Histomorphometrical Analysis following Augmentation of Infected Extraction Sites Exhibiting Severe Bone Loss and Primarily Closed by Intrasocket Reactive Soft Tissue

Ofer Mardinger, DMD, BMed Sc;* Marilena Vered, DMD;† Gavriel Chaushu, DMD, MSc;‡ Joseph Nissan, DMD§

ABSTRACT

Background: Intrasocket reactive soft tissue can be used for primary closure during augmentation of infected extraction sites exhibiting severe bone loss prior to implant placement. The present study evaluated the histological characteristics of the initially used intrasocket reactive soft tissue, the overlying soft tissue, and the histomorphometry of the newly formed bone during implant placement.

Materials and Methods: Thirty-six consecutive patients (43 sites) were included in the study. Extraction sites demonstrating extensive bone loss on preoperative periapical and panoramic radiographs served as inclusion criteria. Forty-three implants were inserted after a healing period of 6 months. Porous bovine xenograft bone mineral was used as a single bone substitute. The intrasocket reactive soft tissue was sutured over the grafting material to seal the coronal portion of the socket. Biopsies of the intrasocket reactive soft tissue at augmentation, healed mucosa, and bone cores at implant placement were retrieved and evaluated.

Results: The intrasocket reactive soft tissue demonstrated features compatible with granulation tissue and long junctional epithelium. The mucosal samples at implant placement demonstrated histopathological characteristics of keratinized mucosa with no residual elements of granulation tissue. Histomorphometrically, the mean composition of the bone cores was – vital bone 40 ± 19% (13.7–74.8%); bone substitute 25.7 ± 13% (0.6–51%); connective tissue 34.3 ± 15% (13.8–71.9%).

Conclusions: Intrasocket reactive soft tissue used for primary closure following ridge augmentation is composed of granulation tissue and long junctional epithelium. At implant placement, clinical and histological results demonstrate its replacement by keratinized gingiva. The histomorphometrical results reveal considerable bone formation. Fresh extraction sites of hopeless teeth demonstrating chronic infection and severe bone loss may be grafted simultaneously with their removal.

KEY WORDS: bone augmentation, histomorphometric evaluation, infected extraction site, ridge preservation

INTRODUCTION

Hopeless teeth because of root fracture, perforations, and combined endodontic periodontal problems, are associated with infection, which conventionally contraindicated immediate bone grafting following their removal. The fear of graft contamination and failure led to the recommendation of delaying the grafting procedure by 6 to 8 weeks.[1–8] On the other hand, a number of studies have demonstrated that the survival rate of implants placed immediately following extraction of teeth in infected sites with simultaneous augmentation, after socket debridement and prophylactic antibiotic

*Lecturer, Department of Oral and Maxillofacial Surgery, Tel Aviv University, Tel Aviv, Israel; †senior lecturer, Department of Oral Pathology, Tel Aviv University, Tel Aviv, Israel; ‡senior lecturer, Department of Oral and Maxillofacial Surgery, Tel Aviv University, Tel Aviv, Israel; §senior lecturer, Department of Oral Rehabilitation, The Maurice and Gabriela Goldschlager School of Dental Medicine, Tel Aviv University, Tel Aviv, Israel

Reprint requests: Dr. Ofer Mardinger, Department of Oral and Maxillofacial Surgery, School of Dental Medicine, Tel Aviv University, Tel Aviv, Israel; e-mail: ofer.mardinger@gmail.com

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treatment, is similar to that of implants placed in healed ridges.[2,9,10] Marcaccini and colleagues[10] investigated the issue histologically on periodontally infected sites in dogs. They concluded that periodontal disease does not affect bone remodeling around immediately placed implants. Although the healing in periodontally infected sites was slower initially, it reached the level of the non-diseased sites after 12 weeks.

Key factors to ensure successful grafting bone into extraction sites besides asepsis include complete removal of reactive granulation tissue, adequate blood supply, and primary soft tissue closure.[11] Therefore, socket augmentation in sites with severe bone loss and chronic infection seems to be even more challenging. Large amounts of bone have to be regenerated; the chronic infection requires treatment, and primary closure is more difficult because of the fragility of the surrounding soft tissue.

Bone regeneration may be adversely affected by lack of primary wound closure during the healing period.[12] Recently,[13] a method of using the intrasocket reactive soft tissue for primary closure following augmentation of infected extraction sites with severe bone loss prior to implant placement was described. The purpose of the present study was to evaluate the histological features of the intrasocket reactive soft tissue at the time of tooth extraction, the overlying soft tissue at the time of implant placement, and histomorphometry of the newly formed tissue in the grafted area.

MATERIALS AND METHODS

Study Population and Design

This prospective, single-cohort study included 36 patients (19 women, 17 men), ranging in age from 24 to 75 years (mean 50.75 years), with severe bone loss in 43 sites. All the subjects were treated at the Tel Aviv University School of dental medicine and private practices from 2004 to 2008. The study was conducted with the approval of the institutional Helsinki Committee. The study group comprised of 30 cases where biopsies were taken, enabling histologic and histomorphometric evaluation.

Inclusion criteria

1. >18 years of age with hopeless teeth.
2. Implant-supported restoration is the required treatment plan.
3. Reasons for extraction included severe periodontal disease, tooth crack, or fracture.
4. Preoperative periapical and panoramic radiographs demonstrating extensive bone loss. The envelope of bone (clinical and radiological examination) is not intact and a future implant placed in the site would not be completely surrounded by bony walls (Class II extraction site according to Tinti and Parma-Benfenati 2003[14]).

Exclusion criteria

1. History of systemic disease that would contraindicate surgical treatment.
2. Patients taking medications that are associated with a compromised bone healing response (ie, diabetes, autoimmune dysfunction, prolonged cortisone therapy, long-term nonsteroidal anti- inflammatory drug therapy, or chemotherapy).
3. Failure to sign an informed consent.
4. Extraction site with less than two bony wall defects.
5. Noninflamed sites.
6. Unwillingness to return for follow-up examination.
7. The use of >10 cigarettes per day.

Surgical Protocol

The detailed surgical protocol was recently published[13]: the main point of the surgical protocol was the administering of oral antibiotics of 1,000 mg amoxicillin (Moxypen Forte, Teva Pharmaceutical Ltd, Petach Tikva, Israel) and 600 mg Etodolac (Etopan, Taro Pharmaceutical Industries Ltd, Haifa Bay, Israel). Antiseptic mouthwash, 0.2% chlorhexidine gluconate (Tarodent, Taro Pharmaceutical Industries Ltd.), was used immediately prior to surgery. The patients were treated under local anesthesia. Most of the teeth were slightly mobile: discharging pus and the surrounding gingiva bled easily. The teeth were gently separated from the intrasocket reactive soft tissue and bony wall. Sockets were irrigated with saline after extraction. The intrasocket reactive soft tissue was elevated from the bony walls in a subperiosteal plane and rotated while leaving it connected to the alveolar gingiva. The intrasocket reactive soft tissue remained outside the socket.

In all patients, porous bovine xenograft bone mineral (Bio-Oss, Geistlich Pharma, Wolhusen, Switzerland) was used as a single bone substitute. No membranes were used to cover the extraction sites. The grafting material was placed in the extraction site designed to create the previous architecture of the ridge.

The intrasocket reactive soft tissue was sutured over the grafting material to seal the coronal portion of the socket. The suture was not tight and only served to achieve soft tissue adaptation without harming the local blood supply. Amoxicillin (Moxypen Forte) 500 mg three times a day (tid) and 600 mg Etodolac (Etopan) twice a day were prescribed orally for 5 days postoperatively. As an antiseptic solution, 0.2% chlorhexidine gluconate mouthwash (Tarodent) was used for 45 seconds, tid for 2 weeks. No provisional restorations were used. The healing process was monitored clinically every week for the first 4 weeks and then monthly for the next 5 months. Periapical radiographs were taken at the 3- and 6-month follow-up. Panoramic radiographs and dental computerized tomography were taken before implant placement. Six months following surgery, an implant of appropriate size was placed in the healed ridge. At the time of elevation of the mucoperiosteal flaps a biopsy was taken from the site were the reactive tissue was placed. In ten of the cases during the augmentation phase, a biopsy from an excessive reactive soft tissue proliferation from the socket was submitted to histopathologic examination. At the time of implant site preparation, the template was placed and a core of bone 2.0×5.0 mm long was obtained with a trephine from 30 consecutive sites. The core and the gingival specimens were coded and sent to the laboratory of histopathology at the Tel Aviv University Dental School. Dental implants (Seven MIS Implants Technologies Ltd, Shlomi, Israel; Tapered Screw-Vent, Zimmer Dental, Carlsbad, CA, USA; Screwplant Implant Direct, Calabasas Hills, CA, USA; Osseotite® 3i/Implant Innovations, Biomet®, Palm Beach Gardens, FL, USA) were placed into the augmented sockets. All implants were rehabilitated with fixed partial denture.

Histomorphometric Evaluation

Submitted specimens were fixed in 10% buffered formalin for 24 hours. Soft tissue specimens were routinely embedded in paraffin and further prepared for hematoxylin and eosin staining procedure. The bony cores first underwent rapid decalcification for ~72 hours (Ethylenediaminetetraacetic acid, pH = 6). Afterwards, they were routinely embedded in paraffin and hematoxylin and eosin stained slides were prepared. Sections were prepared along the long axis of the core. Using a light microscope (Olympus BH-2, Tokyo, Japan) with a mounted digital camera (Olympus DP-70, Tokyo, Japan), each bony core was photographed. The entire area of the cores representing the bone grafted zones was covered-contained in five consecutive, nonoverlapping photomicrographs performed at ×200. The photomicrographs were saved as .jpeg files. A power point presentation was prepared in which each case was represented by five successive slides, each showing at full screen the photomicrographs copied from the corresponding jpeg files of the case. A 10×10 graphical square grid was prepared, where the center of each square was marked by a "+". This grid was superimposed on each photomicrograph in the power point presentation.

Histomorphometric evaluation of the photomicrographs was performed using a modified point-counting methodology, which was previously applied in histomorphometric studies.[15] The parameters evaluated in the study were bone, bone substitute and connective tissue. Each time one of these parameters overlapped the "+" mark, it was awarded one point. Whenever a "+" fell outside the tissue, that point was extracted from the total points counted for each photomicrograph (ie, 100 points), allowing only the "effective" points for the final calculations. After all five fields from each photomicrograph were examined; the sum of the points overlying each parameter was calculated and divided by the total "effective" points.[16] This allowed calculation of the mean volume fraction (Vv) for each of the evaluated parameters, because each fraction is equal to the mean volume fraction occupied by the parameter to which it relates. The results are expressed as the mean Vv (%) of each evaluated parameter.

RESULTS

The main cause for tooth extraction was root fracture resulting in severe bone loss (36/43) followed by perioendo destructive lesion (7/43) (Figure 1). Sites were mostly in the mandible (35) compared with the maxilla (8). The reactive soft tissue demonstrated features compatible with granulation tissue with long attachment epithelium (Figure 2).

Healing progressed uneventfully. No leakage or infection of the grafting material was recorded and minimal postoperative side effects, mainly swelling and pain. After 4 weeks, maturation of the soft tissue was noted. The newly formed soft tissue on the alveolar crest showed clinical characteristics of keratinized gingiva. At the time of implant placement (6 months), the alveolar bone architecture was evaluated. All grafted sites allowed

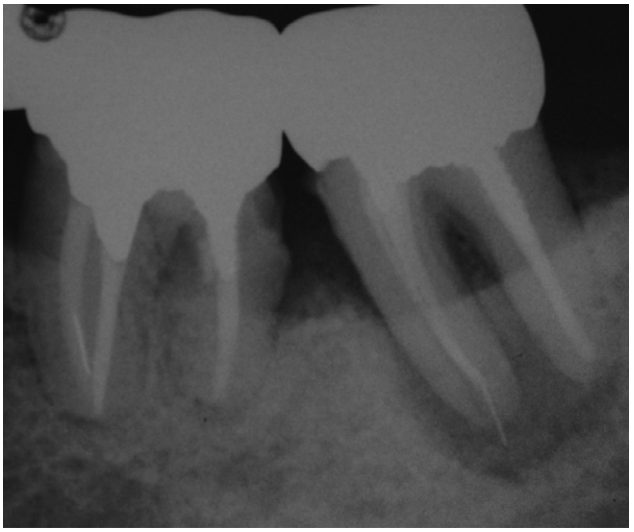

Figure 1 Preoperative periapical radiograph demonstrating bone loss.

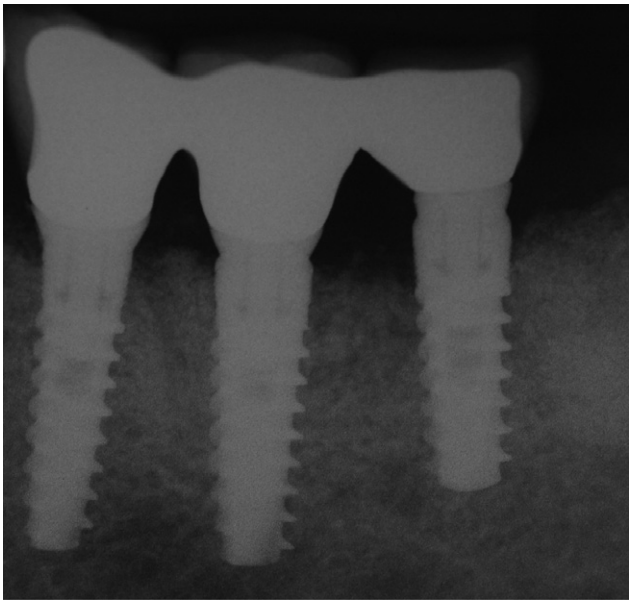

Figure 3 Periapical radiograph demonstrating bone density at 12-month follow-up.

for implant placement. Supplemental bone augmentation was not needed. Fixture installation could be performed according to the standard protocol with initial stabilization. Implant diameter ranged from 3.7 to 5 mm (mean 4.45 ± 0.25 mm). No implant failed (Figure 3). Mean follow-up following loading was 18 ± 12 months (6–42 months).

In the core biopsies, the first observable change at the host connective tissue – bone substitute interface was the slight accumulation of fibroblast-like cells and emergence of a delicate basophilic line that marked the boundary zone between these two elements (Figure 4). The subsequent phase was the emergence of a delicate strip of eosinophilic material, reminiscent of calcified osteoid, but still devoid of cells (Figure 4). In more advanced phases, the eosinophilic material became thicker and entrapped connective tissue fibroblast-like cells, denoting the evolving tissue the appearance of woven bone (Figure 4). Rarely, plump connective tissue cells with an osteoblast appearance were seen rimming the new bone formation in limited areas (Figure 5). As the bony areas extended, they tended to coalesce and form a continuous surface of intermixed bone and fragments of bone substitute (Figure 6).

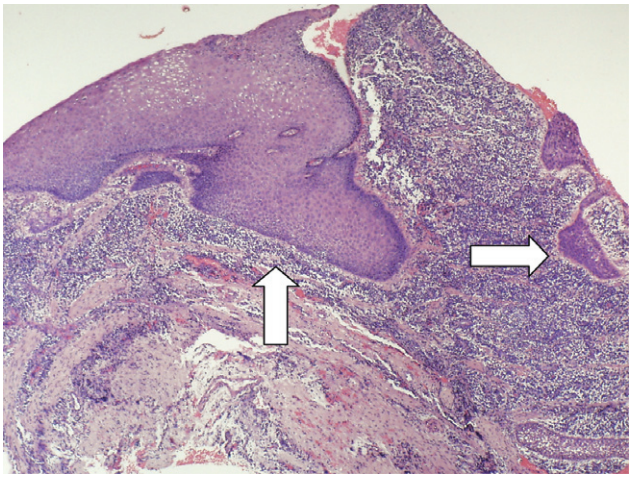

Figure 2 Granulation tissue with long attachment epithelium, gingival mucosa (upper left), long attachment epithelium (right).

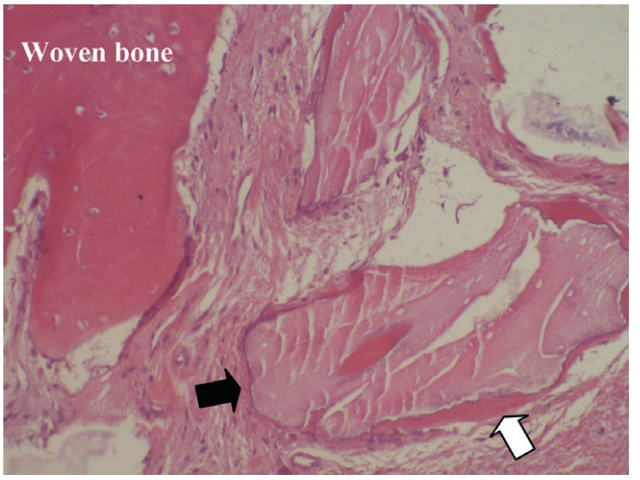

Figure 4 Bone substitute interface was the slight accumulation of fibroblast-like cells with basophilic strip of eosinophilic material, reminiscent of calcified osteoid (down right) and woven bone (upper left).

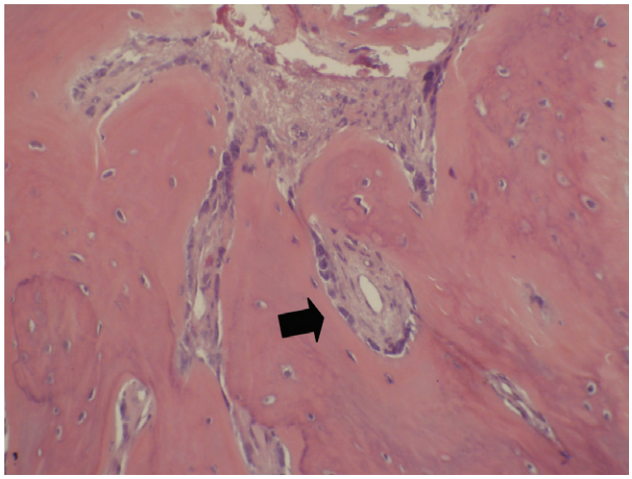

Figure 5 Rarely, plump connective tissue cells with an osteoblast appearance were seen rimming the new bone formation in limited areas (center).

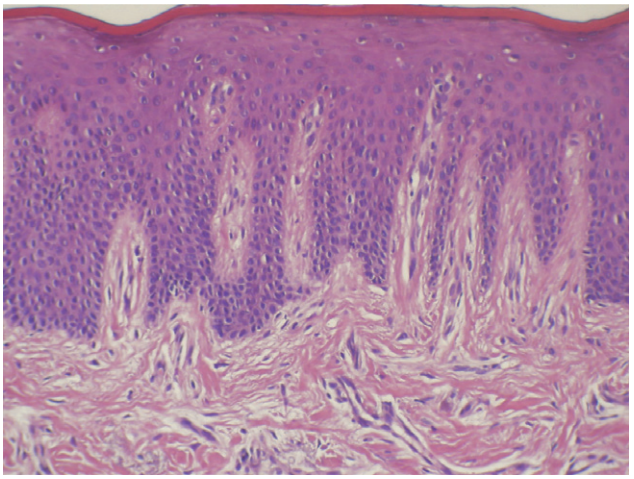

Figure 7 The overlying soft tissue demonstrated keratinized stratified squamous epithelium with elongated, thin rete ridges and a fibrous connective tissue with no residual elements of the granulation tissue.

Histomorphometrically, the mean Vv of the bony tissue was 40.0 ± 19% (range: 13.7 to 74.8%), that of the bone substitute 25.7 ± 13%% (range: 0.6 to 51%) and of the connective tissue 34.3 ± 15% (range: 13.8 to 71.9%).

The overlying soft tissue demonstrated keratinized stratified squamous epithelium with elongated, thin rete ridges and a fibrous connective tissue. A mild-to-moderate chronic inflammatory infiltrate was usually present. These histopathological findings are featured by keratinized gingival/alveolar mucosa with no residual elements of the granulation tissue (Figure 7).

DISCUSSION

Advanced periodontal, endodontal lesions, or lesions caused by root fractures, develop mainly as a result of the immunological response to continuous antigenic stimulation from the root canal or periodontal pocket creating a chronic inflammatory process.[6,17] According to the literature[17–20] and the present study, the reactive tissue, composed of granulation tissue covered by long junctional or "pocket" epithelium is one of the local defensive response to this chronic inflammatory process.

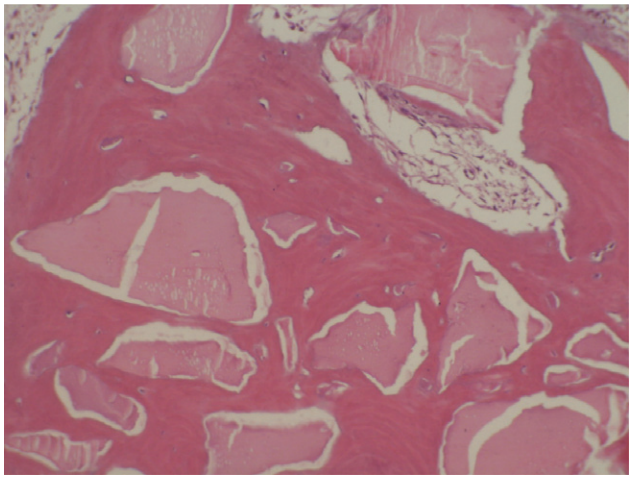

Figure 6 As the bony areas extended, they tended to coalesce and form a continuous surface of intermixed bone and fragments of bone substitute.

Junctional epithelium is a rapidly proliferating tissue. Its turnover time is estimated 50 to 100 times faster compared with the oral epithelium. Regeneration appears as epithelial downgrowth producing the long junctional epithelium. The high rate of proliferation activity strongly suggests that the epithelium is a non-differentiating tissue.[21]

The chronically inflamed granulation tissue develops from the connective tissue surrounding the damaged area and contains fibroblastic cells that reach the blood supply and inflammatory cells. Maeda and colleagues[22] cultured fibroblastic cells derived from human apical periodontitis granulation tissue and demonstrated that these tissues include osteogenic cells, which have the potential to differentiate into mature cells and produce calcified deposits in vitro. It was concluded that some cells in granulation tissue may play an important role in osseous healing. Since this ability is age related, it could take longer to acquire osseous healing in elderly patients.

Accordingly, it can be postulated that preserving and using the granulation tissue and the attached long junctional epithelium as a complex flap would have an

TABLE 1 Mean Histomorphometric Analysis of Different Augmentation Procedures and Graft Materials

Author	Augmentation	Graft Material	Vital Bone (%)	Residual Filler (%)	Connective Tissue (%)
Mardinger et al.[13]	Ridge preservation	Porous bovine*	40.0	25.7	34.3
Barone et al.[23]	Ridge preservation	Corticocancelous- porcine bone	35.5	29.2	36.6
		Without graft	25.7		59.1
Froum et al.[24]	Socket preservation	DFDBA	34.7	13.5	51.6
		Bioactive glass	59.5	5.5	35.3
Geurs et al.[25]	GBR	DFDBA and Corticocancelous	21	36	43
Proussaef et al.[26]	GBR	Autogenous (50%)	36.47	14.35	49.18
		Porous bovine* (50%)			
Norton et al.[27]	GBR and socket preservation	Porous bovine*	26.9	25.6	47.4

*Bio-Oss, Geistlich Pharma, Wolhusen, Switzerland.
DFDBA = demineralized freeze-dried bone allograft; GBR = guided bone regeneration.

advantage in bone grafting procedures. The rapid proliferation rate of the epithelium creates a fast closure of the socket opening. The present study shows that the initial complex of granulation tissue and long junctional epithelial cells was replaced by clinically and histologicaly healthy keratinized gingiva.

A literature review regarding histomorphometric results in socket preservation sites and bony defect augmentation of atrophic ridges shows a difference in the percentage relations between the vital bone and the augmentation graft materials although our results shows high percentage of newly formed vital bone regarding to socket preservation and guided bone regeneration procedures using porous bovine or other augmentation materials[23–27] (Table 1).

According to our clinical and histological results it can be speculated that the regeneration potential of inflamed extraction sites is high. The main reason might be the rich blood vascular bed and the inflammatory status with its cytokines and mediators. The blood supply is from a large area of exposed medullar bone by the destructive resorption of the lamina dura and from the previous inflammatory process that formed the complex flap above. It should be highlighted that the histomorphometrical results demonstrating a high volume of new vital bone were obtained without the use of a barrier membrane.

CONCLUSIONS

Histological results of a surgical approach for bone grafting in the inflammatory phase were presented. Intrasocket reactive soft tissue used for primary closure following ridge augmentation is composed of granulation tissue and long junctional epithelium. At implant placement, clinical and histological results demonstrate its replacement by keratinized gingiva. The histomorphometrical results reveal considerable bone formation. Fresh extraction sites of hopeless teeth demonstrating chronic infection and severe bone loss may be grafted simultaneously with their removal. Further clinical follow-up and histological data are needed to support the concept and promote our knowledge of the biological processes involved.

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