Mineralized Allograft Implant Site Preservation in 3-Wall Post-Extraction Sockets in Ten Subjects: Clinical, Radiographic and Histologic Evaluation

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Abstract: *Objective*: The purpose of the present investigation was to evaluate the efficacy of a surgical technique using mineralized human allograft and a collagen membrane on counteracting dimensional changes of 3-wall post-extraction socket.

Materials and Methods: In 10 patients, 10 single extraction sites without the labial bone plate in the anterior area were treated by means of mineralized human allograft and a collagen membrane. Intraoral radiographs and clinical measurement were taken at baseline and after 4 months. At surgical reentry, prior to implant placement, a biopsy core was obtained for histological analysis.

Results: After 4 months of healing the intraoral radiographs showed a mean difference in bone level height of 0.77 mm (SD ± 0.63), while clinical measurements showed a mean reduction of 1.1 mm (SD ± 0.46) in width, allowing for normal diameter implants placement without the need of further augmentation procedures. The specimens harvested showed bone formation, composed of particles of mineral human bone allograft and newly formed bone trabeculae.

Conclusion: Alveolar ridge preservation using mineralized human allograft and a collagen membrane allows for preserving height and width of a 3 wall extraction socket, suggesting that it may be useful prior to dental implant placement, especially in the esthetic region.

Keywords: Allograft, bone regeneration, histomorphometry, membrane, microradiography, socket.

INTRODUCTION

The alveolar process is a tooth dependent structure and significant morphological changes will occur after extraction procedures, mainly during the first 6 months of healing [1-5]. The greatest amount of bone loss occurs in the horizontal dimension and mainly in the buccal aspect of the ridge, as well as vertical loss of the ridge height, which is most pronounced on the labial aspect [6].

This reduction of the alveolar volume may interfere with future implant placement, due to relocation of the ridge to a more lingual/palatal position [7], especially in the anterior maxilla, where bone volume is significant both for biologic and esthetic reasons [8]. The implant intrabony position may not meet the ideal prosthetic position of the implant. This may compel the surgeon to place tilted implants [9], thus leading to occlusal loads featuring oblique vectors [10]. The prosthodontist should then choose angulated abutments to compensate for the discrepancy between the implant and prosthesis axis, choose a reliable implant-abutment connection to withstand off-axis loads [11] and provide the most suitable occlusal pattern [12] in order to optimize the stress state at the bone implant interface [13]. Therefore, many surgical techiques and biomaterials have been tested in order to maintain the alveolar ridge dimensions, resulting in clinical [14-16] and histological [17] benefit. The main techniques range from a careful flapless tooth extraction to an immediate implant placement, to filling the socket with different grafting materials, with or without barrier membranes, such as GBR procedures [18].

In different recent studies mineralized human bone allograft was used in combination with a collagen membrane to preserve post extraction alveolar ridge dimensions. The authors concluded that such surgical procedure limited the loss of hard tissue ridge width and provide a gain in hard tissue height when compared to extraction alone [19]. Furthermore, no statistically significant differences have been founded in the amount of new bone growth or residual graft particles 6 months after extraction or ridge preservation using mineralized human bone allograft and a collagen membrane [19], neither compared specimens obtained at 6 or 3 months of healing [20]. In these studies, no bone plates of the post-extraction sites were missing, i.e. a four-wall post-extractive alveolus were treated. However, the labial bone plate could be absent after extraction, due to both pre-operative or post-operative reasons, as the presence of a periodontal compromised tooth to be extracted or a traumatic extraction procedure. So the aim of the present pilot

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study was to assess the possibility of preserving a three-wall alveolar bone crest from resorption using a mineralized human allograft and a collagen membrane, and to evaluate histological outcomes of such procedure after 4 months of healing.

MATERIALS AND METHODS

Patient Selection

A prospective pilot study was performed on 10 patients consecutively treated at the Department of Clinical Sciences and Translational Medicine, Section of Dentistry, University of Rome “Tor Vergata”, Italy. Each patient required the extraction of one single-rooted tooth and the reasons for extraction included root fractures, periodontal involvement, caries and endodontic treatment failures.

The inclusion criteria were as follows: good general health, good oral hygiene, 3-wall (buccal plate missing) post extraction socket in esthetic region (incisors, canines and premolars), no active periodontal disease, presence of tooth in the mesial and distal aspect of the extraction socket, minimally atraumatic extraction and no flap elevation.

The exclusion criteria were as follows: active local infection or severe inflammation in the areas intended for treatment, implant in the adjacent region, smoking more than 20 cigarettes per day, radiotherapy on the maxillary region, chemotherapy, diabetes, pregnancy and treatment with bisphosphonates or immunosuppressants.

The present study was performed following the principles outlined in the Declaration of Helsinki on experimentation involving human subjects and all patients provided an informed consent.

Surgical Technique

Before surgery, each patient underwent to a non-surgical periodontal treatment in order to achieve both full mouth bleeding and plaque scores < 30%.

Local anesthesia was induced by using a 4% articaine solution with epinephrine 1:100 000 (Ubistein; 3M Italy SpA, Milan, Italy) and each tooth was gently luxated and an atraumatic extraction with forceps was performed without flap elevation. The socket was freed from the granulation tissue. Following, a collagen membrane (Socket Membrane Repair ®, Zimmer DentalInc, Carlsbad, CA, USA) was adapted to the alveolus shape in order to build the missing labial plate and the sockets were filled with mineralized human allograft (Puros Cancellous Particulate®, Zimmer Dental Inc, Carlsbad, CA, USA). At last the membrane was folded on top of the graft and firmly fixed to the soft tissue with 5-0 detached sutures (Vicryl; Ethicon, Johnson & Johnson, New Brunswick, NJ, USA) (Figures **1**, **2**). Antibiotics (amoxicillin/clavulanate potassium, Augmentin; GlaxoSmithKline, Verona, Italy) 1g twice a day for 6 days, non-steroidal anti-inflammatory drugs when needed, and chlorhexidine digluconate 0.2% mouthwash avoiding brushing the surgical site for 2 weeks were prescribed as postoperative care for all participants. Sutures were removed 10 days after the surgery.

Figure 1: Initial situation: **a**) occlusal view; **b**) lateral view.

Evaluation Methods

The alveolar ridge was evaluated by calibrated examination of periapical radiographs supported by a customized silicon device. The vertical change, i.e. the distance between a line tangent to cemento-enamel junction of proximal teeth and the center of the alveolar margin was measured immediately after tooth extraction (T0) and after 4 months of healing (T1).

The width of the residual alveolar bone was further clinically assessed with a calibrated periodontal probe

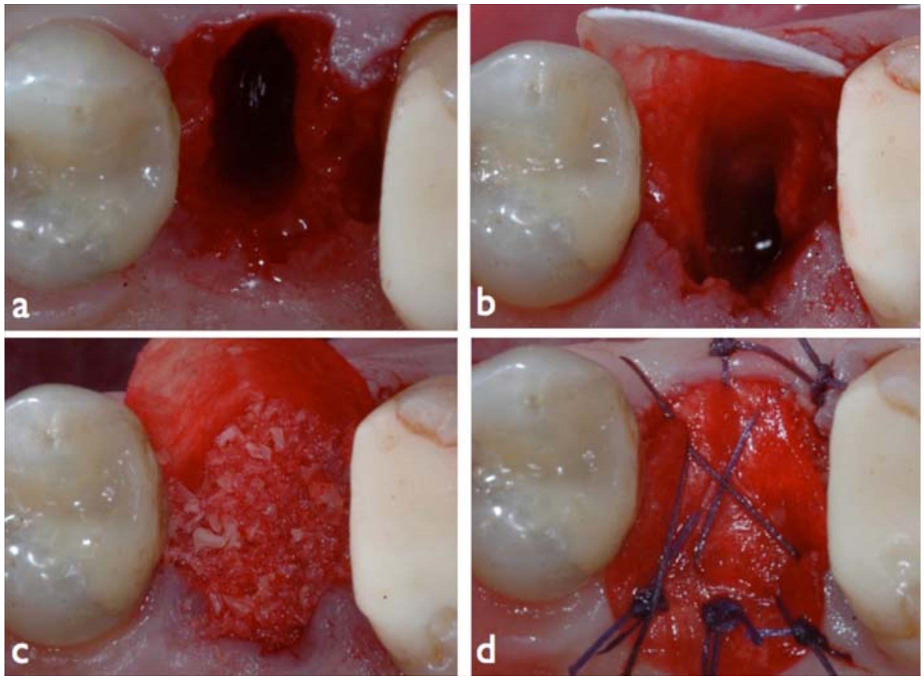

Figure 2: Surgical procedures: **a**) flapless atraumatic tooth extraction; **b**) collagen membrane adaptation to the alveolus shape in order to build the missing labial plate; **c**) post-extraction sockets filled with mineralized human allograft; **d**) suture.

at the baseline (T0) and at the re-entry surgery performed 4 months later (T1) with a flap elevation in order to place an implant (Figure **3**).

The changes in mm of bone height and width (ΔT) was calculated by subtracting the baseline values (T0) recorded immediately after each extraction, with values detected after 4 months of healing (T1), i.e. ΔT= T0 – T1.

Histological Processing

A 3 mm diameter trephine, under a saline irrigation, was used at 600 rpm (up to 10 mm) (Figure **4**) in order to collect bone core biopsy specimens before implant insertion (Tapered Screw Vent, Zimmer Dental Inc. Carlsbad, CA, USA). The bone specimens were immediatley fixed in 4% paraformaldehyde (all reagents from Fluka, Sigma-Aldrich Schweiz, Buchs SG, Switzerland) in 0.1 M sodium phosphate buffer pH 7.2 for 4 h at room temperature. Then the samples were dehydrated through ethanol series at 4 °C and subsequently embedded in methyl methacrylate (PMMA) using a water bath at 4°C as described elsewhere [21]. Finally they were serially sectioned parallel the longitudinal axis of the cylindrical bone sample to its center using a diamond saw microtome (SP1600; Leica Microsystem, Nußloch, Germany). Some sections were perfectly polished with emery paper and alumina and then X-ray microradiographed (3K5; Italstructures, Riva del Garda TN, Italy) at 15 kV and 10 mA on high-resolution film (SO 343; Eastman Kodak Co, Rochester, NY, USA). Some were stained with toluidine blue or trichrome Gomori stain, others were stained with total alkaline phosphatase (TAP)

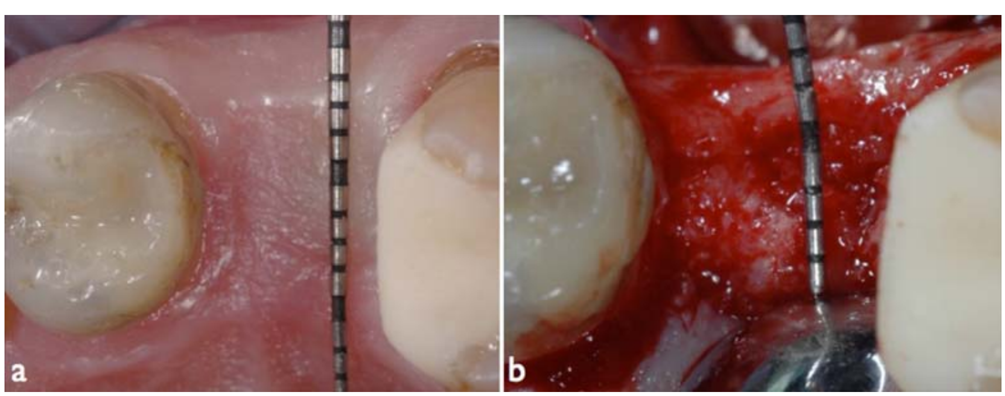

Figure 3: Clinical width 4 months after surgery: **a**) pre-operative measurement; **b**) intra-operative measurement.

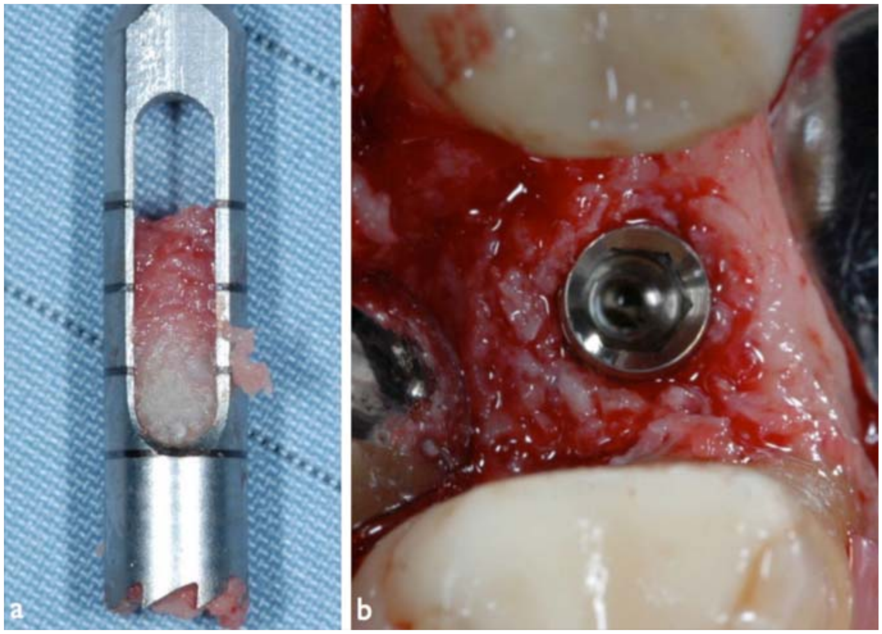

Figure 4: Bone core biopsy specimen collected at the re-entry surgery after 4 months of healing and implant placement with adequate thickness of the buccal plate.

histochemical method, a marker of osteogenesis (azo dye = fast blue BB), or tartrate resistant acid phosphatase (TRAP) histochemical method, a marker of osteoclasts (azo dye = Fast Red RC) [22,23]. The microradiographs and the sections were analyzed and photographed using a light microscope at 150x with a 10x objective and 15x reticle eyepiece (Axiophot; Carl Zeiss AG, Oberkochen, Germany). The amount of bone tissue and graft per tissue volume were evaluated on microradiographs (MR) using image analysis software (AnalySIS, Soft Imaging System GmbH, Munster, Germany).

Statistical Methods

The data distribution of average bone level changes was plotted in a box-plot and mean values with standard deviations (SD) were calculated. The Student t-test (confidence level: 99%, $p < 0.01$) was selected to identify differences in horizontal and vertical bone level changes between measurements performed before surgery and after 4 months of healing.

RESULTS

Ten patients, 4 males and 6 females, aged between 25 and 56, mean 38 years were recruited for this study. Each of them had one tooth extracted for a total of 10 post-extraction socket samples. Two incisors, 1 canine and 7 premolars were extracted, one per patient. No patients withdrew from the study. No signs of inflammation and no signs of graft material loss were detected in any socket during healing.

Table 1: Vertical Alveolar Bone Height at Baseline T(0) mm and after 4 Months T(1) mm

Patient	Sex	Age	Smoker/ Non Smoker	Tooth	T(0) mm	T(1) mm	ΔT= T0 – T1
1	M	37	S	44	2	2	0
2	F	26	NS	34	2	0.5	1.5
3	M	42	NS	12	2.5	2	0.5
4	M	32	NS	35	2	1	1
5	F	39	NS	12	2.5	1,5	1
6	F	45	S	13	2	1.5	0.5
7	F	25	NS	25	0	0	0
8	M	56	NS	15	1.5	1	0.5
9	F	30	S	14	2	0	2
10	F	48	NS	14	1.8	1.1	0.7
Mean ± SD		38			1.83 ± .71	1.06 ± 0.72	+0.77 ± 0.63 (p = < 0.05)

During the re-entry surgery (T1) performed four months after the extraction, ten core biopsies were collected and ten implants were placed, one per patients, achieving primary stability (torque insertion range: 35 to 55 Ncm).

At baseline (T0) the mean distance between the line tangent to cement-enamel junction of proximal teeth and the center of the alveolar margin was 1.83 ± 0.71 mm (range 0 to 2 mm). After 4 months of healing (T1) it decreased to a mean of 1.06 ± 0.72 mm (range 0 to 2 mm), resulting in a non significant (p= < 0.05) gain of 0.77 mm (SD ± 0.63) (Table **1**).

After tooth extraction (T0) the mean width of the alveolar bone crest was 7.35 ± 0.80 mm (range 6 to 8.5 mm), and after 4 months (T1) the mean width was 6.25 ± 0.75 mm (range 5 to 7 mm); therefore a significant (p= < 0.05) bone resorption of 1.1 mm (SD ± 0.46) occurred (Table **2**).

Histologic sections showed bone formation, composed of particles of mineral human bone allograft and newly formed bone trabeculae. The majority of grafted material was surrounded by new bone, with few particles that were totally surrounded by fibrous tissue. The structure of the newly formed bony trabeculae was the woven as the parallel-fibered or lamellar structure (Figure **5**).

DISCUSSION

The aim of the present prospective pilot study was to evaluate bone resorption of 3-wall post extraction socket by means of mineralized human bone allograft and a collagen membrane. Recent systematic reviews [14-16] reported that alveolar ridge preservation techniques may aid in reducing the bone dimensional changes following tooth extraction. In fact, even if some degree of bone modelling and remodelling will occur, such procedures resulted in significantly less vertical and horizontal contraction of the alveolar bone crest. However, these reviews did not stratified results between post-extraction alveolus with and without the presence of the labial bone plate, but one could speculate that the absence of the labial bone plate makes the resorption process greater and the ridge preservation procedure more difficult. In this study only post-extraction alveolus without labial bone plate were treated, resulting in a bone gain in height (0.78 mm) and a bone loss in width (1.1 mm). Even if a non statistically significant vertical bone increase was found, it could be explained by the additional extrasocket augmentation procedure in the coronal portion of the alveolus, similar to Iasella *et al*. [19], where an increase of 1.3 was obtained mid-buccally. On the other hand the significant bone loss in width could be explained by the absence of the buccal bone plate in the treated post-extractive alveolus. In fact a four-wall alveolus obviously contains the blood clot better than a three-wall alveolus, where the absence of the vestibular bone obviously plays a critical role in the resorption process. In this study, a collagen membrane has been adapted to the vestibular portion of the alveolus, in order to substitute the absent wall and maintain the graft. In this way the horizontal resorption has been less pronounced respect those reporting in recent systematic reviews analyzing spontaneous healing. In fact a mean of 3.87 mm bone resorption in width has been reported by Van der Weiden *et al*, and a very recent systematic review with a total of 20

Table 2: Horizontal Alveolar Bone width at Baseline T(0) mm and after 4 Months T(1) mm

Patient	Sex	Age	Smoker/Non Smoker	Tooth	T(0) mm	T(1) mm	ΔT= T0 – T1
1	M	37	S	44	8	7	1
2	F	26	NS	34	8	6.5	1.5
3	M	42	NS	12	6	5	1
4	M	32	NS	35	7.5	6.5	1
5	F	39	NS	12	6	5	1
6	F	45	S	13	7.5	6.5	1
7	F	25	NS	25	7	7	0
8	M	56	NS	15	8.5	7	1.5
9	F	30	S	14	8	6.5	1.5
10	F	48	NS	14	7	5.5	1.5
Mean ± SD		38			7.35 ± 0.8	6.25 ± 0.75	-1.1 ± 0.44 (p = < 0.05)

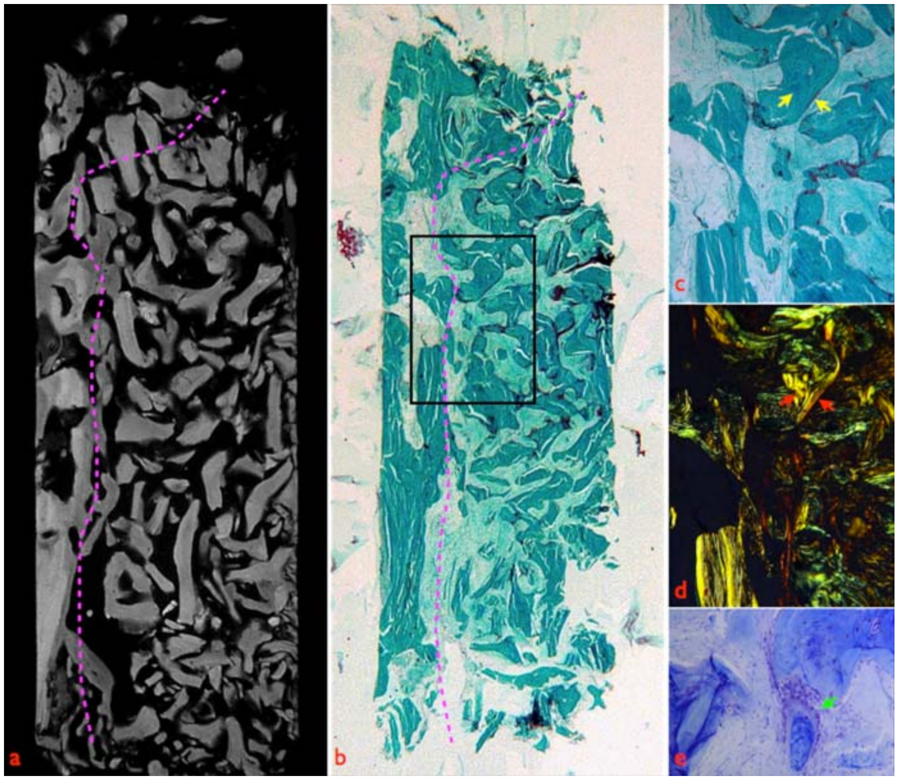

Figure 5: Radiographical and histological examination of the post-extraction socket preserved using mineralized human allograft and a collagen membrane 4 months after surgery. Figures **a** and **b** show microradiograph and trichrome Gomori stain under ordinary light of the same section of the biopsy respectively. The superimposed broken line marks the alveolar bone wall. Figures **c** and **d** shows, at higher magnification, trichrome Gomori stain under ordinary and polarized light respectively of the same section of the biopsy highlighted by the rectangle in figure **b**. The mineral human bone allograft seems to be surrounded by newly formed bone trabeculae, even if few particles seem to be totally surrounded by fibrous tissue. The structure of the newly formed bony trabeculae has a woven structure but some parts of the bone become lamellar (yellow and red arrows). In figure **c**, toluidine blue staining under ordinary light shows a core of calcification into connective tissue. The green arrows points to a band of osteoblasts which constitute a front of centrifugal bone apposition. The connective tissue surrounding new bone appears rich in fibroblasts.

included studies demonstrated a horizontal bone loss of 3.79 ± 0.23 mm, (29–63% of the total) after 6–7 months [6]. This could mean that the use of an allograft and a collagen barrier adapted to the alveolus shape might help to interfere in the normal sequence of biologic events leading to resorption in wound healing.

The scientific evidence reported a significant positive effect of the flapped surgery in alveolar ridge procedures. However it could be argued that loss of keratinized mucosa occurred, when a flap is raised for primary intention healing. In this study a flapless surgery has been performed, and the inserted graft has been covered by the adapted collagen membrane. Even if there are no data, one could speculate that more keratinized tissue would be present after healing, avoiding the necessity of any soft tissue augmentation procedures during the following implant placement step. Histologically, both cellular (vital) and acellular (non vital) bone has been founded. The acellular portion has been considered residual graft particles, often surrounded by new vital woven or lamellar bone or encapsulated in fibrous connective tissue. Similar results have been reported by Iasella *et al*. [19] and Beck *et al*. [20], even if in such studies a histomorphometric analysis had been conducted.

The major limitation of this pilot study is the absence of a controlled group (naturally healed) and, to our knowledge, there are no studies in literature which provide a comparison since all previous similar studies investigated 4-wall extraction socket preservation technique. In addition, the bi-dimesional radiographical evaluation (periapical radiographs) does not evaluate differences in height between the buccal and lingual bone wall. Even if a clinical analysis of horizontal dimensions has been conducted during the re-entry surgery, such information could be important, mostly in cases of an absence of the labial bone wall (three-wall post-extractive alveolus). Within the limitation of this study, it could be concluded that the use of a mineralized human allograft and a collagen membrane allows for preserving height and width of the extraction socket even when the labial bone plate is missing. This procedure may be useful prior to dental implant placement, especially in the esthetic region.

CONFLICT OF INTEREST

All the authors state that they have no conflict of interest relevant to the content of the submission.

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