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ORIGINAL ARTICLE

Contour changes after guided bone regeneration of large non-contained mandibular buccal bone defects using deproteinized bovine bone mineral and a porcine-derived collagen membrane: an experimental in vivo investigation

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Abstract
Objective The objective of this study was to evaluate soft tissue contour changes after three different regenerative therapies in chronic ridge defects.
Material and methods Buccal bone defects were created in the mandible of nine beagle dogs. Augmentation procedures were performed 3 months later using a bone replacement graft (BRG), resorbable collagen membrane (MBG), or a combination of both procedures (CBG). Silicone impressions were taken before tooth extraction (T1), before the augmentation procedure (T2), and 3 months after the regenerative surgeries (T3). Casts were optically scanned and stereolithography files were superimposed to analyze the horizontal changes in ridge contours.
Results After defect creation, most part of the horizontal changes occurred 4 and 6 mm below the gingival margin. In the mesial defect (D1) at T3, the mean horizontal gain in MBG amounted to 0.47 ± 0.34 mm, 0.79 ± 0.67 mm in the BRG, and 0.87 ± 0.69 mm for the CBG. In the middle defect (D2), the mean changes for the MBG were 0.11 ± 0.31, 1.01 ± 0.91 for the BRG, and 0.98 ± 0.49 for the CBG. The mean changes in the distal defect (D3) amounted to 0.24 ± 0.72 for the MBG, 1.04 ± 0.92 for the BRG, and 0.86 ± 0.56 for the CBG. The differences reached significance in all defects for the comparison MBG-BRG and MBG-CBG, while similar parameters were observed for the comparison BRG-CBG.
Conclusion BRG and CBG were equally effective and superior to MBG in increasing the horizontal tissue contours. The augmentation seldom reached the values before extraction.
Clinical relevance Scaffolding materials are needed for contour augmentation when using resorbable collagen membranes.

Keywords Bovine bone mineral · Collagen membrane · Bone regeneration · Experimental study · Wound healing

Introduction

In humans, the loss of horizontal ridge contour as a consequence of tooth loss may account for more than 50% of the ridge width [1, 2], and the resulting lack of adequate crestal bone availability may significantly affect the successful implant placement in an ideal, prosthetically driven position [3]. Bone augmentation procedures are, therefore, aimed to compensate these changes and to reconstruct deficient alveolar ridges to permit the accurate placement of dental implants. Different regenerative interventions, such as the use of autogenous bone grafts, distraction osteogenesis, "split" ridge osteotomy, and guided bone regeneration (GBR), have shown efficacy in augmenting the alveolar ridge [4].

GBR with barrier membranes is based on the biological principle of compartmentalized healing by preventing the ingrowth of cells from the overlying mucosa into the membrane-protected space and allowing the colonization of competent

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osteogenic cells [5, 6]. The survival rate of implants placed with GBR procedures has been shown to be similar to that of implants placed in native bone [7]. Barrier membranes of different designs and compositions have been tested in pre-clinical and clinical models to provide evidence that GBR predictably results in bone regeneration when applied over critical size osseous defects [8, 9]. Residual crests, however, usually result in non-contained bone defects where the use of barrier membranes, mainly those being resorbable, will collapse into the defect and will reduce the space available for the colonization of osteogenic cells [10]. In addition, the blood clot tends to shrink during healing, what amplifies this effect [11].

Current GBR approaches, therefore, combine the use of barrier membranes with bone grafts and bone substitutes, which serve as scaffolds to fill the defect volume and to stabilize the blood clot, thus preventing its tendency to shrinkage. Moreover, the current understanding of bone biology and the biological behavior of modern biomaterials have resulted in less invasive surgical approaches and the attainment of better clinical results [12]. A recent systematic review from our research group has reported that the combination of a xenogeneic bone replacement graft with a resorbable native collagen membrane is the GBR procedure most widely used and the one that achieves more consistent results [13].

In spite of this body of evidence on the efficacy of GBR combining bone replacement grafts and bio-absorbable membranes [14], their respective wound healing patterns and their specific tissue response when used either alone or in combination are still partially known. This existing knowledge has been mainly derived from pre-clinical studies using histological outcomes to evaluate the healing patterns [15]. These studies have demonstrated the ability of these biomaterials of guiding new bone formation (osteoconduction) and being gradually replaced by new bone, although there is high variability in the degree of biomaterial bioabsorption and its replacement by new bone formation [16]. Histological methods, however, are unable to assess the reconstruction of the whole alveolar process since they can only focus on selected sections of varying thicknesses depending on the method of histologic processing.

The use of digital image analysis has been recently introduced in implant dentistry to study changes in contours and tissue volume. This has been particularly useful in the assessment of the tissue changes after bone augmentation procedures, either simultaneous or staged with implant placement [17, 18]. The outcome of implant supported reconstructions is not assessed anymore solely on the basis of implant survival but on how peri-implant tissues are in harmony with its adjacent structures [19].

It is therefore the aim of this pre-clinical in vivo investigation to evaluate the changes in tissue contour occurring after a GBR procedure combining a xenogeneic bone replacement graft and a natural collagen resorbable membrane for the lateral augmentation of critical size defects.

Material and methods

This pre-clinical in vivo investigation was designed following the modified ARRIVE guidelines for pre-clinical research [20] with a randomized block, examiner-blind experimental study evaluating four stages of healing in two groups of dogs. This investigation reports the results from a subset analysis of the specimens whose histological results are reported in a separate publication [21].

Sample and facilities

The experimental phase of the study was carried out at the "Veterinary Teaching Hospital" in the University of Santiago in Lugo, Spain after the study protocol was approved by the Ethical Committee of the Rof Codina Foundation (Lugo, Spain) (Ref AE-LU-001/12/INVMED (02)/Outros/04). Nine female beagle dogs, between 1.5 and 2 years old, with a weight ranging between 10 and 20 kg, were used. This investigation was conducted according to Spanish and European Union regulations (European Communities Council Directive 86/609/EEC) on experimental in vivo experimentation. All animals were fed on a soft pellet diet and maintained in individual kennels in a 12:12 light/dark cycle and 22–21 °C as well as daily monitored during the entire course of the experiment by an experienced veterinarian.

Surgical procedure

After animal sedation with propofol (2 mg/kg/i.v., Propovet, Abbott Laboratories, Kent, UK), general anesthesia was maintained under mechanical induced respiration of 2.5–4% of isoflurane (Isoba-vet, Schering-Plough, Madrid, Spain). The animals were pre-medicated with acepromazine (0.05 mg/kg/i.m., Calmo Meosan, Pfeizer, Madrid, Spain), and morphine (0.3 mg/kg/i.m., Morfina Braun 2%, B. Braun Medical, Barcelona, Spain) was administered as analgesic medication. Lidocaine 2% with epinephrine 1:100,000 (2% Xylocaine Dental, Dentsply, York, PA, USA) was infiltrated locally to reduce bleeding during surgery.

Defect preparation and augmentation procedures

The experimental model used in this study is outlined in Fig. 1. On both sides of the mandible, buccal and lingual mucoperiosteal flaps were raised. The second, third, and fourth lower pre-molars (P) and the first molar (M) were hemisected by means of a Lindemann bur. The mesial root of M1, the mesial root of P4, the distal root of P3, and the booth roots of P2 were extracted. A pulpotomy was made with a sterile bur and a pulp cap with calcium hydroxide was applied (Dycal, Dentsply, York, PA, USA) and a glass-ionomer filling (Ketac, 3M ESPE, Berkshire, UK) in each of the

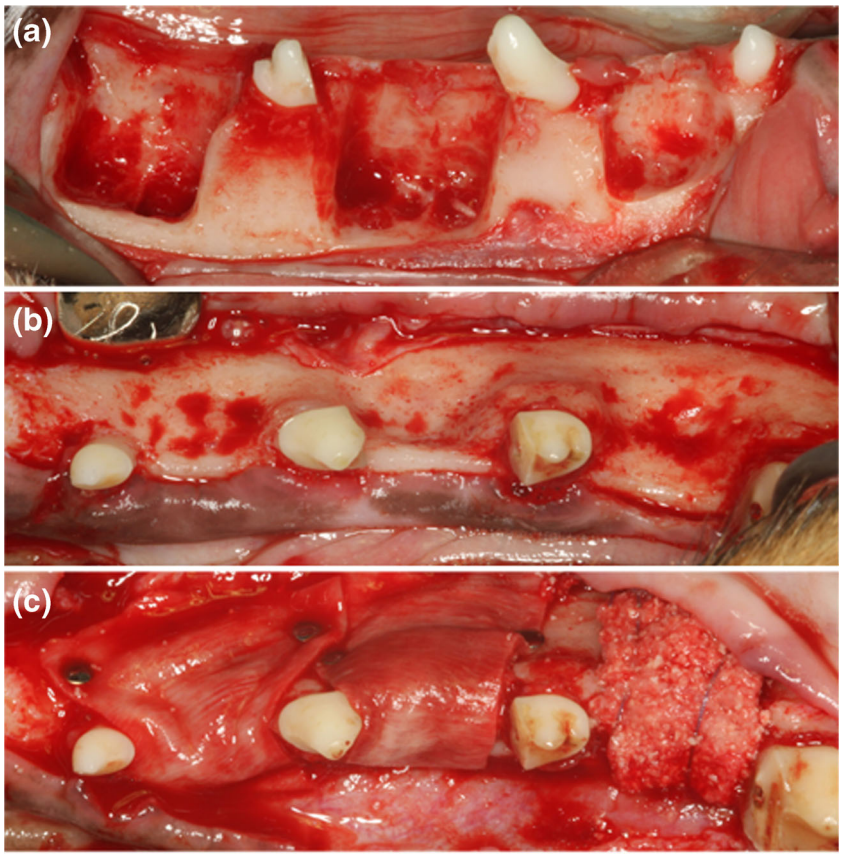

Fig. 1 **a** Facial view of box shaped defects after the extraction of the mesial root of M1, the mesial root of P4, the distal root of P3, and booth roots of P2. **b** Occlusal view 3 months after defect creation. **c** Augmentation procedures after randomization mesial defect (D1) received the collagen native membrane alone. Central defect (D2) received the combination therapy while the distal defect (D3) received the bone replacement graft

residual roots. In the three edentulous regions of each side of the mandibular buccal bone, defects were created with diamond burs under copious sterile saline irrigation (Fig. 1a). The defect sizes were about 10 mm in height (apico-coronally), 10 mm in width (mesio-distally), and 5 mm in depth (buco-lingually). Flaps were then repositioned and sutured (Vicryl″ 5.0, Johnson & Johnson, Sint-Stevens-Woluwe, Belgium). A period of 3 months was given for healing of the surgically created defects. Then, the augmentation procedures were carried out with the elevation of full thickness flaps from 1M1 to 1P1 fully exposing the bone defects (Fig. 1b).

Each defect was randomly allocated to one of three augmentation procedures using a computer-generated list. In the bone replacement group (BRG), the defect was filled with a bone replacement graft composed of 90% of deproteinized bovine bone mineral with 10% collagen (DBBM-C) (Geistlich Bio-Oss® Collagen; Geistlich Pharma AG, 6110 Wolhusen, Switzerland). This bone replacement graft was hydrated with saline and well adapted to fill the residual crest defect by means of resorbable sutures (Vicryl" 4.0, Johnson & Johnson, St-Stevens-Woluwe, Belgium). In the membrane alone group (MBG), the defect was covered with an absorbable native bilayer collagen membrane (NBCM) (Geistlich Bio-Gide®; Geistlich Pharma AG, Wolhusen, Switzerland). The membrane was trimmed and adapted over the ridge to completely cover the defect and extended beyond the defect margins by 2–3 mm. The NBCM was secured by attaching four titanium pins (Frios® membrane tacks, Dentsply, York, PA, USA) in the buccal and lingual bone. In the combination group (CBG), both interventions were combined and the defect was filled with the DBBM-C and the NBCM was adapted to cover the defect and extended beyond the defect margins by 2–3 mm. The membrane was secured as previously described (Fig. 1c).

Releasing incisions were made in the periosteum at the base of the buccal and lingual flaps, and the augmented defects were carefully covered by tension-free flaps and secured by horizontal internal mattress sutures alternated with interrupted 4/0 e-PTFE sutures (Goretex Suture, W. L. Gore & Associates Inc. Newark, DE, USA). For postoperative pain control, morphine (0.3 mg/kg/i.m.) was administered for the first 24 h and meloxicam (0.1 mg/kg/s.i.d./p.o., Metacam, Boehringer Ingelheim España, Barcelona, Spain) for three following days. Amoxicillin (22 mg/kg/s.i.d./s.c., Amoxoil retard, Syva, León, Spain) was used as postoperative antibiotic therapy for 7 days. During 2 weeks postoperatively, the animals were fed with water-softened food and surgical wounds were cleaned three times a week using gauzes impregnated with a chlorhexidine solution (0.12%). The sutures were removed after 14 days.

Soft tissue contour changes

Impressions of the lower jaws were obtained before the extractions (T1), prior to the augmentation surgery (T2) (i.e., 3 months after the extractions) and 3 months after the augmentation procedure (T3). For this purpose, a one-step/two-viscosity technique with silicone impression materials (Express 2 Putty Soft/Express 2 Light Body, 3M Espe, St. Paul, MN, USA) and individualized acrylic impression trays were used. Dental stone casts were fabricated (Elite Model, Zhermack, Rome, Italy), resulting in a total of 27 casts, 9 casts for each of the three different timelines (T1, T2, T3). Models were evaluated for the presence of irregularities such as porous areas, undefined gingival margins, broken cusps, or undefined vestibulum.

The cast models were optically scanned with a desktop 3D scanner (Zfx Evolution Scanner, Zimmer Dental, Bolzano, Italy) resulting in individual stereolithography (STL) files for each time period (Fig. 2), which were uploaded to an image analysis software (Swissmeda Software, Swissmeda AG, Zürich, Switzerland) (Fig. 3a). To match the STL files, three clear and visible common reference points were selected in both the baseline and follow-up casts. After the selection of these references, the software automatically superimposed the three models using a series of mathematical algorithms. In those sites where inproper fitting occured, manual adjustments were performed until the matching was deemed adequate (Fig. 3b).

Once the matching was deemed adequate, a longitudinal slice perpendicular to the ridge that divided defect into two equal parts was selected. A line coinciding with the axis of tooth at baseline was then drawn in the transversal images of the sections. A screenshot was then exported to an image processing software to perform the horizonal measurements (ImageJ, National Institutes of Health, Maryland, USA).

Since the three defects had distinct anatomical characteristics, data were analyzed separetely. Defect D1 corresponded to the most mesial defect and was created after the extraction of P2 and had mesialy to it P1 and distally to it the mesial root of P3. Defect D2 was created after the extraction of the distal root of P3 and the mesial root of P4 and had mesially to it the mesial root of P3 and distally to it the distal root of P4. Defect D3 was the most distal defect, was created after the extraction of the mesial root of M1, and had mesial to it the distal root of P4 and distal to it the distal root of M1.

The following linear measurements were performed by a blinded calibrated examiner (JA), independent from the investigator undertaking the analysis (Fig. 4):

i) The *horizontal soft tissue changes* were assessed 2, 4, and 6 mm below the gingival margin (GM) by measuring the distance between the line coinciding with the axis of the tooth at baseline and the buccal soft tissue outline at the three diferent timelines (T1, T2, and T3) [22, 23]. Once the horizontal measurements (HM) were calcualted at the three different heights (2, 4, and 6 mm) at T1, the horizontal changes (HC) were calculated by substracting the HM at T1 from the HM at T2 to obtain the HC from T1 to T2 which gave information on the degree of ridge collapse. To assess the changes in horizontal measurements after the augmentation procedure, the values at T3 were substracted from those at T2 to obtain the horizontal changes from T2 to T3. Horizontal measurements at T1 were substracted from those at T3 to obtain the horizontal changes from T1 to T3 which assessed the differences between the regenerated and the baseline tissue contours.

ii) The *vertical soft tissue changes* were assessed by measuring the distance between two lines perpendicular to the axis of the tooth. The first line was coinciding with

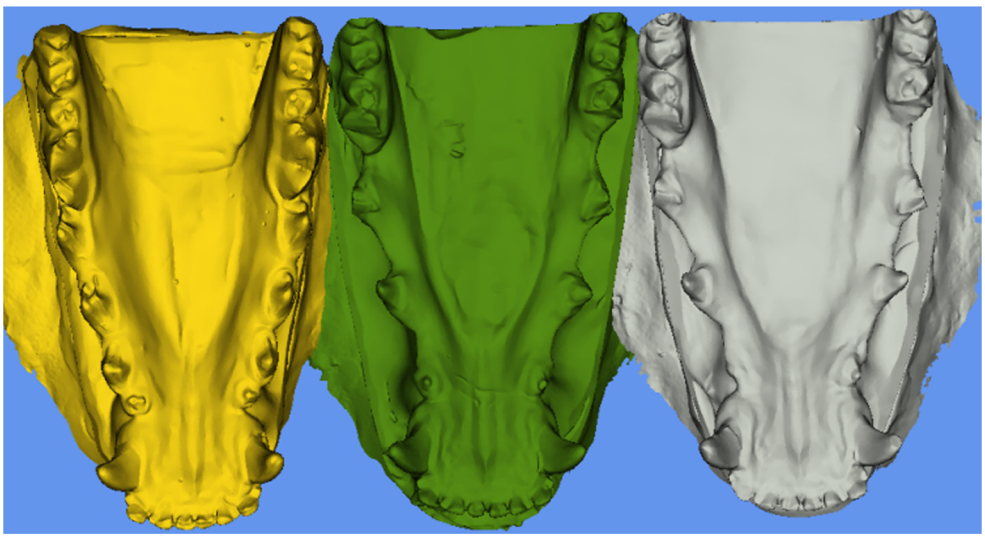

Fig. 2 Three-dimensional reconstructions of STL files after optical scanning of models before tooth extraction (T1/yellow), before augmentation procedure (T2/green), and 3 months after the augmentation therapy (T3/gray)

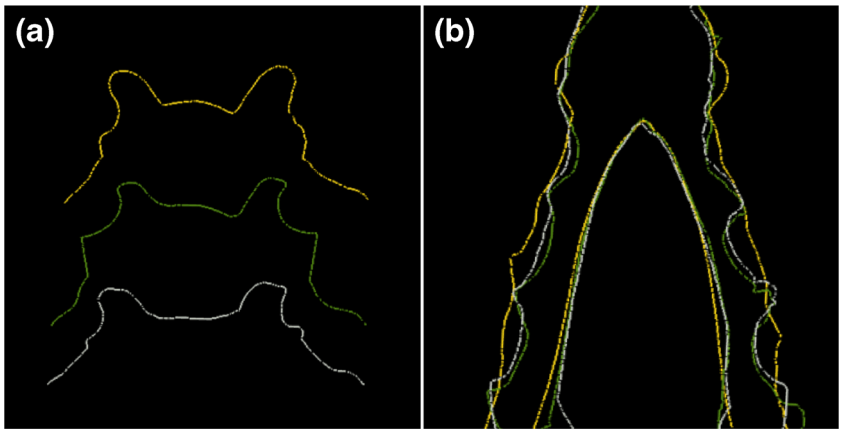

Fig. 3 **a** Outline of models before tooth extraction (T1/yellow), before augmentation procedure (T2/green), and 3 months after the augmentation therapy (T3/gray). **b** STL image superimposition with the aid of image analysis software. Notice the difference in ridge with from T1 to T2 and T3

the buccal gingival margin of the tooth at T1, and the other lines were coinciding with the edentulous crest at T2 and T3 (VC T1-T2, VC T1-T3).

iii) *Mean linear changes* between T2 and T3 provided information on the mean distance between the two surfaces in a selected area of interest. For this purpose, an area that encompassed the center of each defect was selected by means of a dedicated software (Swissmeda Software, Swissmeda AG, Zürich, Switzerland). The area extended 10 mm mesio distally and had an apico-coronal height of 5 mm. The software then calculated, by mean of a series of mathematical algorythms, the mean distance between T2 and T3 surfaces in each defect.

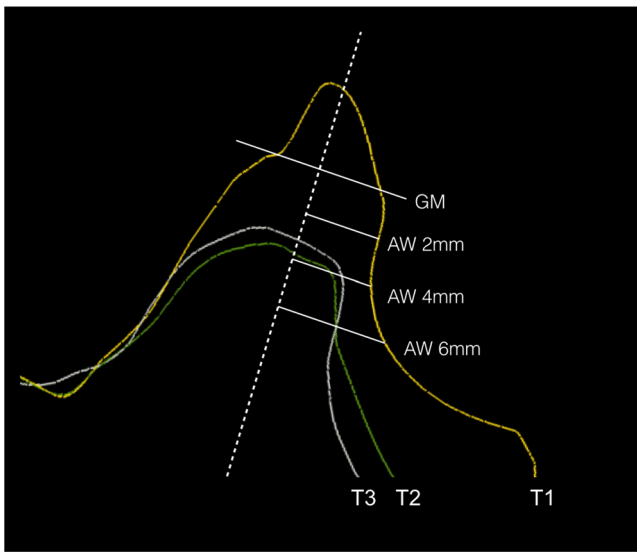

Fig. 4 Linear measurements performed to evaluate soft tissue changes. The dotted line represents the axis of the tooth, the most coronal line perpendicular to it links the facial and lingual gingival margin. Horizontal linear measurements are taken at the level of the gingival margin: 2, 4, and 6 mm below it

Statistical analysis

Descriptive statistics (means, standard deviations) of continuous variables were computed for each site separately using a statistical software program (SPSS version 18.0, IBM Corporation, New York, USA). The data was tested for normality by means of a Shapiro-Wilk test and found to be non-normally distributed. The Kruskall-Wallis test was used to determine differences at baseline and to analyze if the regenerative treatment had an impact in the continuous variables. Post hoc analysis was further performed with the Kruskall-Wallis test to check for pairwise comparisons between the three regenerative approaches. Statistical significance was set at the alpha level of 0.05.

Results

All animals healed uneventfully after both surgical interventions without occurrence of infections or evident membrane dehiscences after the regenerative procedures.

Changes in horizontal and vertical measurements after defect creation (T1-T2)

Table 1 depicts the baseline (T1) horizontal widths (BW) at the level of the GM and 2, 4, and 6 below the GM, the horizontal and vertical changes from T1 and T2 at 4 and 6 mm from the GM and the percentage of loss that occurred from baseline values. At baseline, there were no significant differences in horizontal measurements between the three treatment groups in each of the three defects at the level of the GM and 2, 4, and 6 mm below. In the most distal defect (D3), the baseline horizontal measurements at the level of the GM and 2 mm below presented marked differences between the three treatment groups.

After the extraction and defect creation, major changes occurred in the alveolar ridge. All groups had a mean loss in height that ranged from 2.04 to 3.22 mm from the GM with no

Table 1 Vertical and horizontal measurements at baseline (T1) and changes between T1 and T2 (n = 9)

	BW GM T1	BW 2 mm T1	BW 4 mm T1	BW 6 mm T1	HC 4 mm T1-T2	HC 6 mm	VC	% Loss 4 mm	% Loss 6 mm
Defect 1 (D1)									
MBG	4.34 ± 0.24	6.10 ± 0.35	7.07 ± 0.41	8.56 ± 0.65	3.07 ± 0.25	1.73 ± 0.42	3.22 ± 0.51	43.42	20.21
BRG	5.02 ± 0.50	6.52 ± 0.89	7.27 ± 0.24	8.15 ± 0.45	2.18 ± 0.43	1.70 ± 0.27	2.98 ± 0.38	29.98	20.85
CBG	5.30 ± 0.88	6.72 ± 0.43	7.47 ± 0.14	7.54 ± 1.32	4.32 ± 0.70	1.53 ± 0.40	2.57 ± 0.52	57.83	20.29
p value (MBG-BRG/MBG-CBG/ BRG-CBG)	0.729/0.245/0.300	0.353/0.246/ 0.758	0.426/0.146/0.384	0.657/0.288/0.459	0.425/0.487/0.130	0.988/0.909/0.922	0.519/0.555/0.948		
Defect 2 (D2)									
MBG	5.05 ± 1.7	7.36 ± 0.56	8.12 ± 0.46	8.91 ± 1.19	4.03 ± 0.43	3.76 ± 1.22	2.57 ± 0.35	49.63	42.19
BRG	5.05 ± 1.6	6.83 ± 1.09	7.61 ± 0.57	7.87 ± 0.39	4.52 ± 0.78	2.04 ± 0.6	2.66 ± 1.76	59.39	25.92
CBG	5.6 ± 0.16	8.20 ± 0.94	9.40 ± 1.16	10.61 ± 0.62	3.35 ± 0.15	3.75 ± 0.2	2.08 ± 0.21	35.63	35.34
p value (MBG-BRG/MBG-CBG/ BRG-CBG)	0.989/0.688/0.696	0.494/0.345/0.151	0.416/0.103/0.101	0.191/0.080/0.065	0.400/0.828/0.579	0.234/0.994/0.236	0.778/0.439/0.319		
Defect 3 (D3)									
MBG	8.80 ± 1.7	9.91 ± 1.3	10.07 ± 0.72	10.26 ± 1.07	6.5 ± 2.25	3.34 ± 0.92	2.86 ± 1.84	64.54	32.55
BRG	5.15 ± 2.1	8.25 ± 0.85	9.07 ± 0.30	9.69 ± 0.77	5.43 ± 3.47	3.24 ± 2.02	1.99 ± 2.34	59.86	33.43
CBG	6.09 ± 1.27	8.27 ± 0.93	9.56 ± 0.14	10.09 ± 0.87	5.04 ± 2.88	3.49 ± 0.77	2.41 ± 0.82	52.71	34.58
p value (MBG-BRG/MBG-CBG/ BRG-CBG)	0.086/0.066/0.494	0.081/0.101/0.975	0.073/0.257/0.312	0.535/0.831/0.662	0.746/0.625/0.907	0.933/0.833/0.830	0.576/0.740/0.788		

MBG membrane group, *BRG* bone replacement graft group, *CBG* combination group, *BW GM T1* baseline width at gingival margin in T1, *BW 2, 4, 6 mm T1*, baseline width 2, 4, 6 mm below gingival margin at T1, *HC 4–6 T1-T2* horizontal changes 4 and 6 mm below the gingival margin from T1 to T2, *VC T1-T2* vertical changes from T1 to T2, *% Loss 4, 6 mm* percentage of loss from the baseline horizontal values

significant differences between the treatment groups. The majority of the horizontal changes occurred at 4 and 6 mm below the gingival margin with greater changes occuring in the most posterior sites (D2 and D3) than in the anterior defects (D1). The horizontal changes at 4 and 6 mm below the GM were similar for the three groups, ranging in losses between 30 and 60%. Six millimeters below the GM the percentage bone loss was more homogeneous among the defects (D1 20%, D2 26 to 42%, and D3 32–34%).

Changes in horizontal measurements from defect healing to 3 months after the regenerative intervention (T2-T3)

Since vertical gains did not occur in any of the three augmentation procedures, these values were not reported. In fact, in all groups, there was a mean loss in height ≥ 2 mm, which obliged us to report the horizontal changes at the levels 4 and 6 mm below the GM. In the three defects (D1, D2, and D3), the amount of tissue augmentation was greater in the BRG and CBG compared to the MBG groups, at both 4 and 6 mm levels, although these differences were not statistically significant.

The mean horizontal contour changes in millimeters (HC T2-T3) and mean percentages of regenerated contours are reported in Table 2 for the three defect sites. At the 4 mm level in the intermediate defect (D2), they were 0.57 ± 0.19 mm (14%) in the MBG; 1.26 ± 0.89 mm (27%) in the BRG; and 1.18 ± 0.51 mm (35%) in the CBG groups. At the 6 mm level, these changes were respectively for the MBG, BRG, and CBG groups 0.40 ± 1.19 mm (14%), 1.14 ± 1.67 mm (55%), and 1.05 ± 0.07 mm (48%).

The mean linear changes between T2 and T3 are also reported in Table 2 for the three defect sites. These values provide more comprehensive information regarding the changes that occured between T2 and T3 as they report on the mean horizontal changes that occur in the selected area of interest as opossed to the horizontal changes which report on values from a single sagittal slide. In D2, the mean changes for the MB, BRG, and CBG groups were respectively 0.11 ± 0.31, 1.01 ± 0.91, and 0.98 ± 0.49 mm. The pairwise analysis showed statistically significant differences between MBG-BRG and MBG-CBG in all three defect sites, while no differences were observed for the comparison BRG-CBG.

Comparisons between baseline and postregenerative horizontal and vertical measurements (T1-T3)

The mean horizontal contour changes in millimeters (HC T1-T3) are reported in Table 3 for the three defect sites. Positive values indicate that the values in T1 were greater than in T3 while negative values indicate the opposite. In D1, since the initial loss was less pronounced than in D2 and D3, the horizontal changes at T3 was closer to baseline values. At the 4 mm level, the horizontal measurements did not reach the baseline values in any of the three augmentation modalities although in the BRG, the differences between T1 and T3 were closer to the baseline values when compared to the MBG and CBG.

At the 4 mm level in the intermediate defect (D2) horizontal changes, values were 3.40 ± 0.63 mm in the MBG, 3.59 ± 2.27 mm in the BRG, and 2.17 ± 0.66 mm in the CBG groups. At the 6 mm level, these changes were

Table 2 Vertical and horizontal changes from T2 to T3 (n = 9)

	HC 4 mm	HC 6 mm	Mean changes	% Gain 4 mm	% Gain 6 mm
Defect 1 (D1)					
MBG	0.59 ± 0.69	0.75 ± 0.08	0.47 ± 0.34	19.21	43.35
BRG	1.76 ± 1.18	2.25 ± 1.37	0.79 ± 0.67	80.73	132.35
CBG	1.89 ± 1.60	1.75 ± 0.69	0.87 ± 0.69	43.75	114.37
p value (MBG-BRG/MBG-CBG/BRG-CBG)	0.586/0.323/0.590	0.152/0.229/0.736	0.049*/0.025*/0.546		
Defect 2 (D2)					
MBG	0.57 ± 0.19	0.54 ± 0.58	0.11 ± 0.31	14.14	14.36
BRG	1.26 ± 0.89	1.14 ± 1.67	1.01 ± 0.91	27.87	55.88
CBG	1.18 ± 0.51	1.05 ± 0.07	0.98 ± 0.49	35.22	28
p value (MBG-BRG/MBG-CBG/BRG-CBG)	0.166/0.246/0.896	0.397/0.492/0.934	0.001*/0.002*/0.833		
Defect 3 (D3)					
MBG	0.41 ± 1.18	0.40 ± 1.19	0.24 ± 0.72	6.3	11.97
BRG	1.18 ± 0.17	0.58 ± 1.29	1.04 ± 0.92	21.73	17.9
CBG	1.79 ± 0.55	1.68 ± 0.41	0.86 ± 0.56	35.51	48.13
p value (MBG-BRG/MBG-CBG/BRG-CBG)	0.358/0.098/0.458	0.848/0.172/0.276	0.026*/0.044*/0.507		

MBG membrane group, *BRG* bone replacement graft group, *CBG* combination group, *HC 4–6 T2-T3* horizontal changes 4 and 6 mm below the gingival margin from T2 to T3, *% Gain 4, 6 mm* percentage of gain from the initial loss

Table 3 Vertical and horizontal changes from T1 to T3 ($n = 9$)

	HC 4 mm	HC 6 mm	VC
Defect 1 (D1)			
MBG	2.48 ± 0.43	0.98 ± 1.50	2.97 ± 0.15
BRG	0.42 ± 0.74	−0.52 ± 1.1	2.15 ± 1.03
CBG	2.43 ± 1.20	−0.22 ± 1.74	2.51 ± 1.03
p value (MBG-BRG/MBG-CBG/BRG-CBG)	0.045*/0.955/0.031	0.219/0.397/0.614	0.169/0.500/0.370
Defect 2 (D2)			
MBG	3.4 ± 0.63	3.22 ± 1.81	2.91 ± 0.66
BRG	3.59 ± 2.27	0.57 ± 1.14	2.32 ± 1.90
CBG	2.17 ± 0.66	2.71 ± 0.05	2.16 ± 1.61
p value (MBG-BRG/MBG-CBG/BRG-CBG)	0.491/0.861/0.435	0.246/0.746/0.189	0.934/0.693/0.640
Defect 3 (D3)			
MBG	6.11 ± 2.23	2.94 ± 0.94	2.94 ± 1.56
BRG	1.72 ± 1.66	2.66 ± 0.73	2.48 ± 2.46
CBG	3.08 ± 2.3	1.81 ± 0.75	1.95 ± 0.36
p value (MBG-BRG/MBG-CBG/BRG-CBG)	0.074/0.147/0.520	0.730/0.157/0.312	0.753/0.456/0.713

MBG membrane group, *BRG* bone replacement graft group, *CBG* combination group, *HC 4–6 T1-T3* horizontal changes 4 and 6 mm below the gingival margin from T1 to T3, *VC T1-T3* vertical changes from T1 to T3

respectively for the MBG, BRG, and CBG groups 2.94 ± 0.94, 0.57 ± 1.14, and 2.71 ± 0.05 mm.

The vertical changes from T1 to T3 were very similar from the values obtained from T1 to T2 proving minimal vertical gains after the regenerative procedures without significant differences between the three interventions.

Discussion

The present experimental in vivo investigation measured the soft tissue contour changes occurring after tooth extraction and defect creation and then assessed the efficacy of three augmentation procedures to reconstruct the soft tissue contour. Extraction and defect creation caused a marked reduction in ridge height and width. Horizontal loss ranged from 30 to 60% at 4 mm below the GM and from 20 to 40% at 6 mm. The horizontal changes after the augmentation surgeries favored the bone replacement graft and the combination of bone replacement graft and membrane. The BRG recovered 42% of the loss that occurred after defect creation at 4 mm below the GM and 69% at 6 mm. The CBG recovered 37 and 63% at the 4 and 6 mm levels, respectively. In the MBG, theses values were of 13 and 22%.

The ridge collapse that occurred after defect creation is well beyond what may be expected after tooth extraction and therefore these chronified defects may resemble those that may be encountered in long-term edentulous patients. This must be taken into consideration when interpreting the partial recovery obtained with the different regenerative techniques.

The analysis of the mean changes in tissue contours in all defects (D1, D2, and D3 combined) before and after the augmentation surgeries showed significantly higher gains for the BRG and CBG when compared to the MBG (0.94, 0.90, and 0.27 mm, respectively). The lesser gains observed in the MBG can be explained by the lack of bone replacement graft which prevented space maintenance and appropriate clot stabilization. This effect was also due to the native collagen membrane utilized, which has scarce memory and tends to collapse over the surrounding tissues. On the other hand, this barrier membrane easily adapts to the contours provided by the bone replacement grafts when used as scaffolds [24, 25]. In similar defects in experimental studies, the use of scaffolds giving support to collagen barriers prevented the ridge collapse when compared with sham operated areas [26].

The lack of differences between the BRG and CBG groups could be explained by the proven slow bioabsorbability of DBBM [27, 28]. In this study, the bone replacement graft was able to prevent soft tissue collapse, irrespective of whether it was covered by a membrane or not.

Interestingly, the histologic findings reported on a separate manuscript [21] revealed that only in the largest non-contained defects the use of the bone replacement graft, either alone or in combination with the membrane, was significantly superior for restoring the dimensions of the ridge when compared to the use of a barrier membrane alone. In the smaller defects, however, differences among the three groups were not statistically significant. Moreover, despite of the differences in the regenerative potential of the three defects, it was found that

larger proportions of mineralized tissue were obtained in the groups where a resorbable membrane was used what clearly highlights the need of barrier membranes for bone regenerative procedures with particulate bone substitutes.

Clinical studies have shown that the combination of resorbable membranes and particulate bone grafts was able to predictably reconstruct the alveolar ridge in single tooth defects and achieve esthetically pleasing tissue contours that remained stable at the 6-year follow-up [29]. The present investigation dealt with large non-contained defects with wide mesio-distal edentulous spaces, which probably compared better to defects after extraction of multiple teeth, rather than single tooth anterior defects. Although the objective of the regenerative interventions was not to achieve vertical growth but to attempt horizontal augmentation solely, complete horizontal reconstruction at 4 and 6 mm below the GM was rarely achieved. This partial reconstruction of the alveolar ridge was probably due to the use of non-space maintaining membranes. Space-containing non-resorbable membranes have demonstrated in clinical and experimental studies their ability to attain both vertical and horizontal regenerations [30–32], although their use may lead to more postoperative and soft tissue complications, mainly if the membranes become exposed during healing [33].

Recently published reviews have concluded that the use of space maintaining non-resorbable membranes or devices such as titanium meshes may be needed when the aim is to regenerate vertical defects or horizontally defects with a significant collapse [34]. The use of ridge preservation techniques has proven to minimize the dimensional changes that occur after tooth extraction and may be considered clinically to avoid more advanced regenerative therapies and the complications associated with them [35, 36].

In terms of methodology, the modified healed defect model used in this pre-clinical study is a validated experimental mode to test alveolar ridge regenerative interventions [37, 38]. Similarly, the methodology used to assess the changes in tissue contours is a well-established method to investigate the impact of different regenerative therapies in mucosal contours [39–41]. In fact, soft tissue assessment by means of optical scanning of dental impressions in combination with image analysis software is the method of choice for the three dimensional assessment of the soft tissue changes after implant placement and augmentation procedures [23, 42]. This analysis, however, focuses on the soft tissue changes, which does not allow drawing inferences on the interplay with the hard tissues changes, which may impact tissue thickness [43].

In fact, although the histologic analysis reporting on the hard tissue behavior and soft tissue contour changes appear to indicate similar findings, one of the limitations of the present investigation is the inability to correlate the hard tissue findings with soft tissue contour changes and soft tissue anatomy. Future research should focus in the development of methodology that can better understand the soft and hard tissue interplay.

The high variability observed in the horizontal measurements is in line with other pre-clinical investigations utilizing similar methodology [44]. Similarly, human clinical studies, utilizing comparable image analysis technology, have also reported high variability when evaluating the changes that occur in ridge contours after ridge augmentation procedures with autologous block grafts in the maxillary anterior region [17, 45]. In this study, the changes in tissue contours were evaluated before grafting, before implant placement, before abutment connection, at crown placement, and 1 and 5 years later. The authors observed a marked increase in ridge width after the augmentation procedure and after crown insertion, which clearly indicated that the implant supported restorations, had a significant influence on the final horizontal ridge contours. In this investigation, only the changes before and after different bone augmentation procedures were assessed without evaluating the impact of dental implants or restorations, what makes the comparisons with the previously mentioned studies impossible. Moreover, in light of the inherent limitations with the present animal model resulting in defects of different sizes from mesial to distal and on marked changes after the healing period, the obtained results should not be fully extrapolated to clinical situations. Our randomized experimental design, however, assured that all treatment strategies were equally distributed in the different sites. Furthermore, the data extracted from the image analysis was stratified according to the different defects locations to allow for site-specific comparisons. The methodology used for image analysis has shown a high reproducibility and excellent accuracy for measuring contour changes in a methodological study [46]. This method has been widely used in a variety of clinical and experimental investigations proving to be a non-invasive and reliable technique to assess changes in soft tissue after reconstructive therapy [47–49].

Conclusion

The gains in horizontal tissue contours achieved by bone replacement grafts or by the combination of bone replacement grafts with resorbable collagen membranes were superior to the membrane group alone, although none of the treatment strategies were able to completely restore the ridge width to baseline values before tooth extraction. Additional therapy may be required if the goal is to completely reestablish the alveolar tissue contours.

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Compliance with ethical standards This investigation was conducted according to Spanish and European Union regulations (European Communities Council Directive 86/609/EEC) on experimental in vivo experimentation.

Conflict of interest Dr. Sanz-Martín, Dr. Ferrantino, Dr. Vignolettu, Dr. Nuñez, Dr. Baldini, and Dr. Duvina report no conflict of interest. Dr. Sanz reports to have received research grants through the University Complutense of Madrid and lecture fees from Geistlich Pharma.

Ethical approval The study protocol was approved by the Ethical Committee of the Rof Codina Foundation (Lugo, Spain) (Ref AE-LU-001/12/INVMED (02)/Outros/04).

Informed consent No informed consent was obtained since the present was an animal study.

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