was calculated in all sections of the same sample, and the measurements were performed at 63× magnification. Values reported were the percentage of vital bone (VB) and the percentage of connective tissue (CT). The total amount of VB and CT corresponded to the complete tissue volume.

Radiographic Examination

The CBCT scans within this study were performed with a device dedicated to dental and maxillofacial imaging (Gendex GXCB-500; Gendex Dental Systems). Free i-CAT Vision viewing and sharing software was applied; 1-mm-thick scans were obtained at 120 kV and 30.89 mAs, with a resolution of 0.2 voxels for 23 seconds, and a diameter of 8.5 × 8.5 mm. A CBCT cross-sectional projection was extrapolated and analyzed at the longitudinal midportion of each examined defect. Cone beam units use a divergent cone/pyramid-shaped beam to obtain multiple planar projections in a single rotation. These cone-shaped...
beams are similar to those of X-ray units used for two-dimensional radiography. This cone beam unit functions with the patients sitting.

CBCT examinations were performed before tooth extractions and 3 months later (Fig 2). The alveolar bone levels were assessed from the most coronal, palatal bone in which the alveolar crest was identified. For measurements of defect depth, one straight line was drawn from most coronal point perpendicular to the palatal surface and another parallel at the bottom of the defect before extraction; perpendicular to these lines, a straight line was drawn to measure the distance between them (Fig 2e). Three months later, one line was drawn at same previous coronal point of defect and the second parallel at the most coronal point of bone mineralization (Fig 2f). Before and 3 months after tooth extractions, measurements were acquired, and changes were evaluated. For buccal-palatal width assessment, two lines were drawn, from the palatal to the buccal side at the coronal and apical portion of the sockets (Figs 2g and 2h). The two CBCT scans for each patient were then transferred to a blind examiner radiologist for evaluation.

**Statistical Analysis**

Dedicated software was used for all statistical analysis (SPSS 13.0, IBM). Data were presented as means ± standard deviations. Comparisons between bone level values before and after extraction were performed by a Student t test (P < .05 was considered the threshold for statistical significance).

**RESULTS**

Twenty-six patients, 15 women and 11 men with a mean age of 55.9 years (range, 37 to 69 years), were included in the clinical study. In all cases, after tooth extraction, the granulation tissue was left in the defect.

Thirty-one teeth were extracted; defect sites are reported in Table 1.

During the healing period, no signs of inflamed tissue or exposed bone were observed.

**Radiographic Evaluation**

Three months after tooth extractions, CBCT revealed bone changes. Before extractions, large bone defects presented mean bone levels of –9.24 ± 2.53 mm; 3 months after extraction, 2.89 ± 3.38 mm were reported with a mean bone gain of 12.13 ± 3.91 mm. In all examined defects, mean vertical bone levels showed a statistically significant increase (P < .01) at 3 months after extraction.
All defects observed in this study presented bone growth in buccolingual width; nevertheless, volume dimensional changes were reported after tooth extraction (Figs 2f and 2g). After 3 months, a mean volume loss of –2.76 ± 0.87 mm for the apical section and –4.84 ± 0.66 mm for the coronal section was observed.

**Histologic Evaluation**

Histologic evaluation of all examined sections revealed the presence of vital lamellar bone and some areas of woven bone surrounding highly vascularized tissue, with the presence of connective tissue and the absence of inflammatory cells. Trabecular bone assessment showed no differences among the apical, mesial, and coronal portions of the specimens (Fig 3). Areas of immature bone were observed, especially in the core of biopsy specimens (Fig 3). Data from the histomorphometric analysis are reported in Table 2. Mean VB measurements were 41.59% ± 5.80%, and CT percentages averaged 50.37% ± 7.73%.

**DISCUSSION**

In the present study, CBCT reported a statistically significant increase \( (P < .01) \) of vertical bone volume at 3 months in large defects after tooth extraction. Biopsy specimens obtained in 15 patients confirmed radiographic data, showing the presence of vital bone in the defects 3 months later. Reactive soft tissue was
maintained in the defects, and collagen sheets were grafted. In a clinical study, buccal bone plate recontouring in maxillary fresh sockets with buccal bone loss using CBCT was evaluated. It was reported that bone growth in buccolingual width, in particular in monoradiculars, had a mean bone gain of 5.36 ± 2.65 mm after 3 months, and in pluriradiculars, a mean bone gain of 5.89 ± 2.88 mm. The study concluded that in the first months, it is possible to observe the formation of buccal bone plate in sockets with previous buccal bone loss.

Dental infection, due to periodontal, endodontic lesions, or root fractures, develops mainly as a result of the immunologic response to continuous antigenic stimulation from the root canal or periodontal pocket, creating a chronic inflammatory process.19,20 This reactive tissue composed of granulation tissue represents local defensive response to this chronic inflammatory process.20,21

Reactive soft tissue left in the defects after tooth extraction is an example of fibrovascular proliferation; it contains new small blood vessels, fibroblasts, and mononuclear cells in an edematous extracellular matrix, and it can form in connective tissue during wound healing, chronic inflammation, and certain pathologic conditions. Interestingly, the granulation tissue fibroblasts from both chronically inflamed periodontal lesions and healing wounds behaved similarly in vitro.22 Therefore, the chronically inflamed periodontal tissue that is usually removed during surgery might also contain stem cells for wound healing.

Recent evidence suggests that reactive soft tissue obtained from intrabony defects during surgery contains mesenchymal stem cell populations that express the STRO-1 marker.23 To this extent, the cell cultures established from these granulation tissues expressed a panel of embryonic stem cell markers, including Oct4, Rex-1, and Sox2, which are crucial for the pluripotent capacities of stem cells.24-27 This provides evidence that reactive soft tissue contains cells with embryonic stem cell properties.

Nevertheless, the present findings imply that surgical removal of granulation tissue inevitably results in removal of pluripotent stem cells that might potentially contribute to the healing of the tissue, once the infection is controlled.

For this reason, surgical trauma was minimized and the original soft tissue contour preserved because no soft tissue flap with releasing incisions was raised, and periosteal releasing incisions that could jeopardize the blood supply were avoided.

No attempt was made to elevate a flap for primary soft tissue closure. Therefore, the amount of attached gingiva and the original apicolar position of the

Table 2  Histomorphometrical Data of 15 Defects

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Defect tooth position1</th>
<th>VB (%)</th>
<th>CT (%)</th>
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<tr>
<td>1</td>
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<td>M</td>
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<tr>
<td>2</td>
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<td>F</td>
<td>47</td>
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<td>57.7</td>
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<td>41</td>
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<td>38.8</td>
</tr>
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</table>

1 FDI tooth-numbering system.

VB = vital bone; CT = connective tissue.

Fig 3  (a) Histologic section of bone obtained 3 months later. Vital mature lamellar bone (VT), with the presence of numerous CT spaces. (b) Vital bone containing osteoblasts, osteocytes, and many blood vessels. No osteons are present. (D.I.C. Toluidine blue staining; original magnification: a: 10×; b: 32×). (c) The bone formation process was well identified by the presence of osteoblasts (D.I.C. Toluidine blue staining; original magnification: 250×).
mucogingival junction relative to the neighboring teeth remained unchanged.

In this study, collagen was placed over granulation tissue and gingival flaps were sutured alongside collagen sponge, promoting a wound healing process by secondary intention.

Collagen provides structural and mechanical support to tissues and organs and fulfills biomechanical functions in bone, cartilage, skin, tendons, and ligaments.

Forms of collagen type I, commonly extracted from bovine tendon, are biocompatible and adequate scaffolds for tissue engineering in terms of mechanical properties, pore structure, permeability, hydrophilicity, and in vivo stability. Several immunologic studies (animal models) of injectable collagen gels and implanted collagen sponges confirm that few or no antibodies to collagen type I are detected. Collagen type I has been shown to support osteoblast and osteoclast proliferation and differentiation in vitro as well as in vivo. Collagen is easily degraded and resorbed by the body and allows good attachment to cells.

In this context, it must be pointed out that both stabilization of the blood clot and early angiogenesis were considered to be important factors strongly influencing wound healing, because experimental studies in animals have indicated that formation of blood capillaries precedes the formation of new bone since osteogenic cells were observed to arise from pericytes adjacent to the connective tissue of small blood vessels. Blood clots fill the bony defect; neovascularization and maturation of the fibrin clots support formation of a new connective attachment, and subsequently, new bone formation starts from open bone marrow spaces of the adjacent defect borders.

However, great variability exists in humans with respect to hard tissue formation within extraction sockets. Thus, whereas a provisional connective tissue consistently forms within the first weeks of healing with bone volume formation in the first months; successively, the interval during which mineralized bone is laid down is much less predictable later.

These results may be important in suggesting implant placement in the first months of bone healing, avoiding bone resorption later.

CONCLUSIONS

This study reported that reactive soft tissue left in situ, with collagen sponge grafted in large bone defects, may be helpful for significant vertical bone gain. However, these results are not supported by biologic explanation, and further laboratory and clinical research will be required to determine that both collagen graft and granulation tissue maintenance are available for bone healing.

ACKNOWLEDGMENTS

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REFERENCES