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Three-dimensional analysis of bone remodeling following ridge augmentation of compromised extraction sockets in periodontitis patients: a randomized controlled study.

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ABSTRACT

Objectives: The aim of this study was to analyze linear and volumetric hard tissue changes in severely resorbed alveolar sockets after ridge augmentation procedure and to compare them with spontaneous healing using three-dimensional cone beam computed tomography (CBCT).

Material and methods: Thirty patients (mean age 53.2 ± 6.3 years) requiring tooth extraction for advanced periodontitis were randomly allocated to test and control groups. The test sites were grafted using a collagenated bovine-derived bone (DBBM-C) covered with a collagen membrane, while control sites had spontaneous healing. Both groups healed by secondary intention. Linear and volumetric measurements were calculated on superimposed CBCT images obtained after tooth extraction and 12 months later.

Results: Greater horizontal shrinkage, localized mainly in the crestal zone, was observed in the control group (4.92 ± 2.45 mm) compared to the test group (2.60 ± 1.24 mm). While both groups presented a rebuilding of the buccal wall, it was most pronounced in the grafted sockets (2.50 ± 2.12 mm versus 0.51 ± 1.02 mm). A significant difference was also registered in the percentage of volume loss between grafted and non-grafted sites (9.14% versus 35.16%, P -value <0.0001).

Conclusion: Alveolar sockets with extensive buccal bone-deficiencies undergo significant three-dimensional volumetric alterations following natural healing. The immediate application of a slow-resorbing xenograft with a covering collagen membrane seems to be effective in improving alveolar ridge shape and dimensions, thus potentially reducing the need for adjunctive regenerative procedures at the time of implant placement.

1. INTRODUCTION

Following tooth extraction the residual alveolar bone undergoes marked qualitative and quantitative changes (Araújo & Lindhe, 2005; Pietrokovski, Starinsky, Arensburg, & Kaffe, 2007). The analysis of this clinical phenomenon and of its biological basis started in 1967 (Pietrokovsky & Massler, 1967), but the experimental study by Schropp et al. in 2003 reopened this field of research with the radiographic analysis of bone remodeling of more than 40 human alveolar sockets. They found a mean vertical reduction of 0.8 mm orally and a collapse of more than 50% of the bucco-lingual ridge dimension during the first year after tooth extraction. A similar magnitude of alveolar bone loss was reported in recent systematic reviews on post-extraction sockets with intact 4-wall configuration (Tan, Wong, Wong, & Lang, 2012; van der Weijden, Dell'Acqua, & Slot, 2009).

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 in the animal model (Lee et al., 2015). If compared with a 35% reduction in intact extraction sites over a 6-month period (Araújo & Lindhe, 2009), it could be expected that compromised alveolar sockets undergo more pronounced atrophy as a result of the natural remodeling process.

In such cases, critical-size alveolar ridge defects are most likely to occur leading to increased difficulty in placing the implant fixture in a prosthodontically suitable position. To restore the lost volume and to facilitate implant insertion several ridge reconstructive techniques including guided bone regeneration (GBR), use of particulate and block grafting materials, and distraction osteogenesis have been proposed (Chiapasco, Zaniboni, & Rimondini, 2007; Sbordone et al., 2012; Wang & Lang, 2012). 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.

Recent clinical studies in humans reported favorable outcomes when fresh extraction sockets showing buccal bone dehiscence > 5 mm or partial buccal wall deficiency were filled with hydroxyapatite or cortico-cancellous bone combined with a collagen membrane (Barone et al., 2015; Sisti et al., 2012). Although periodontal disease is a primary cause of tooth loss in adults, no information is available about

the immediate reconstruction of severely compromised alveolar sockets in periodontitis patients. The advanced resorption of the interproximal bone peaks, the uncontained anatomy and the inadequate soft tissue volume makes it difficult to ensure grafting material stability and to obtain primary wound closure. This may jeopardize the hard tissue volume reconstruction.

The use of deproteinized bovine bone covered with a collagen membrane left intentionally exposed may be a promising combination. The literature shows that intentional exposure of resorbable membranes does not seem to jeopardize the preservation procedure (Darby, Chen, & Buser, 2009). Resorbable membranes displayed also greater soft tissue compatibility compared with non-resorbable barriers in bone augmentation procedures (Urban, Nagursky, Lozada, & Nagy, 2013; Zitzmann, Naef, & Schärer, 1997). Among resorbable membranes, collagen barriers have specific physiochemical properties such as semipermeability, chemotactic effect over gingival fibroblasts, and early wound stabilization (Rothamel 2004) but require bone graft materials to maintain space for regeneration. Deproteinized bovine bone mineral coated with 10% porcine derived collagen (DBBM-C) seems to be an appropriate material for alveolar reconstruction (Heberer, Al-Chawaf, Hildebrand, Nelson, & Nelson, 2008; Jung et al., 2013). Collagen would seem to render the bone mineral surface more attractive for cell adhesion (Schwartz et al., 2007).

Nowadays, dimensional changes in post-extraction sockets after alveolar ridge preservation or augmentation techniques have been mainly described by vertical and horizontal linear measurements obtained clinically or radiographically on intraoral radiographs or cone beam computed tomography (CBCT) (Barone et al., 2013; Sisti et al., 2012; Jung et al., 2013). Horváth, Mardas, Mezzomo, Needleman and Donos (2012) emphasized the inability of bi-dimensional images to provide accurate data on hard tissue alterations due to their distortion and the need to standardize CBCT images. This is even more relevant when considering severely adsorbed alveolar sockets that exhibit complex three-dimensional (3D) anatomy due to the buccal/lingual wall deficiency. Hence, there is a need of 3D volumetric analyses to assess the efficacy of different ridge augmentation procedures in compromised sockets.

In view of these considerations, the aim of the present randomized controlled trial was to analyze hard tissue modifications of severely resorbed alveolar sockets grafted with DBBM-C and a collagen

membrane by means of 3D volumetric analysis on standardized CBCT images compared with spontaneous healing in chronic periodontitis patients.

2. MATERIAL AND METHODS

This report has been prepared in accordance with the guidelines outlined in the CONSORT statement. A copy of the checklist has been included.

2.1 Patient population

This present single-center prospective randomized controlled clinical study was conducted in accordance with the revised World Medical Association Declaration of Helsinki and was approved by the Institutional Ethics Committee (Protocol n° 695/2015). Adult patients requiring tooth extraction for advanced periodontitis were consecutively recruited in the trial between January and June 2015 at the Section of Periodontology, C.I.R. Dental School of the University of Turin (Italy). All patients were informed about possible risks and benefits and 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 (Armitage 1999) who completed the etiological periodontal therapy. All extraction sockets had to display pronounced buccal bone resorption with a discrepancy between the buccal and lingual bone walls of at least 35% (Jung et al., 2013). Preliminary screening was performed on the basis of clinical examination and intraoral radiography.

Patients were excluded from the study if any of the following were present: systemic diseases precluding surgical procedures (such as bone metabolic and hematologic disorders, uncontrolled diabetes) head or neck radiotherapy in the past 12 months, 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 (Hämmerle, Araujo, & Simion, 2012). Hopeless teeth due to trauma, endodontic problems or prosthetic reasons were excluded from the study.

2.2 Randomization and allocation concealment

All patients who met the inclusion criteria were consecutively enrolled in the trial. Each participant received a number in ascending order and 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 in order to avoid unequal balance between two treatments. To conceal assignment, forms with the treatment modality were put into identical and opaque envelopes with the patient number on the outside. The sealed envelopes were placed into the custody of a clinician not involved in the study. He opened the sealed envelope just after tooth extraction and informed the surgeon on the treatment to provide. The operators involved in the periodontal maintenance and in the radiographic analysis as well as the statistician were blinded.

2.3 Pre-surgical treatment

One week before extraction all patients underwent one session of oral hygiene in order to provide a more favorable oral environment to wound healing. An impression was taken so as to create a custom-made template in radio-transparent acrylic resin with aluminum radiopaque markers (high-precision balls, diameter 5 mm, volume 65.5 mm³, Martin & C. Srl, Italy).

2.4 Surgical protocol

Prophylactic antibiotic therapy (2 g amoxicillin and clavulanic acid) was administered 1 h prior to the surgery. Intra-oral antiseptis was performed with 0.2% chlorhexidine digluconate (CHX) rinse for 2 minutes. One experienced surgeon carried out all the surgical procedures. Following the administration of local anesthesia teeth were extracted trying to minimize surgical trauma to the alveolar bone walls. The sockets were thoroughly curetted and copiously irrigated with sterile saline solution. On the 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) to the most coronal bone peak level and covered by a double layer of collagen membrane (Bio-Gide[®], Geistlich Pharma AB) secured with pins on the buccal aspect. The flap was repositioned at the presurgical level and sutured by horizontal mattress sutures. On the control sites, no augmentation procedure was performed. Healing was by secondary intention in 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.

2.5 Postoperative care and follow-up

All patients received oral and written postoperative instructions. They were prescribed amoxicillin 1g and 600 mg ibuprofen to be taken after the surgical session, and 0.12% CHX mouthwash to be used twice daily for 2 weeks. Patients were advised to continue with ibuprofen only if they experienced pain. Sutures were removed 14 days post-surgery after decontamination with 0.12% CHX solution. Patients were recalled weekly within the first month and every three months for the maintenance periodontal treatment until the end of the study.

2.6 Radiographic methodology and volumetric measurements

CBCT scans were obtained immediately after tooth extraction and 12 months later (New Tom/NTVG; field of view = 153.3 mm width x 109.8 mm height; thickness slice = 0.3 mm; voxel size = 0.3 mm; voltage = 110 kV; 2 mA; 10 s) for the test and control groups using standardized positioning. Data were converted to DICOM (Digital Imaging and Communications in Medicine) format and imported into Mimics 17.0 software (Mimics Innovation Suite®, Materialise NV, Belgium). Three-dimensional reconstructions were created using a threshold-based semi-automatic segmentation. On an axial image, the threshold level was determined individually for each CBCT data set on the basis of a profile line made along the bone adjacent to the alveolar bone defect (Linderup, Kuseler, Jensen, & Cattaneo, 2015). Using this profile line, it was possible to visualize the profile of the grey values, or pseudo Hounsfield Units (pHU). Based on the minimal and maximal threshold values, the hard tissue (included teeth and bone tissue) and the background (included soft tissue) were semiautomatically defined and color-coded. The threshold values for hard tissue ranged between 436.6 and 1041.6.

This procedure required the manual contouring of the compromised buccal bone wall in the hard tissue mask. Then, by applying the Boolean algebraic subtractive operation between the two masks, it was possible to identify a third mask of the region-of-interest (ROI), corresponding to the segmented socket region (Fig. 2). All voxels with similar intensity were identified iteratively by the Region Growing algorithm and appended gradually inside it. The ROI was identified by a polyline, a colored line marking the socket outline.

The 3D-rendered images of each of these volumes were then generated and exported as STL file. The volumes in cubic millimeters (mm^3) were assessed using the “Calculate 3D” tool in Mimics. To

compare the pre- and post-operative alveolar bone volumes of the same region it was necessary to superimpose the corresponding CBCT images. The matching was based on the realignment of the entire CBCT dataset by inserting six reference points on the CBCT images that were manually checked. The middle and posterior reference points (tips of nasal bone, anterior and posterior nasal spine, and clivus) were identified according to Alsufyani, Dietrich, Lagravère, Carey, and Major (2014), while the anterior landmarks corresponded to the center of the aluminum spheres. The 3D superimposition and fusion of the alveolar sockets was obtained by aligning the polylines on the post-operative CBCT dataset, which was appropriately rotated by the software. A Boolean subtractive operation was made to identify the augmented or resorbed area within each grafted and non-grafted sockets, respectively, in relation to the preoperative height and width of the alveolar crest. By means of the rendering operation it was possible to display the volumetric 3D reconstruction of the alveolar socket and to calculate the volumetric measurements of the desired volume of interest (VOI) on the screen (Fig. 3a). The volume and percentage of mineralized bone tissue loss were calculated at three zones (Fig. 3b): 1) the region from the most coronal part of the preoperative crest to 1 mm apical to that plane (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). In a previous study (Manavella et al., 2017) this image acquisition protocol led to a volumetric error of no more than 4.5%.

2.7 Linear measurements on CBCT images

Linear measurements were made as previously described by Jung et al. (2013) on superimposed CBCT images (Fig. 4). All measurements were referred to the following reference lines: a vertical line drawn in the center of the extraction socket and crossing its most apical point, and two horizontal lines perpendicular to the vertical line and crossing the most apical point of the socket and its most coronal portion, respectively. 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).

A masked engineer performed all the volumetric and linear measurements. In order to assess the intra-examiner reproducibility, 30% of the cases were randomly reanalyzed in two different occasions. The duplicate measurements differed by <6%.

2.8 Patient-centered outcomes

At the end of the surgical procedure patients received a 10-cm horizontal visual analogy scale (VAS) to quantify postoperative pain intensity (0= no pain; 10=extreme pain) at week 1, 2 and 3. A study assistant not involved in the randomization process recorded any adverse event during the first post-operative month.

2.9 Sample size calculation

Each patient provided a single alveolar socket to be treated. Sample size calculation was performed on the results of a previous study where ridge changes were evaluated comparing ridge preservation versus extraction alone (Barone et al. 2008) and resulted in 12 patients per group (12 in the test and 12 in the control group). It was assumed that the minimum clinically relevant difference between groups in horizontal width change would amount to 1.5 mm with a standard deviation of 1.2 mm. The Type I error probability was set at 0.05 and the statistical power at 80%. To compensate for possible dropouts the sample size was adjusted to 30 patients.

2.10 Statistical analysis

The primary outcome measurement of the study was the horizontal width. Secondary outcomes included all the other linear measurements, volumetric measurements and patient-centered outcomes. Data were expressed as mean \pm standard deviation. The statistical unit was the patient. The Shapiro–Wilk test confirmed the normal 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 and VAS scores were assessed by means of the Chi-square test. The Mann-Whitney *U*-test was used to evaluate pain-related VAS values. All statistical tests were two-tailed and conducted at a 5% level of significance using a statistical tools package (SPSS version 19, IBM, Chicago, IL, USA).

3. RESULTS

The study was conducted from March 2015 to September 2016. Figure S1 summarizes the flow chart of the study. Forty-two subjects were assessed for their eligibility. Of these 12 were excluded: 8 did not meet the inclusion criteria, while the other 4 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.

3.1 Patient-centered outcomes

Data concerning patient-reported outcomes are described in Table 2. No post-surgical adverse events were recorded during the first postoperative month and all post-extraction sites healed without complications. Pain-related VAS values collected during the first postoperative week were significantly higher in the test group compared to the control one ($P < 0.001$). A greater improvement was observed in the control group during the second week of healing. At week 3, pain was no longer reported. Only three patients of the test group claimed some interference with daily activities during the first week.

3.2 Radiographic outcomes

As reported in Tables 3 and 4, 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 sites at -3 mm level, with a mean VT-3 of 0.29 ± 0.33 mm and 0.44 ± 0.76 mm, respectively. It appeared relatively intact at the more apical portion in most of the extraction sites. The mean VT-5 was 0.63 ± 0.48 mm in the test and 0.48 ± 0.59 mm in the control group.

Both groups showed a significant horizontal width reduction from baseline to the 12-month follow-up (Table 3) 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 were more pronounced in the cervical alveolar region (4.92 ± 2.45 mm versus 2.60 ± 1.24 mm, HW-1), while decreasing at the -5 mm level (0.61 ± 1.14 mm versus 0.34 ± 0.87 mm, HW-5).

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 ($P < 0.0001$).

The volumetric analysis (Table 4) showed a statistically significant decrease in volume (all $P = 0.001$) in both groups with a mean difference of 18.61 ± 17.93 mm³ (9.14%) in the grafted sockets that increased to 62.09 ± 25.81 mm³ (35.16%) in the non-grafted sites (Figs 5 and 6). As reported in Figure 7, the most pronounced differences were observed in the first millimeter below the bone crest (zone 1) where the control group displayed an average loss of bone volume of 18.14 ± 12.13 mm³ (42.96%) compared to 6.20 ± 6.34 mm³ (16.24%) in the test group ($P = 0.001$). Approximately a mean bone volume loss of 29% and 10% occurred at 1-3 mm apical to the bone crest (zone 2) in the grafted and non-grafted sockets, respectively. The percentage decreased to 20.41% and 4.87% at 3-5 mm apical to the bone crest (zone 3).

4. DISCUSSION

The aim of this randomized controlled trial was to evaluate the 12-month linear and volumetric bone changes in alveolar sockets augmented with DBBM-C and covering collagen membrane compared with spontaneous healing on CBCT images. 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 in the present study.

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 (Hürzeler et al., 1998; Nevins & Mellonig, 1992), there is scant information on dimensional changes in compromised alveolar sockets following immediate augmentation procedures (Barone et al., 2015; Crespi, Capparé, & Gherlone, 2014; Sisti et al., 2012). Furthermore, these studies reported direct intra-surgical or linear measurements on periapical radiographs or CBCT images or volumetric measurements of scanned study casts.

In the present study, the VOI of the experimental sockets in the preoperative CBCT images was segmented and digitally superimposed on the corresponding postoperative VOI. This allowed a qualitative and quantitative evaluation of 3D changes of hard tissue volume on 3D superimposed images and not just a mathematical subtraction between them (Linderup et al., 2015).

The main problem encountered in CBCT superimposition is the patient head orientation during images acquisition that may influence measurements accuracy (Alsufyani et al., 2014). In literature two main methods have been suggested to overcome this problem. The first relies on a computer-aided superimposition of the first image on the second one by a repeated best-fit algorithm (Cevidane, Styner, & Proffit, 2006; Park et al., 2012). In the second method the spatial orientation of serial 3D CBCT images is standardized and optimized by transforming the global coordinate system into a new Cartesian coordinate system based on reliable anatomic landmarks (Lagravère, Major, & Carey, 2010). A combination of these methods was applied in the present study. Cranial-base landmarks are stable anatomical structures and can be easily recognized on the CBCT images. Alsufyani et al. (2014) demonstrated in orthodontic patients that tip of nasal bone, anterior and posterior nasal spine, and clivus produced reliable landmarks for new coordinate transformation for CBCT imaging. As suggested by Lagravère, Secanell, Major, and Carey (2011) we used 6 landmarks instead of 4 to optimize the coordinate system and we added to the aforementioned reference points the center of the spherical markers as anterior coordinates. In addition, Mimics software ensures that angular and linear distances between landmark points are maintained when superimposing different images with a mean relative error of 0.21%. Importantly, the baseline CBCT images were taken immediately after tooth removal. This eliminated the need of digital subtraction of pre-extraction target teeth, as reported in previous studies (Abdelhamid, Omran, Bakhshalian, Tarnow, & Zadeh, 2016; Crespi et al., 2014; Sbordone et al., 2016), and had the advantage to avoid the superimposition of bone and dental tissue.

A limitation of the present volumetric technique was the image acquisition protocol using medium-large field of view (FOV) and 0.3-mm voxel size. Although this setting may impact on the quality of the images, it was selected to obtain the superimposition of CBCT images based on craniofacial anatomic landmarks (Alsufyani et al., 2014). Nevertheless, as regards image resolution, Menezes, Janson, da Silveira Massaro, Cambiaghi, and Garib (2016) found comparable accuracy in linear measurements of

alveolar bone crest levels on dried human mandibles comparing to anatomical measurements for voxel dimensions of 0.2, 0.3, and 0.4 mm. Similar results were obtained by de Rezende Barbosa, Wood, Pimenta, Maria de Almeida, and Tyndall (2016) in the calculation of jaw cleft volume and by Maret et al. (2013) for tooth volumetric measurements (using micro CBCT) using different FOV and voxel sizes. Kamburoğlu et al. (2015) do not find any difference in detection and volume measurements of simulated buccal marginal alveolar peri-implant defects by reducing the FOV.

Reduced voxel sizes are needed for detecting and measuring periodontal and peri-implant defects of much smaller dimensions than the post-extraction alveolar socket assessed in the present study (Pinheiro et al. 2015). Kolsuz, Bagis, Orhan, Avsever, and Demiralp (2015) identified a voxel size of 0.15 mm as the cut-off point for overall detection of a periodontal bony defect, while Kamburoğlu et al. (2015) a voxel size of 0.1 mm for furcation defects. Finally, Thönissen et al. (2015) supported the need for improved CBCT image resolution for anatomic structures less than 0.5 mm in width. However, the possibility of using smaller FOVs and smaller voxel sizes to increase image resolution and to limit the radiation dose should be considered in the 3D volumetric analysis of alveolar sockets. As image artifacts are most likely to occur in the peripheral regions of selected smaller FOVs, it is necessary to validate an accurate and reliable superimposition technique based on fixed landmarks located in the central position of the FOV to facilitate images comparison over time (Nackaerts et al., 2011).

Another aspect to be considered is the effect of the threshold settings on the ROI identification. In the present study bone threshold values was selected case by case based on the method described by Linderup et al. (2015). Although a manually defined threshold setting for each CBCT data set may be less reproducible than the use of an automatically approach, it was demonstrated to generate fewer errors in volume determination (El & Palomo, 2010; Linderup et al., 2015). As reported in the literature, CBCT grey values (pHU) cannot be as accurately calibrated as HU in CT scanning procedure due to the cone beam geometry, the size of the field of view, the relatively large amount of noise and different types of artefacts (Lagravère, Carey, Ben-Zvi, Packota, & Major, 2008; Pauwels et al., 2013).

This volumetric quantitative method was applied in the clinical setting to analyze the 12-month dimensional changes of severely damaged alveolar sockets after a ridge augmentation procedure. The 3D ridge contour analysis showed a dome-shaped configuration of the edentulous crest in the test group

as described in previous histological studies on dogs (Araújo, Liljenberg, & Lindhe, 2010; Araújo, Linder, Wennstrom, & Lindhe, 2008). The placement of Bio-Oss collagen in fresh extraction sockets seemed to counteract the bone remodeling 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 (Araújo et al., 2008).

A significant 3D volumetric bone loss of about 35% occurred in the non-grafted sockets. In agreement with previous studies on non-grafted sockets with buccal dehiscence defects (Abdelhamid et al., 2016; Crespi et al., 2014) bone loss occurred primarily in the 0-3 mm zone apical to the alveolar crest (from 29% to 43%). 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 crestal portion of the crest (10%) and decreased to 5% in its middle part. These data are consistent with findings by Araujo and Lindhe (2005) 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 vertical and horizontal 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 unfavorable anatomy, augmented sockets exhibit a pattern and a degree of bone resorption comparable with grafted intact sockets. In the literature review by Avila-Ortiz, Elangovan, Kramer, Blanchette, and Dawson (2014), the weighted mean difference between filled and unfilled sites was 1.89 mm (95% CI: 1.41, 2.36; $P < 0.001$) in terms of horizontal ridge width reduction, and 2.07 mm (95% CI: 1.03, 3.12; $P < 0.001$) in mid-buccal height loss.

Of note, all the sockets included in this study presented a significant vertical increase of the buccal bone

plate, despite being more pronounced in the augmented group. In a study by Crespi et al. (2014), unfilled extraction sockets covered with a collagen sponge displayed a vertical buccal bone growth after 3 months of spontaneous healing. 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 (Amler 1969). However, the peculiar anatomy of compromised sockets should be considered as they are uncontained defects in their coronal portion but provide a bone envelope with high regenerative potential in their most apical part.

In contrast, Jung et al. (2013) reported the inability of DBBM-C to preserve the buccal wall height in intact 4-wall sockets because of the impossibility of the graft material to mineralize at its more coronal portion. This was corroborated by histological outcomes in an experimental study using DBBM following tooth extraction (Mardas, Chadha, & Donos, 2010). Keeping in mind that data are not directly comparable to the present findings, it is clear that they are not in conflict. Jung et al. (2013) carried out a flapless socket preservation technique. DBBM-C was placed into the intact socket and covered with a collagen matrix to counteract socket shrinkage. In the current study, a flap surgery was performed to augment damaged sockets. A collagen membrane was tucked under the lingual flap to replace the missing bone wall and secured to the buccal bone plate by means of pins in order to prevent clot disruption and DBBM-C particles loss during socket healing. The fixation of the membrane plays a pivotal role in maintaining socket volume (Hämmerle, Jung, Yaman, & Lang, 2008) and in improving osteogenesis (Kim et al., 2016; Lee et al., 2015). Since the healing was by secondary intention, the collagen membrane was applied in a double layer to prolong its barrier function (Kim et al., 2009; Von Arx & Buser, 2006). In addition, the use of a collagen membrane might have enhanced wound healing via the induction of angiogenesis or fibroblast cell migration (Rothamel et al., 2004).

The present encouraging results may be also attributable to the long degradation time of the grafting material and to the subsequent lower turnover of the remodeling process in the extraction sockets that might have reduced contraction of the alveolar crest. The uncontained anatomy of the socket defects requires the application of a secured membrane barrier in association with a biomaterial capable of maintaining its initial shape and size in the early healing phase (Hämmerle et al., 2012). The DBBM-C is a demineralized bovine bone mineral within a 10% porcine type I collagen matrix. The collagen

undergoes fast resorption leaving space for clot formation that entraps the graft particles. This larger portion occupied by the blood clot allows for the incorporation of the DBBM particles by the new-formed bone tissue (Araújo et al., 2010). However, this delay in healing requires an increased length of time before implant placement, especially when existing bone was not present at the time of extraction, to gain adequate implant primary stability. At 12 months postoperatively the regenerated bone allowed for the insertion of dental implants without the need for further augmentation procedures. This may represent the most important limiting factor of the present reconstructive procedure. Nevertheless, GBR techniques, in presence of severe atrophy, are complex and technically demanding and require a long healing time to allow the correction of ridge defects and to enable adequate implant-supported restorations (Urban et al., 2013).

When considering the present results, it is important to take in account the relatively homogeneous enrolment of sites, which included only single-rooted teeth extracted due to advanced periodontal disease. Therefore, due to the stringent inclusion criteria the subjects are not representative of all the cases encountered in the clinical practice. Other limitation includes the quite small sample of the enrolled patients. As there was no previous similar study in the literature, the sample size was calculated using variations in clinical linear alveolar ridge measurements in ridge preservation procedures. However, a certain degree of variability was observed in the volumetric dimensional changes. Further studies with greater enrolment numbers in each specific subgroup are required to further investigate the clinical potential utility of this procedure and the long term survival and success rate of implants inserted in the augmented bone.

In conclusions, 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 in the aesthetic zone.

Conflict of Interest and Sources for Funding

No financial support was received for this study. The authors report no conflicts of interest related to this study.

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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

^aUnpaired *t*-test^bChi-square test**Table 2. Patient-related outcomes**

Variables		Test Group (n=15)		Control Group (n=15)		P-value
		Mean ± SD	Range	Mean ± SD	Range	
Pain/Discomfort (VAS score)	<i>week 1</i>	4.3 ± 0.6	3.2-5.3	3.8 ± 0.8	1.9-4.2	<0.001 ^a
	<i>week 2</i>	1.9 ± 0.5	1.1-3.1	0.6 ± 0.7	0-2.1	<0.001 ^a
	<i>week 3</i>	0		0		

VAS units visual analogue scale units (with 0=no pain and 10=unbearable pain), NS not statistically significant (*P*-value > 0.05).

^aMann-Whitney *U*-test

Table 3. Changes in ridge height and width between baseline and 12 months based on CBCT measurements (mean \pm SD).

Variables	Test Group (n=15)	Control Group (n=15)	P-value
H (mm)			
Baseline	6.92 \pm 1.54	6.68 \pm 1.05	0.680
12 months	8.26 \pm 1.59	6.22 \pm 1.12	<0.0001
Difference (mm)	1.34 \pm 1.45	-0.46 \pm 1.35	
Difference (%)	22.11 \pm 24.18	-5.31 \pm 19.81	
P-value	0.003	0.182	
HV (mm)			
Baseline	2.89 \pm 2.27	2.74 \pm 1.49	0.902
12 months	5.39 \pm 1.35	3.25 \pm 1.53	<0.0001
Difference (mm)	2.50 \pm 2.12	0.51 \pm 1.02	
Difference (%)	82.12 \pm 18.73	27.97 \pm 38.47	
P-value	0.002	0.046	
HL (mm)			
Baseline	6.96 \pm 1.60	6.04 \pm 1.11	0.078
12 months	7.23 \pm 1.13	5.83 \pm 1.01	<0.0001
Difference (mm)	0.27 \pm 1.31	-0.21 \pm 0.78	
Difference (%)	7.89 \pm 25.83	-1.88 \pm 16.97	
P-value	0.435	0.326	
W (mm)			
Baseline	8.62 \pm 1.57	7.82 \pm 1.64	0.105
12 months	6.65 \pm 1.41	3.99 \pm 1.30	<0.0001
Difference (mm)	-1.97 \pm 1.55	-3.83 \pm 1.49	
Difference (%)	-21.63 \pm 16.25	-46.32 \pm 17.61	
P-value	<0.0001	<0.0001	
HW_1 (mm)			
Baseline	8.27 \pm 1.50	7.63 \pm 1.48	0.249
12 months	5.68 \pm 1.03	2.72 \pm 2.52	<0.0001
Difference (mm)	-2.60 \pm 1.24	-4.92 \pm 2.45	
Difference (%)	-30.51 \pm 12.05	-64.38 \pm 31.88	
P-value	<0.0001	<0.0001	
HW_3 (mm)			
Baseline	7.84 \pm 1.29	7.04 \pm 1.09	0.077
12 months	6.98 \pm 1.24	5.03 \pm 1.39	<0.0001
Difference (mm)	-0.86 \pm 0.82	-2.01 \pm 0.97	
Difference (%)	-10.67 \pm 9.48	-28.93 \pm 13.86	
P-value	0.001	<0.0001	
HW_5 (mm)			
Baseline	6.91 \pm 2.20	6.13 \pm 1.52	0.654
12 months	7.37 \pm 1.32	5.52 \pm 1.38	<0.0001
Difference (mm)	0.34 \pm 0.87	-0.61 \pm 1.14	
Difference (%)	4.38 \pm 11.16	-8.46 \pm 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.

Table 4. 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 (mm³)			
<i>Baseline</i>	208.80 ± 83.53	183.65 ± 69.32	0.595
<i>12 months</i>	190.19 ± 82.05	121.56 ± 66.96	<0.0001
<i>Difference (mm³)</i>	18.61 ± 17.93	62.09 ± 25.81	
<i>Difference (%)</i>	9.14 ± 8.44	35.16 ± 10.92	
<i>P-value</i>	0.001	0.001	
Zone 1 (mm³)			
<i>Baseline</i>	42.24 ± 16.27	40.20 ± 12.49	0.672
<i>12 months</i>	36.05 ± 18.29	22.06 ± 10.49	0.001
<i>Difference (mm³)</i>	6.20 ± 6.34	18.14 ± 12.13	
<i>Difference (%)</i>	16.24 ± 17.50	42.96 ± 21.78	
<i>P-value</i>	0.002	<0.0001	
Zone 2 (mm³)			
<i>Baseline</i>	85.76 ± 36.63	78.54 ± 30.96	0.564
<i>12 months</i>	77.05 ± 35.91	55.38 ± 25.21	0.066
<i>Difference (mm³)</i>	8.72 ± 8.09	23.15 ± 15.41	
<i>Difference (%)</i>	10.14 ± 8.96	29.26 ± 12.71	
<i>P-value</i>	0.001	<0.0001	
Zone 3 (mm³)			
<i>Baseline</i>	56.26 ± 24.74	46.17 ± 22.57	0.253
<i>12 months</i>	53.36 ± 23.67	36.26 ± 21.22	0.046
<i>Difference (mm³)</i>	2.89 ± 4.92	9.91 ± 12.48	
<i>Difference (%)</i>	4.87 ± 6.82	20.41 ± 22.79	
<i>P-value</i>	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.

Figures Legends

Figure 1. Pictures of clinical case of the augmentation technique (test group): (a) hopeless maxillary central incisor before extraction, (b) probing depth of more than 10 mm and extrusion of the tooth, (c) tooth extraction, (d) intraoperative view of buccal deficiency, (e) socket grafting, (f) membrane placement, (g) sutures, (h) sutures removal, (i) 12 months after surgery.

Figure 2. Segmentation of the DICOM image: (a) green mask identifying the hard tissue, (b) yellow mask identifying the background, (c) fuchsia mask generated by the Boolean subtractive operation between the green and yellow masks.

Figure 3. Rendering of the image of the augmented alveolar socket: (a) changes in the socket volume from baseline (yellow) are represented as a 3D colored-mapped model (augmentation in orange, and resorption in cyan), (b) dark lines at 1 mm, 3 mm and 5 mm from the bone crest.

Figure 4. Linear measurements on baseline CBCT images.

Figure 5. Volumetric analysis of clinical case in Fig. 1 (test group): (a-b) CBCT image of the alveolar socket at baseline and 12 months after the reconstructive procedure, (c) 3D rendering of the augmented alveolar socket, (d-e) 3D rendering of the skull at baseline and 12 months.

Figure 6. Volumetric analysis of clinical case in the control group: (a-b) CBCT image of the alveolar socket at baseline and 12 months later, (c) 3D rendering of the alveolar socket, (d-e) 3D rendering of the skull at baseline and 12 months later.

Figure 7. Box plots for volumetric changes for different zones (zone 1-2-3 = volume at 0-1 mm, 1-3 mm and 3-5 mm from the top of the crest), times (time 0, baseline; time 12, 12 months after the extraction or the augmentation procedure) and procedures employed (collagenated bovine-derived bone xenograft and collagen membrane, test group, vs. spontaneous healing, control group). In box-and-whiskers plot the box line represents the lower, median and upper quartile values. Outliers were data with values beyond the ends of the whiskers.

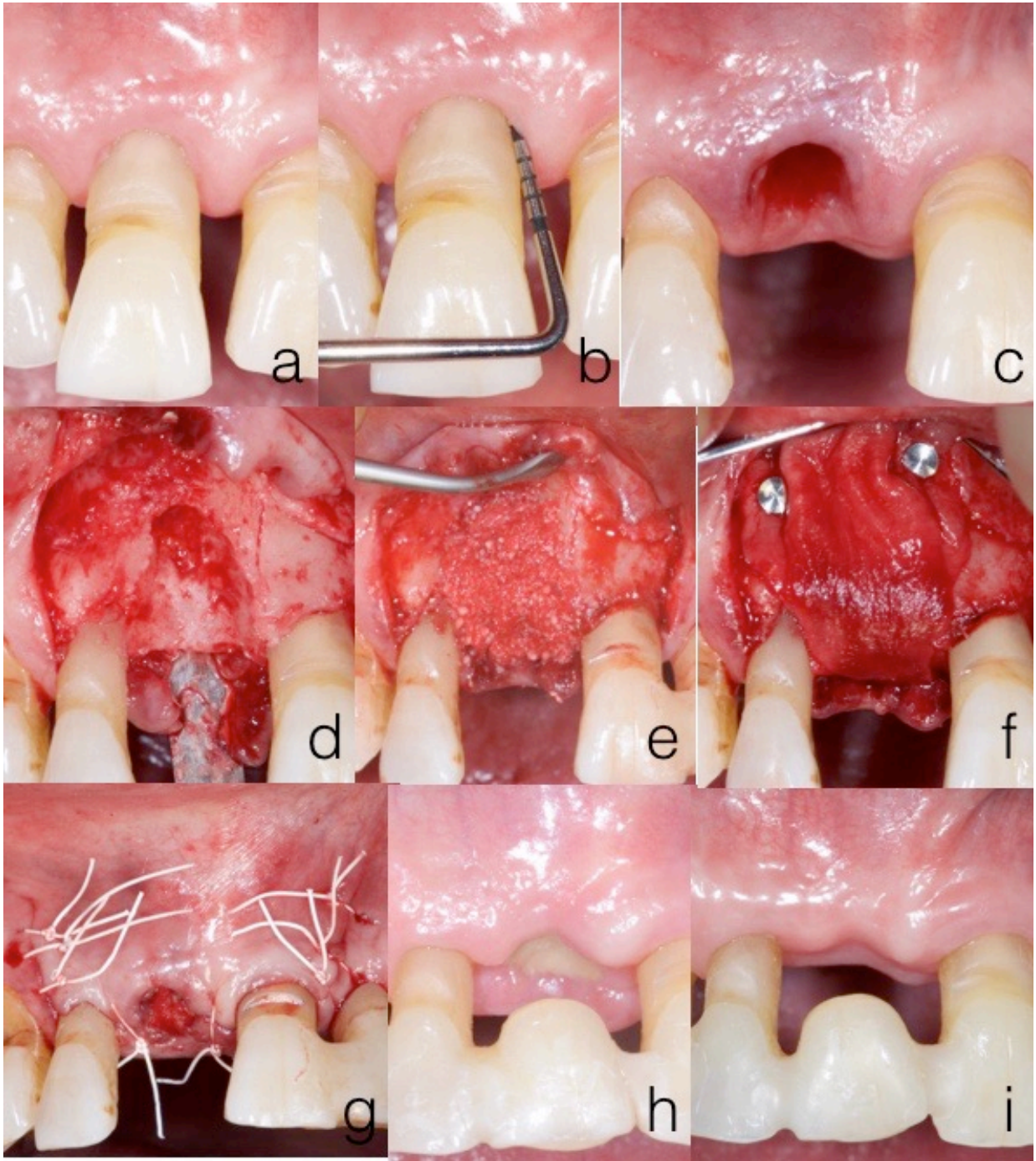


Figure 1.

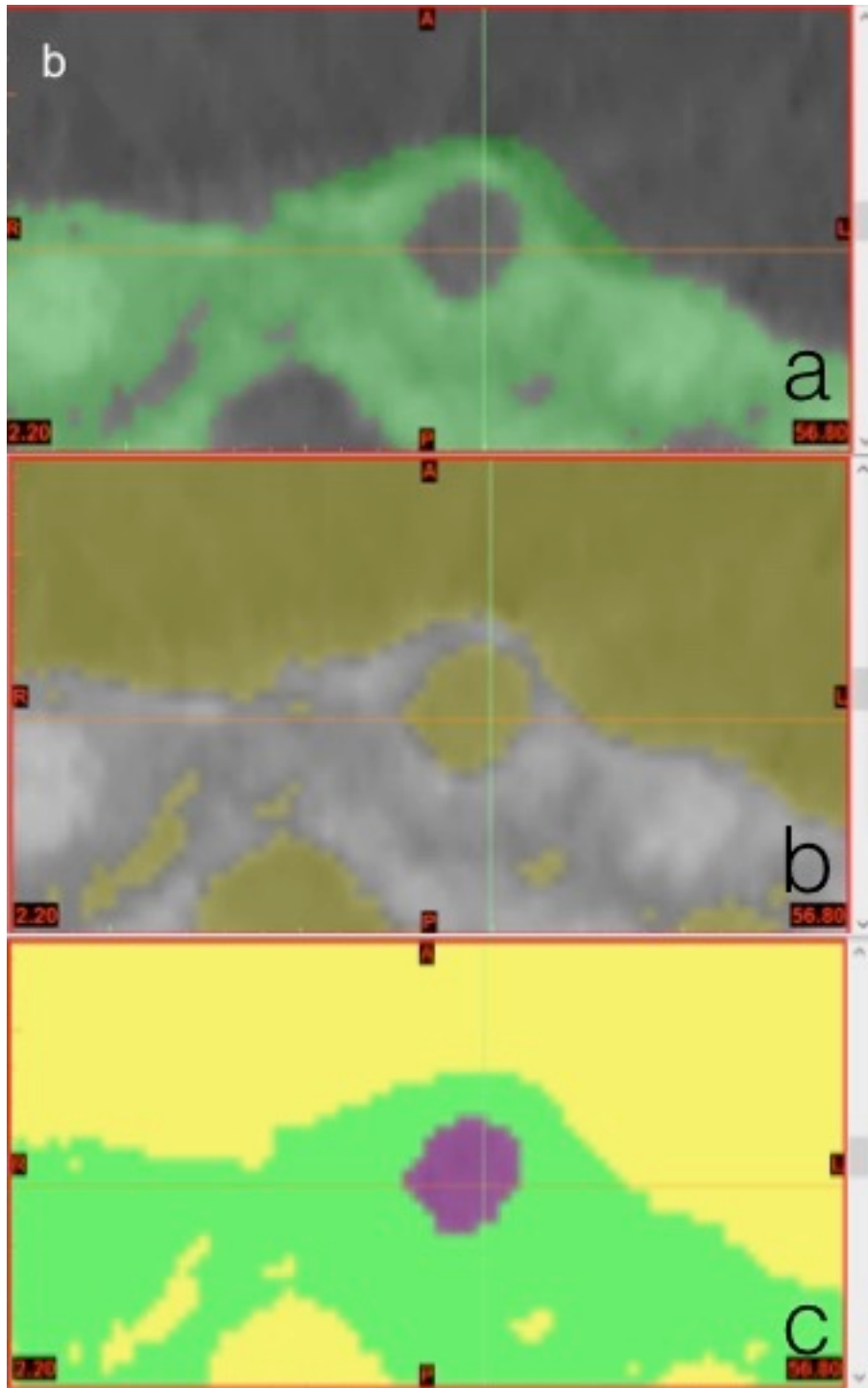


Figure 2.

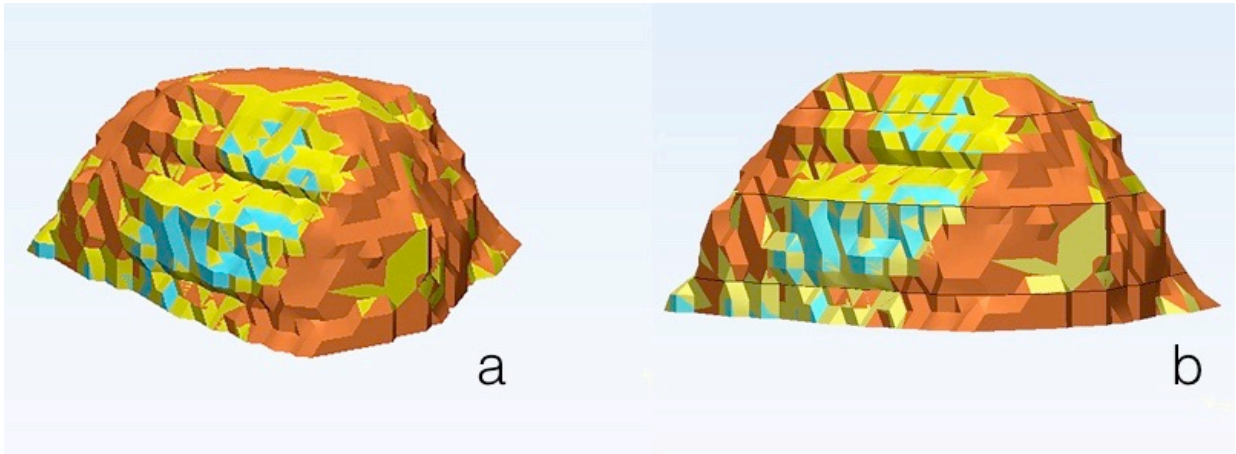


Figure 3.

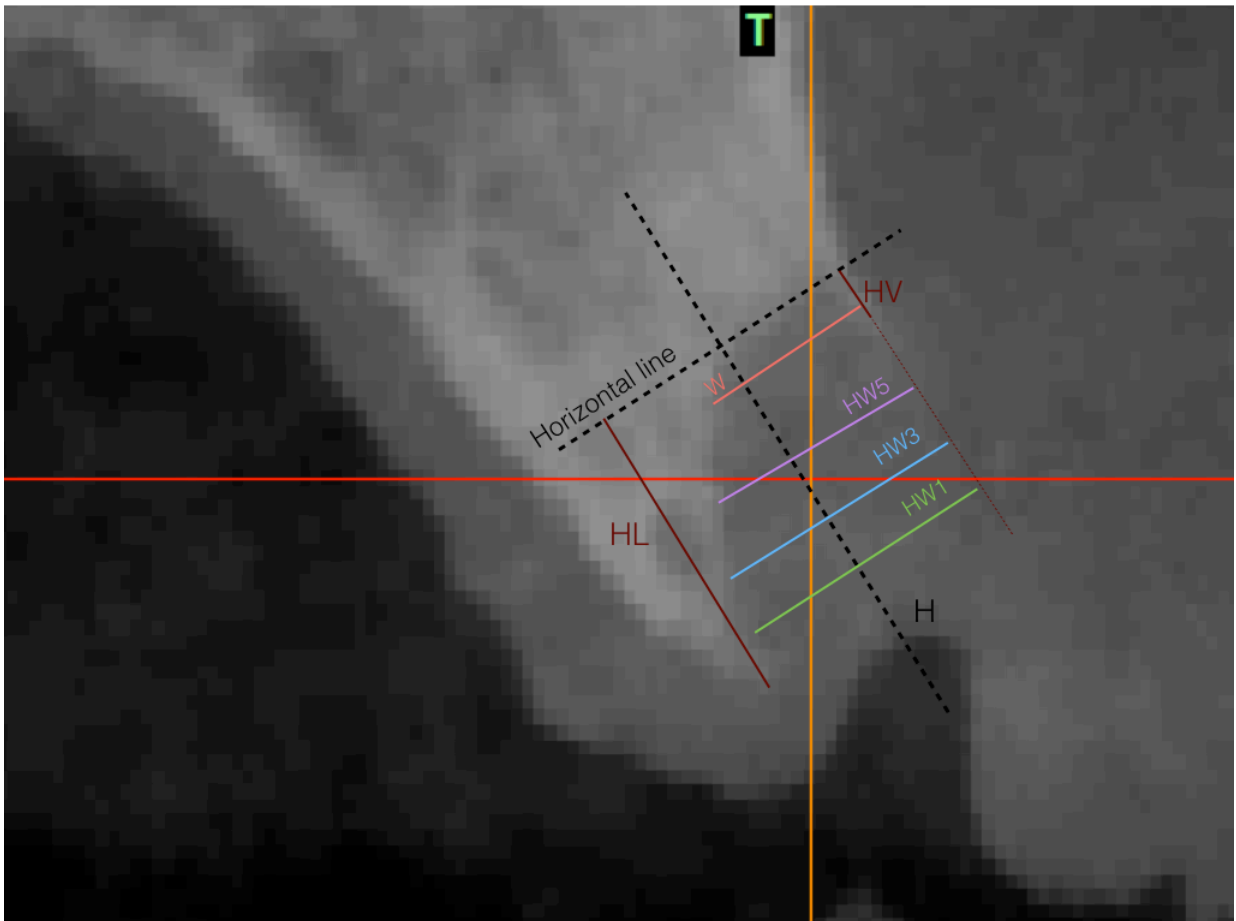


Figure 4.

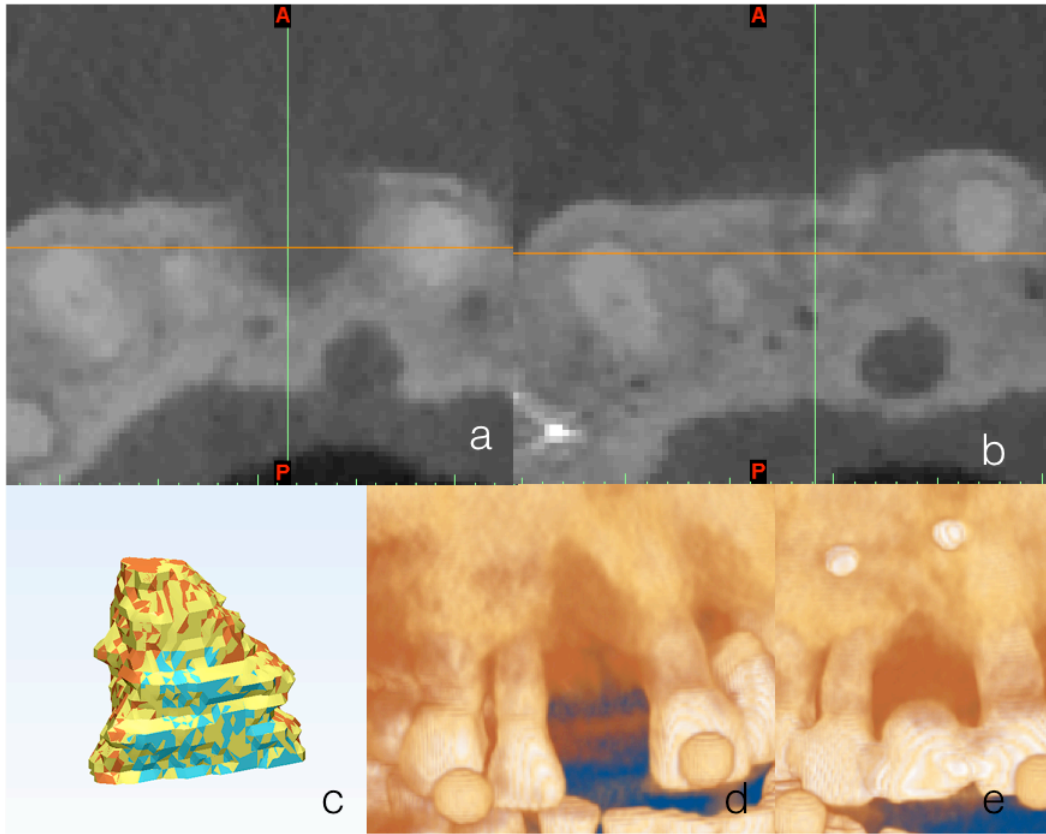


Figure 5.

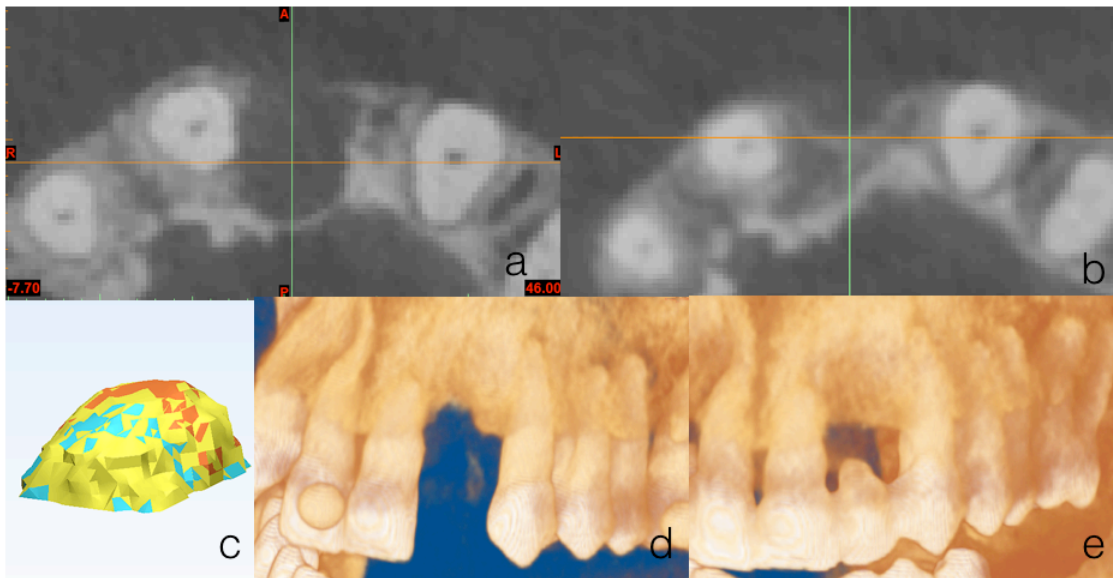


Figure 6.

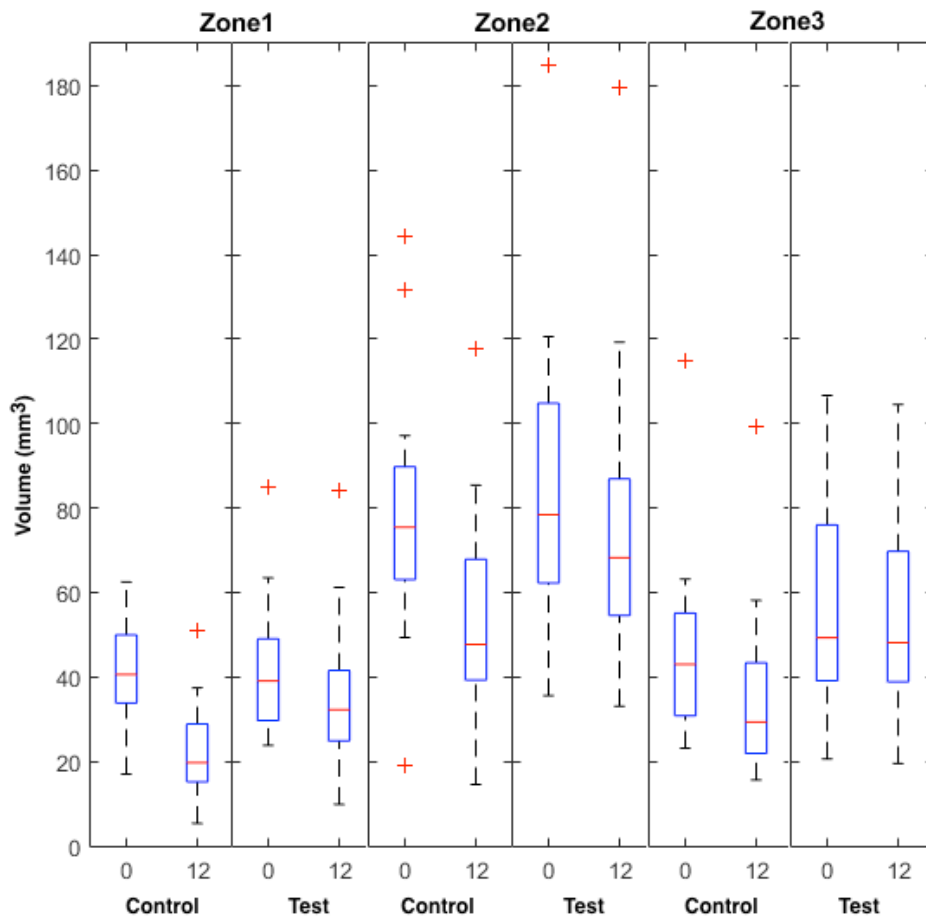


Figure 7.

Supporting information

Figure S1. Flow diagram according to CONSORT 2010.

