VOLUMETRIC AND HISTOLOGIC ANALYSIS OF BONE REMODELLING PATTERN AFTER IMMEDIATE AUGMENTATION OF COMPROMISED EXTRACTION SOCKETS IN PERIODONTITIS PATIENTS: A 1-YEAR RANDOMIZED CONTROLLED STUDY

Analisi volumetrica ed istologica del pattern di rimodellamento osseo dopo ricostruzione immediata di alveoli con pareti riassorbite in pazienti affetti da malattia parodontale: studio controllato e randomizzato con 1 anno di follow-up

Valeria Manavella *, Federica Romano*, Marilisa Toma, Claudia Dellavia**,**

Mario Aimetti *

*Department of C.I.R. Dental School, Surgical Sciences, University of Turin, Turin, Italy

**Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy

Abstract

Objectives: To evaluate linear and volumetric hard tissue changes and new bone formation in severely resorbed alveolar sockets after ridge augmentation procedure compared with spontaneous healing.

Material and methods: Thirty hopeless teeth for advanced periodontitis were randomly allocated to test or control group. After extraction, test sites were grafted with collagenated bovine-derived bone xenograft (DBBM-C) and covering collagen membrane, while control sites had spontaneous healing. Linear and volumetric measurements were calculated on superimposed cone bean computed tomography images obtained after tooth extraction and 12 months later before implant placement. At this time, bone core biopsies were harvested for histological analysis.

Results: Horizontal dimensions decreased by 1.97 mm and by 3.83 mm in the test and control group, respectively. A rebuilding of the buccal wall was observed in both groups, although most pronounced in the grafted sockets (2.50 ± 2.12 mm *versus* 0.51± 1.02 mm). A significant difference was also registered in volume loss between grafted and non-grafted sites (9.14% *versus* 35.16%, P <0.0001), and in new lamellar bone formation.

Conclusion: Immediate augmentation using DBBM-C and collagen membrane is effective in improving alveolar ridge shape and dimensions, reducing the need for further bone regenerative procedures at the time of implant placement.

Riassunto

Obiettivo: comparare le variazioni lineari e volumetriche e la neoformazione ossea in alveoli con pareti riassorbite dopo procedura di aumento di cresta rispetto alla guarigione naturale.

Materiali e Metodi: Trenta elementi dentali da estrarre per motivi parodontali sono stati randomizzati in un gruppo test o controllo. Dopo l'estrazione, gli alveoli test sono stati trattati con inserimento di xenoinnesto bovino collagenato (DBBM-C) e membrana in collagene stabilizzata e in doppio strato, mentre quelli controllo sono andati incontro a guarigione naturale. Le misure lineari e volumetriche sono state calcolare sulle immagini Cone-Beam sovrapposte, ottenute dopo l'estrazione e a 12 mesi. Biopsie di tessuto osseo sono state prelevate per l'analisi istologica.

Risultati: La dimensione orizzontale si è ridotta di 1.97 mm nel gruppo test e di 3.83 mm nei controlli. Si sono osservati un aumento verticale, più pronunciato nel gruppo test, a livello della parete vestibolare (2.50 ± 2.12 mm verso 0.51± 1.02 mm), mentre una maggiore riduzione volumetrica (9.14% verso 35.16%, *P* <0.0001) e neoformazione di osso lamellare nei controlli.

Conclusioni: La ricostruzione immediata mediante DBBM-C e membrana in collagene si è dimostrata efficace nel migliorare la morfologia della cresta ossea, riducendo la necessità di ulteriori procedure rigenerative al momento dell'inserimento implantare.

Introduction

Following tooth extraction the residual alveolar bone undergoes marked qualitative and quantitative changes.[1,2] Schropp et al. described a mean vertical reduction of 0.8 mm and a collapse of more than 50% of the bucco-lingual ridge dimension during the first year after tooth extraction.[3] A similar magnitude of dimensional bone loss was reported in recent systematic reviews on post-extraction sockets with intact 4-wall configuration.[4,5]
Data on the healing pattern of sockets with compromised bony walls are limited. A decrease of about 60% in the horizontal dimensions was observed in buccal-bone-deficient alveolar sockets within the first 8 weeks of healing[6], compared with a 35% reduction in intact extraction sites over a 6-month period in the animal model[7]. Therefore, it can be expected that compromised extraction sites undergo more pronounced additional atrophy than intact sockets as a result of the natural remodelling process.
In such cases critical-sized alveolar ridge defects are most likely to occur leading to increased difficulty in placing the implant fixture at a prosthodontically suitable position. To restore the lost volume and facilitate implant insertion several ridge reconstructive techniques, including guided bone regeneration (GBR), distraction osteogenesis, and use of particulate and block grafting materials, have been proposed.[8-10] Most of these demanding and technically sensitive procedures could not be required if the shape and dimensions of the compromised extraction sockets are restored at the time of tooth extraction.[11] Since damaged extraction sockets are uncontained defects, the GBR technique is recommended to ensure clot protection and space maintenance by positioning a barrier membrane instead of the compromised bony wall.[12]
Recent clinical studies in humans reported favourable outcomes when fresh extraction sockets with a bone dehiscence > 5 mm at the buccal wall or showing partial buccal wall deficiency were treated with the insertion of hydroxyapatite or corticocancellous bone and collagen membrane.[13,14]
No information is available on the immediate reconstruction of alveolar sockets with severely resorbed buccal/lingual plate in patients with severe periodontitis. The association of a

secured resorbable collagen membrane with a bovine xenograft may be a promising combination. Collagen barriers show chemotactic effect over gingival fibroblasts, proangiogenic qualities, and early wound stabilization but require bone graft materials to maintain space for regeneration.[15,16] Deproteinized bovine bone mineral coated with 10% porcine derived collagen (DBBM-C) seems to be an appropriate material for alveolar reconstruction.[17,18] Collagen would seem to render the bone mineral surface more attractive for cell adhesion.[19,20] However, mixed histologic data arise from the literature. While some Authors reported improved new bone formation in intact alveolar sockets, others failed to confirm it.[21,22]

In view of these considerations, the aim of the present randomized controlled trial was to analyse 12-month volumetric and histological tissue modifications of severely resorbed alveolar sockets grafted with DBBM-C and a collagen membrane compared with spontaneous healing in periodontitis patients.

Material and methods

This single-centre randomized controlled clinical study was approved by the Institutional Ethical Committee (Protocol n° 695/2015). Adult patients requiring tooth extraction for advanced periodontitis were consecutively recruited between January and June 2015 at the Section of Periodontology, C.I.R. Dental School, University of Turin (Italy). Each patient signed informed consent form.

Main inclusion criteria were at least one hopeless tooth in the maxillary or mandibular anterior or premolar region to be extracted for periodontal reasons in patients with chronic periodontitis[23] who had completed the etiological periodontal therapy. Only sockets with severely resorbed buccal wall were included. Preliminary screening was performed on the basis of clinical examination and intraoral radiography.

Exclusion criteria were as follows: systemic diseases precluding surgical procedures (such as bone, metabolic disorders, uncontrolled diabetes), current use of steroids and bisphosphonates, smoking > 10 cigarettes/day, pregnancy and lactation, active periodontal disease, full-mouth plaque score (FMPS) and full-mouth bleeding score (FMBS) >20% at the time of tooth extraction.[12] Hopeless teeth due to trauma, endodontic problems or prosthetic reasons were excluded from the study.

Randomization and allocation concealment

Each participant was randomly assigned to receive either the test (DBBM-C and collagen membrane) or the control procedure (natural healing) by a computer-generated table. A balanced randomly permuted block was used to prepare the randomization table to avoid unequal balance between two treatments. Forms with the treatment modality were put into identical and opaque envelopes with the patient corresponding number on the outside and placed into the custody of a clinician not involved in diagnosis or treatment delivery. He opened the envelope just after tooth extraction and informed the surgeon which treatment had to be performed. The operators involved in the radiographic and histological analysis, as well as the statistician were blinded.

Surgical protocol

Prophylactic antibiotic therapy (2 g amoxicillin and clavulanic acid) was administered 1 h prior to surgery. Intra-oral antisepsis was performed with 0.2% chlorhexidine digluconate (CHX) rinse for 2 minutes. After extraction, sockets were thoroughly curetted and copiously irrigated with sterile saline solution. On test sites, two vertical releasing incisions were made beyond the mucogingival junction and a buccal mucoperiosteal flap was elevated (Fig. 1). Lingual tissues were undermined at least 10 mm beyond the alveolar crest margin. The socket was augmented by means of DBBM-C (BioOss® Collagen, Geistlich Pharma AB, Wolhusen, Switzerland) with light compaction to the most coronal bone peak level and covered by a double layer of collagen membrane (Bio-Gide®, Geistlich Pharma AB) secured with pins. The flap was sutured in the presurgical position by horizontal mattress sutures. On the control sites, no augmentation procedure was performed (Fig. 2). Healing was by secondary intention in both test and control sites. A resin bonded provisional pontic was used to replace front teeth taking care to avoid any pressure on the underlying tissue.

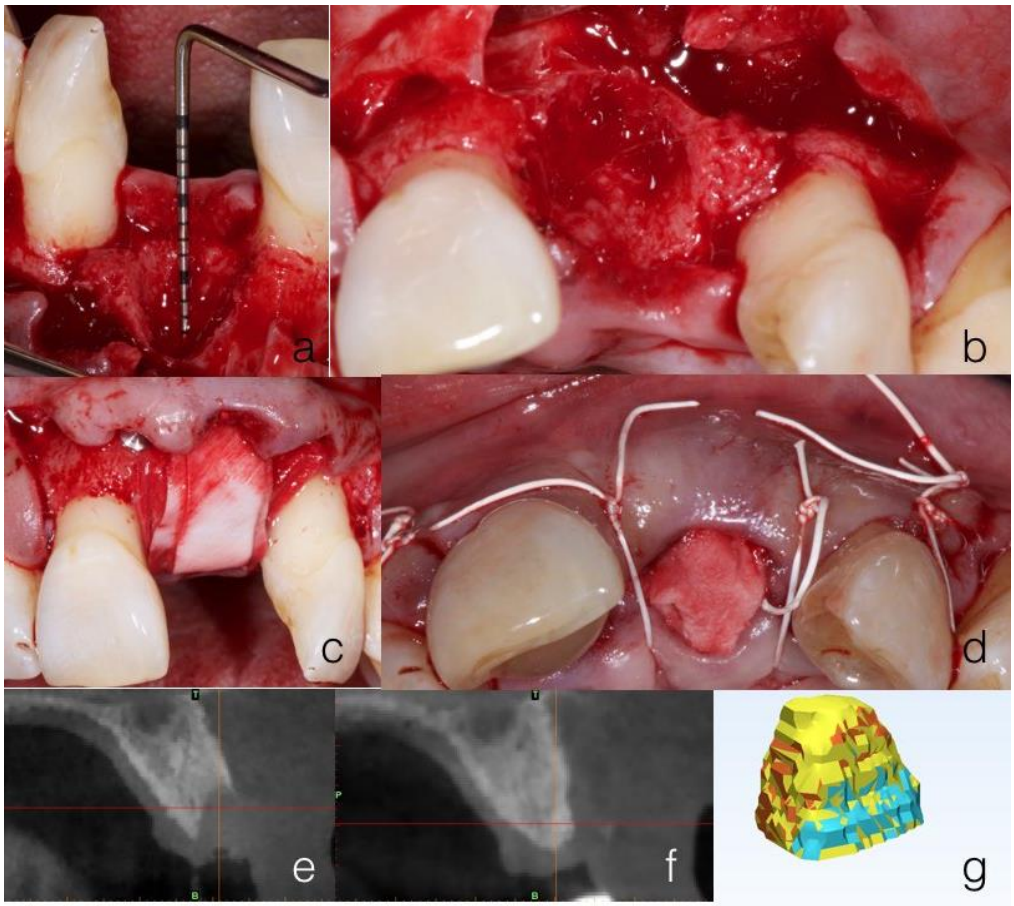

Figure 1. *Pictures of clinical case of the augmentation technique (test group): (a-b) intraoperative view of buccal deficiency following following extraction of hopeless maxillary central incisor, (c) membrane placement, (d) sutures, (e-f) CBCT images of the alveolar socket at baseline and 12 months after the reconstructive procedure, (g) 3D rendering of the augmented alveolar socket. Changes in the socket volume from baseline (yellow) are represented as a 3D coloured-mapped model (augmentation in orange, and resorption in cyan).*

Post-operative care

Patients were prescribed amoxicillin 1g and ibuprofen 600 mg to be taken after the surgical session, and 0.12% CHX mouthwash to be used twice daily for 2 weeks. Sutures were removed at day 14. Patients were recalled weekly within the first month and every three months for the maintenance periodontal treatment.

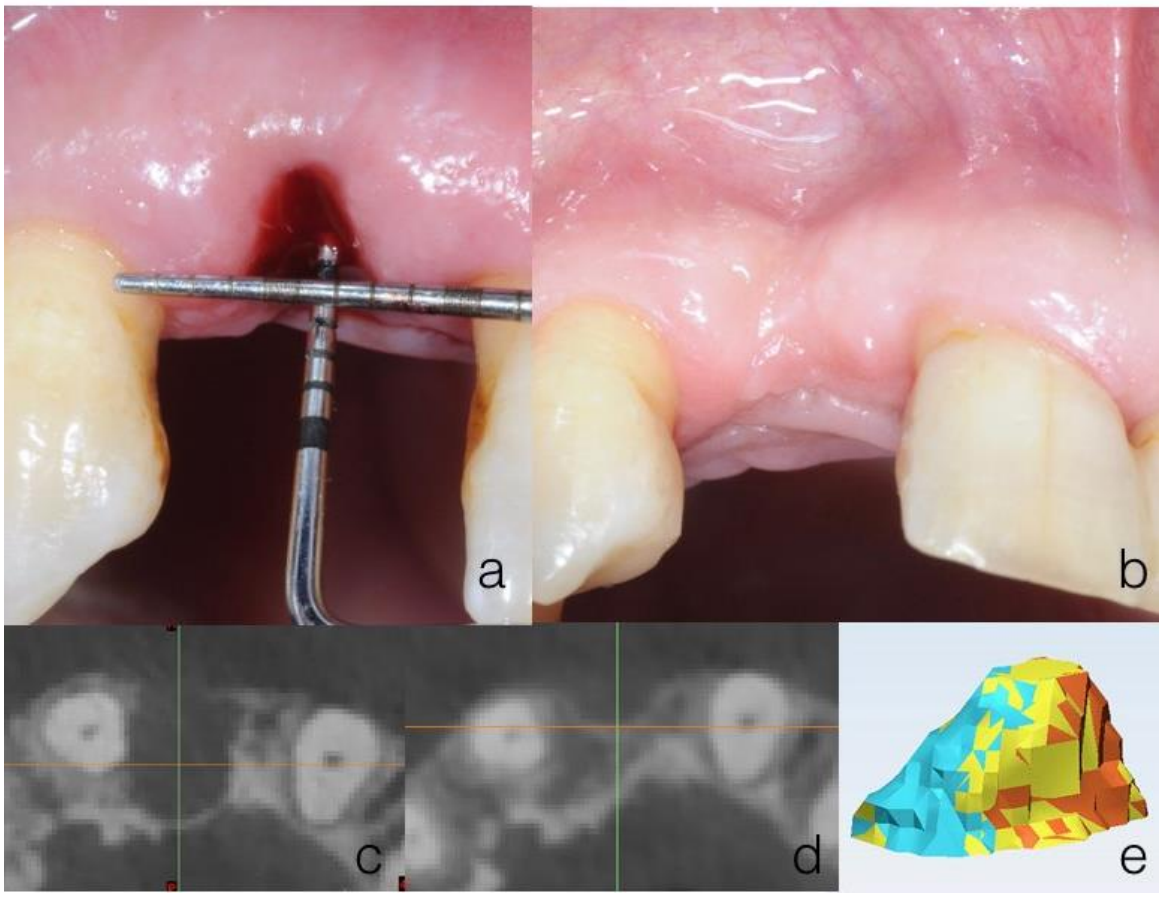

Figure 2. *Pictures of a clinical case in the control group: (a) clinical view following extraction of hopeless maxillary lateral incisor, (b) 12 months after extraction, (c-d) CBCT images of the alveolar socket at baseline and 12 months later, (e) 3D rendering of the alveolar socket.*

Radiographic volumetric and linear measurements

CBCT scans (New Tom/NTVG; field of view = 153.60 mm; thickness = 0.4 mm slice; pixels size = 0.3 mm; voltage = 110 kV; 2 mA; 10 s) were obtained immediately after tooth extraction and 12 months later just before implant placement (Fig. 1 e-f). Patients were asked to wear a custom-made template in radiotransparent acrylic resin with aluminium radiopaque markers (high-precision balls, diameter 5 mm, volume 65.5 mm^3, Martin & C. Srl, Italy). All DICOM (Digital Imaging and Communications in Medicine) images were imported into Mimics 17.0 software (Mimics Innovation Suite®, Materialise NV, Belgium) where bone and teeth were segmented by a mask creation tool, using thresholds corresponding to the greyscale values of these anatomic structures. The 3D-rendered images of the volumetric reconstructions of the alveolar sockets were then generated and exported as STL files. The superimposition of the

volumes of interest (VOI), corresponding to the alveolar sockets, was obtained by inserting some reference points on the pre- and postoperative CBCT images that were appropriately realigned and fused. The middle and posterior reference points were identified according to Alsufyani et al.[23], while the anterior landmarks were placed in the centre of the aluminium spheres. By means of the rendering operation it was possible to calculate 3D volumetric measurements of the VOI (Figs. 1g, 2e) The volume and the percentage of mineralized bone tissue loss were calculated at three zones: 1) from the most coronal part of the preoperative crest to 1 mm apically (0-1 mm zone); 2) from 1 to 3 mm below the alveolar crest (1-3 mm zone); 3) from 3 to 5 mm below the alveolar crest (3-5 mm zone).
On the same CBCT images linear measurements were made according to Jung et al.[17]. Three reference lines were drawn: a vertical line, parallel to the long axis of the socket, from the apex to the centre of the sockets (C–C) and two horizontal lines, perpendicular to line C-C, projecting from the most coronal portion and the most apical point of the alveolar socket. The following parameters were recorded: thickness of the vestibular bone plate at three levels (1 mm, 3 mm and 5 mm) below the lingual bone crest (VT-1, VT-3, VT-5) (only at baseline); height of the socket at the mid-vestibular (HV) and mid-lingual (HL) aspect; height of the socket at the vertical references line (H); horizontal ridge width measured at the horizontal coronal reference line (W); and horizontal ridge width at three levels (1 mm, 3 mm, 5 mm) below the most coronal aspect of the crest (HW-1, HW-3, HW-5).
Radiographic measurements were recorded by a masked engineer. In order to assess the intra-examiner reproducibility, 30% of the cases were randomly reanalysed in two different occasions. The duplicate measurements differed by <6%.

Histological analysis

Histological specimens were obtained from the centre of prior extraction sockets 12 months after extraction, at the time of implant placement, by means of a trephine bur. Bone biopsies were prepared according to the method by Donath & Breuner.[24] Briefly, they were fixed in 10% formalin/0.1 M phosphate buffer saline solution for 10 days, dehydrated in increasing ethanol concentrations and embedded in acrylic resin (Kulzer Technovit 7200 VLC, Bio-Optica, Milan, Italy). Each sample was sectioned along the major axis of the biopsy, and grounded to 70 μm. Sections were stained with toluidine blue/pyronine G (Sigma–Aldrich) and observed using a Nikon light microscope (Eclipse E600) equipped with a calibrated digital camera (DXM1200, Nikon). Histomorphometrical measurements were performed at a magnification of 10x using a stereological method and separate quantifications of areas of mineralized tissue, graft particles, and non-mineralized tissue were performed.

Sample size calculation

Each patient provided one alveolar socket to be treated. The horizontal width was set as the primary outcome. A sample size of 12 patients per group was calculated to detect a minimum difference of 1.5 mm between test and control treatment procedures at 1-year follow-up with an expected standard deviation (SD) of 1.2 mm, a two-sided alpha error of 0.05 and a power of 80%.[25] For compensation of possible dropouts, 30 individuals were recruited.

Statistical analysis

The statistical unit was the patient. The Shapiro–Wilk test confirmed the gaussian distribution of the volumetric and linear variables, except for HV. Pair-wise comparisons were performed by the Wilcoxon-signed-rank test or by the paired *t*-test for matched samples and by the Mann-Whitney *U*-test or the Student *t*-test for independent samples. Differences between groups in qualitative variables were assessed by means of the Chi-square test. All statistical tests were two-tailed and conducted at a 5% level of significance. All analyses were conducted using a statistical tool package (SPSS version 19, IBM, Chicago, IL, USA).

Results

Forty-two subjects were assessed for their eligibility. Of these 12 were excluded: 8 did not meet the inclusion criteria, while the other four refused tooth extraction. As a result, a total of 30 patients with advanced chronic periodontitis (18 females and 12 males, mean age 53.2 ± 6.3 years, range 45-68 years) were enrolled in the study and randomly assigned to the test or control procedures. All 30 participants (15 [test] and 15 [control]) received the allocated procedure and were included in the statistical analyses.

Patient characteristics at baseline were not significantly different ($P > 0.05$) between groups (Table 1). The distributions of hopeless teeth were: 53.3% incisive, 6.7% canine, and 40% premolar for the test group and 60% incisive, and 40% premolar, for the control group.

Table 1. Baseline characteristics of patients

Variables	Test Group (*n*=15)	Control Group (*n*=15)	*P*-value
Age *(years)*	53.6 ± 7.4	52.9 ± 5.1	0.765 [a]
Males/females *(n)*	7/8	5/10	0.709[b]
Light smokers *(n)*	1/15	3/15	0.598[b]
Maxilla/Mandible *(n)*	13/2	11/4	0.651[b]
Incisors/Canines/Premolars *(n)*	8/1/6	9/0/6	0.792[b]

[a]Unpaired *t*-test

[b]Chi-square test

Radiographic measurements

As reported in Tables 2 and 3, no statistically significant difference was detected for any of the baseline defect dimensions between treatment modalities. At baseline the buccal bone plate was detectable on CBCT images in only 4 test and 5 control sockets at -3 mm level, whereas it appeared relatively intact at the more apical portion in most of the extraction sites.

Both groups showed a significant horizontal width reduction from baseline to the 12-month follow-up (Table 2) with an average shrinkage of 3.83 ± 1.49 mm in the control group and 1.97 ± 1.55 mm in the test group (all $P < 0.0001$). The differences between the treatment groups

were statistically significant (*P* <0.0001) and more pronounced in the cervical alveolar region (4.92 ± 2.45 mm *versus* 2.60 ± 1.24 mm, HW-1). At 12 months, no statistically significant vertical changes were observed with respect to the lingual crest (LH) in both test and control sites, whereas it was possible to recognize a height gain on the buccal wall (HV) with values ranging from 0.51 ± 1.02 mm in the control group to 2.50 ± 2.12 mm in the test group. The difference reached statistical significance (*P* < 0.0001).

Table 2. Changes in ridge height and width between baseline and 12 months based on CBCT measurements (mean ± SD).

Variables	**Test Group (*n*=15)**	**Control Group (*n*=15)**	***P*-value**
H *(mm)*			
Baseline	6.92 ± 1.54	6.68 ± 1.05	0.680
12 months	8.26 ± 1.59	6.22 ± 1.12	<0.0001
Difference (mm)	1.34 ± 1.45	-0.46 ± 1.35	
Difference (%)	22.11 ± 24.18	-5.31 ± 19.81	
P-value	0.003	0.182	
HV *(mm)*			
Baseline	2.89 ± 2.27	2.74 ± 1.49	0.902
12 months	5.39 ± 1.35	3.25 ± 1.53	<0.0001
Difference (mm)	2.50 ± 2.12	0.51 ± 1.02	
Difference (%)	82.12 ± 18.73	27.97 ± 38.47	
P-value	0.002	0.046	
HL *(mm)*			
Baseline	6.96 ± 1.60	6.04 ± 1.11	0.078
12 months	7.23 ± 1.13	5.83 ± 1.01	<0.0001
Difference (mm)	0.27 ± 1.31	-0.21 ± 0.78	
Difference (%)	7.89 ± 25.83	-1.88 ± 16.97	
P-value	0.435	0.326	
W *(mm)*			
Baseline	8.62 ± 1.57	7.82 ±1.64	0.105
12 months	6.65 ± 1.41	3.99 ± 1.30	<0.0001
Difference (mm)	-1.97 ± 1.55	-3.83 ± 1.49	

Difference (%)	-21.63 ± 16.25	-46.32 ± 17.61	
P-value	<0.0001	<0.0001	
HW_1 *(mm)*			
Baseline	8.27 ± 1.50	7.63 ± 1.48	0.249
12 months	5.68 ± 1.03	2.72 ± 2.52	<0.0001
Difference (mm)	-2.60 ± 1.24	-4.92 ± 2.45	
Difference (%)	-30.51 ± 12.05	-64.38 ± 31.88	
P-value	<0.0001	<0.0001	
HW_3 *(mm)*			
Baseline	7.84 ± 1.29	7.04 ± 1.09	0.077
12 months	6.98 ± 1.24	5.03 ± 1.39	<0.0001
Difference (mm)	-0.86 ± 0.82	-2.01 ± 0.97	
Difference (%)	-10.67 ± 9.48	-28.93 ± 13.86	
P-value	0.001	<0.0001	
HW_5 *(mm)*			
Baseline	6.91 ± 2.20	6.13 ± 1.52	0.654
12 months	7.37 ± 1.32	5.52 ± 1.38	<0.0001
Difference (mm)	0.34 ± 0.87	-0.61 ± 1.14	
Difference (%)	4.38 ± 11.16	-8.46 ± 17.31	
P-value	0.160	0.061	

H = total height; HL = mid-lingual height; HV = mid-buccal height; W = total (bucco-lingual) horizontal width; HW_1_3_5 = horizontal width at 1-3-5 mm from the top of the crest. Positive values = gain of tissue; negative values = loss of tissue.

The volumetric analysis (Table 3) showed a statistically significant decrease in volume (*P* = 0.001) in both groups with a mean difference of 18.61 ± 17.93 mm^3 (9.14%) in the grafted sockets that increased to 62.09 ± 25.81 mm^3 (35.16%) in the non-grafted sites. The most pronounced differences were observed in the first millimetre below the bone crest (zone 1) where the control group displayed an average loss of bone volume of 18.14 ± 12.13 mm^3 (42.96%) compared to 6.20 ± 6.34 mm^3 (16.24%) in the test group (*P* = 0.001). No additional bone regenerative procedures were needed at the time of implant placement in the grafted sites.

Table 3. Changes in ridge volume between baseline and 12 months based on CBCT measurements (mean ± SD).

Variables	**Test Group (*n*=15)**	**Control Group (*n*=15)**	***P*-value**
Volume *(mm^3)*			
Baseline	208.80 ± 83.53	183.65 ± 69.32	0.595
12 months	190.19 ± 82.05	121.56 ± 66.96	<0.0001
Difference (mm^3)	18.61 ± 17.93	62.09 ± 25.81	
Difference (%)	9.14 ± 8.44	35.16 ± 10.92	
P-value	0.001	0.001	
Zone 1 *(mm^3)*			
Baseline	42.24 ± 16.27	40.20 ± 12.49	0.672
12 months	36.05 ± 18.29	22.06 ± 10.49	0.001
Difference (mm^3)	6.20 ± 6.34	18.14 ± 12.13	
Difference (%)	16.24 ± 17.50	42.96 ± 21.78	
P-value	0.002	<0.0001	
Zone 2 *(mm^3)*			
Baseline	85.76 ± 36.63	78.54 ± 30.96	0.564
12 months	77.05 ± 35.91	55.38 ± 25.21	0.066
Difference (mm^3)	8.72 ± 8.09	23.15 ± 15.41	
Difference (%)	10.14 ± 8.96	29.26 ± 12.71	
P-value	0.001	<0.0001	
Zone 3 *(mm^3)*			
Baseline	56.26 ± 24.74	46.17 ± 22.57	0.253
12 months	53.36 ± 23.67	36.26 ± 21.22	0.046
Difference (mm^3)	2.89 ± 4.92	9.91 ± 12.48	
Difference (%)	4.87 ± 6.82	20.41 ± 22.79	
P-value	0.059	0.008	

Zone 1-2-3 = volume at 0-1 mm, 1-3 mm and 3-5 mm from the top of the crest.

Histomorphometrical measurements

All samples had a normal structure without evident presence of inflammatory infiltrate at 12 months of healing (Figs 3a, 4). In the test group, grafted particles were still present and were surrounded by new woven bone, confirming the process of osseointegration of the graft. At low magnification, lamellar bone tissue appeared organized in trabeculae, and the biomaterial was well integrated with woven bone that was completely organized. At higher magnification, no gaps between regenerated bone and biomaterial particles still existed (Fig. 3b). Histomorphometrical analysis revealed a significant difference only for lamellar bone values between groups (P = 0.02). Overall, a mean of 30.2 ± 8% lamellar bone, 20.8 ± 9.7% woven bone, and 22.2 ± 12.7% connective tissue were detected in the grafted sites compared to 52.8 ± 13.9%, 21.4 ± 9.9% and 23.8 ± 13.8%, respectively, in the non-grafted sites. The residual graft material was 26.8 ± 10.4%.

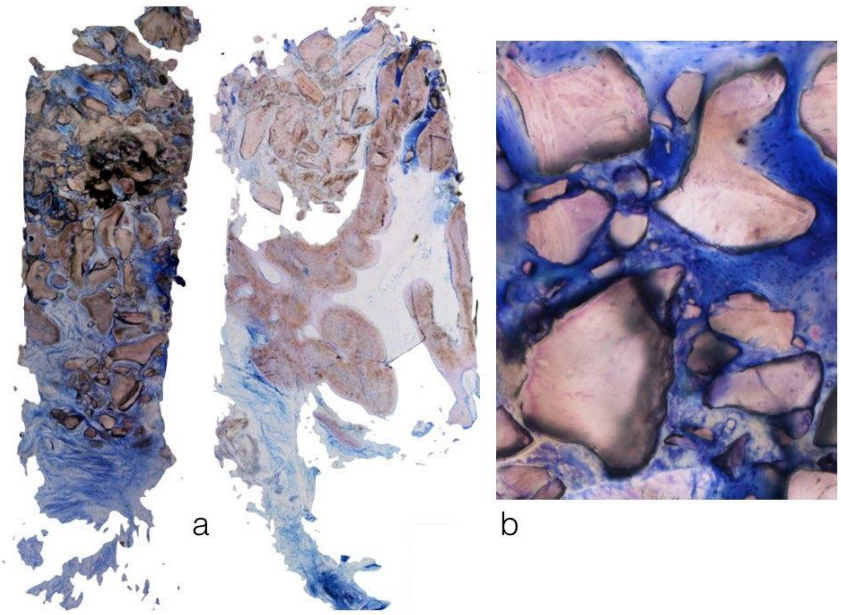

***Figure 3**. Histological analysis at 12 months of healing in test group: (a) overview of two samples. Biomaterial blocks (in dark brown) appeared surrounded by new woven bone and bone marrow (blue) (Toluidine Blue and Pyronine Y staining, total magnification 100x), (b) detail showing that the interface between biomaterial and new bone matrix was closed (total magnification 200x).*

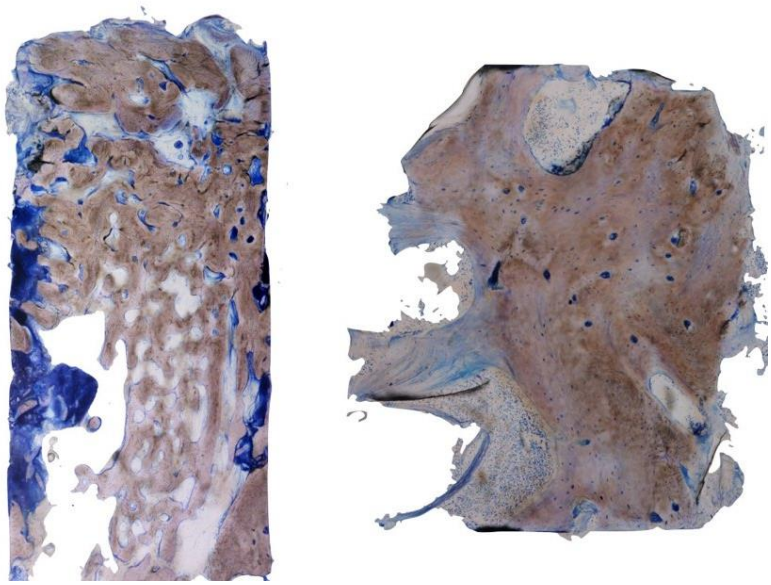

Figure 4. *Histological analysis at 12 months of healing in control group: overview of two samples.*

Discussion

The aim of this randomized controlled trial was to evaluate linear and volumetric hard tissue changes and new bone formation in augmented alveolar sockets with DBBM-C and covering collagen membrane during 12 months of follow-up compared with spontaneous healing. Only fresh alveolar sockets in the upper and lower anterior region with extensive buccal bone loss as a result of advanced chronic periodontitis were enrolled.

Unlike abundant data for peri-implant buccal bone defects demonstrating that they do not repair themselves completely without the use GBR procedures or grafting materials[26,27], there is scant information on dimensional changes that occur in compromised alveolar sockets following immediate augmentation procedures[13,14,28]. Furthermore, these studies mostly relied on direct intra-surgical or linear measurements on periapical radiographs on CBCT images or on volumetric analysis of scanned study cast and provided clinicians only with approximate estimates for volumetric bone ridge changes.[29]

In the present study, the VOI of the experimental sockets in the preoperative CBCT images were segmented and digitally superimposed on the corresponding postoperative VOI, allowing a qualitative and quantitative evaluation of 3D changes of the hard tissue volume and contour on 3D overlay images.[30] As suggested by Lagravère et al.[31], we considered 6 cranial-base landmarks and the centre of the spherical markers to optimize images superimposition. In addition, Mimics software ensures that the angles and distances between landmark points are maintained for different images. Of note, the baseline CBCT images were taken immediately after tooth removal. This eliminated the need of digital subtraction of pre-extraction target teeth, and had the advantage to avoid the superimposition of bone and dental tissue.

The 3D ridge morphology analysis in the present study showed a dome-shaped configuration of the edentulous crest after 12 months of healing as described in previous histological studies

on dogs.[32,33] The placement of Bio-Oss collagen in fresh extraction sockets seemed to counteract the bone remodelling process resulting in a less pronounced change of the buccal profile of the alveolar crest and a better maintenance of the hard tissue volume when compared to the naturally healing control. The non-grafted sockets resulted in a much narrower and irregular shape.[32]

A significant 3D volumetric bone loss of about 35% occurred in the non-grafted sockets. In agreement with previous studies on post-extraction sockets with buccal dehiscence defects, bone loss occurred primarily in the 0-3 mm zone apical to the alveolar crest (from 29% to 43%).[28,34] Conversely, the augmented sockets exhibited a volume deficiency of about 9% with respect to the maximum volume for regeneration. It was slightly more pronounced in the more coronal portion of the crest (10%) and decreased to 5% in its middle part. These data are consistent with findings by Araujo & Lindhe[1] who reported a 12% reduction in the coronal portion, and an increase of 4% in the middle and apical portion of intact sockets grafted with DBBM-C after a healing period of 6 months in the canine model.

With regard to linear hard tissue changes, the proposed ridge augmentation procedure was effective not only in limiting the physiological ridge reduction but also in repairing significant portion of the buccal wall as compared with tooth extraction alone. The mean differences between grafted and non-grafted sockets at 12 months were 1.86 mm in terms of buccolingual width (P <0.0001) and 2.00 mm for midbuccal height (P <0.0001), whereas the midlingual height was nearly unchanged in both groups. Interestingly, these findings were in line with those observed after alveolar ridge preservation techniques demonstrating that, in spite of the unfavourable anatomy, augmented sockets exhibit a pattern and a degree of bone resorption comparable with grafted intact sockets.[35]

Of note, all the sockets observed in this study presented a significant vertical increase of the buccal bone plate that was more pronounced in the augmented group. A vertical buccal bone growth was previously described by Crespi et al.[28] after 3 months of spontaneous healing in unfilled extraction sockets covered only with a collagen sponge. The authors hypothesized the role of the blood clot as a physical matrix amplifying and regulating the migration, proliferation and differentiation of cells involved in angiogenesis and subsequent new bone formation.[36,37] However, it should be considered the peculiar anatomy of compromised sockets that are uncontained defects in the coronal portion, but provide a bone envelope in the most apical part with high regenerative potential.

The present encouraging results may be also due to the long degradation time of the grafting material and to the subsequent lower turnover of the remodelling process in the extraction sockets. The uncontained anatomy of the socket defects requires the placement, in association with a secured membrane barrier, of a biomaterial capable of maintaining stable shape and dimensions in the early healing phase.[12] The fixation of the membrane plays a pivotal role in optimizing bone regeneration from a biological point of view.[6,38,39] Furthermore, the membrane was applied in a double layer to prolong its barrier function.[40,41]

Mixed data are available in the literature on time and amount of new bone formation with DBBM-C in humans. The initial histomorphometrical data on DBBM remodelling in fresh extraction sockets were described by Becker et al.[42] The authors reported that after 3–7 months of healing the bovine bone particles were surrounded by connective tissue with

marginal presence of new woven bone. Lindhe et al. found comparable results at 6-month evaluation. DBBM-C particles were not resorbed but surrounded by a provisional connective tissue in the central part of the grafted site, but the peripheral part showed new bone formation.[43] Conversely, Alkan et al. observed remaining DBBM-C particles mainly in the coronal area of grafted intact sockets after 3 months of healing. The quality and quantity of the bone was clinically sufficient for a correct implant placement.[44]

Due to the anatomy of alveolar sockets and the delayed healing of DBBM-C grafted sites, implants were placed 12 months after the reconstructive procedure to optimize primary stability.[45] The histomorphometrical analysis in this study revealed a high percentage of deproteinized bovine bone particles still remaining in site at 12 months. Despite the high presence of deproteinized bovine bone, a process of osteointegration was evident, and woven bone was observed surrounding grafts, with bridges of newly formed bone between xenograft particles. These findings proved the good osseointegration of graft material and the absence of inflammatory response.

Conclusion

Results from the present study suggest that 3D volumetric dimensional alterations of the hard tissues in severely resorbed alveolar sockets can be quite extensive. The application of a slow resorbing xenograft with a secured covering collagen membrane may prove effective not only in limiting post-extraction crestal ridge bone loss but also in improving alveolar ridge shape and dimensions with the advantage to simplify later implant insertion and to be less technically demanding than more complex GBR techniques. The present study permits also to confirm the osteoconductive properties of DBBM-C, at 12 months of healing, providing an explanation by a biological point of view.

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