CLINICAL ORAL IMPLANTS RESEARCH

Sergio Spinato
Pablo Galindo-Moreno
Davide Zaffe
Fabio Bernardello
Carlo M Soardi

Is socket healing conditioned by buccal plate thickness? A clinical and histologic study 4 months after mineralized human bone allografting

Authors' affiliations:
Sergio Spinato, Private Practice, Sassuolo, Italy
Pablo Galindo-Moreno, Department of Oral Surgery and Implant Dentistry, School of Dentistry, University of Granada, Granada, Spain
Davide Zaffe, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy
Fabio Bernardello, Private Practice, Legnago, Italy
Carlo M Soardi, Private Practice, Brescia, Italy

Corresponding author:
Davide Zaffe
Dipartimento di Scienze Biomediche, Metaboliche e Neuroscienze, Sezione di Anatomia e Istologia
Università di Modena e Reggio Emilia
Via del pozzo 71, Policlinico, 41124 Modena MO, Italy
Tel.:+39 0594224804
Fax: +39 0594224861
e-mail: davide.zaffe@unimore.it

Date:
Accepted 8 October 2012

To cite this article:
Spinato S, Galindo-Moreno P, Zaffe D, Bernardello F, Soardi. C M. Is socket healing conditioned by buccal plate thickness? A clinical and histologic study 4 months after mineralized human bone allografting.
Clin. Oral Impl. Res. **00** 2012, 1-7
doi: 10.1111/clr.12073

Key words: allograft, bone graft, bone regeneration, histomorphometry, microradiography, socket

Abstract

Objective: The aim of this study was to clinically and histologically analyze the healing of grafted sockets by mineralized human bone allograft (MHBA) and nongrafted sockets, correlating the results with buccal plate thickness.

Material and methods: Thirty-one sockets were randomly split into control (CG) and treatment (MHBA grafted) (TG) groups and, subsequently, into four subgroups according to buccal plate thickness: a $\leq$ 1 mm and b > 1 mm. Ridge thickness, depth, and height were monitored. Four months after, at implant placement, a bone core biopsy for histologic and morphometric analyses was taken.

Results: The differences of buccal height (TG-a -0.27 and CG-a -1.17 mm) and width (TG-a 0.55 and CG-a 2.67 mm, TG-b 0.12 and CG-b 1.17 mm) were statistically significant. The increase in bone amount CG-b (28.17%) compared with CG-a (16.98%) was statistically significant. Soft tissue amount of TG-b (54.21%) and TG-a (56.91%) was lower than that of CG-b (71.83%) and CG-a (83.01%), both being statistically significant (P = 0.002).

Conclusions: The results proved that thin buccal plates had a worse outcome on socket healing and that network formation by MBHA not only predisposes a successful implant insertion but also acts as size keeper.

A recent meta-analysis on socket preservation therapies (Ten Heggeler et al. 2011) emphasized the scarcity of data on socket preservation therapies in humans. These authors concluded that socket preservation techniques do not prevent bone resorption but may reduce bone resorption with consequent superior results to those recorded in cases of natural healing.

Several studies on socket healing have been performed both in dogs (Cardaropoli et al. 2005; Fickl et al. 2008a,b, 2009a,b; Araújo et al. 2009, 2010; Vignoletti et al. 2012) and humans (Carmagnola et al. 2003; Norton et al. 2003; Nevins et al. 2006; Wang & Tsao 2007, 2008; Cardaropoli & Cardaropoli 2008; Serino et al. 2008; Trombelli et al. 2008; Pelegrine et al. 2010; Heberer et al. 2011; Neiva et al. 2011) in the last 10 years.

Studies on bone dynamics in human sockets adopting preservation techniques (Trombelli et al. 2008) revealed a great variability in hard tissue regeneration processes in humans. However, different mean new bone amounts (from 42% to 56%) recorded with natural healing of human sockets by other authors (Carmagnola et al. 2003; Serino et al. 2008; Pelegrine et al. 2010; Heberer et al. 2011; Neiva et al. 2011) do not support this result. The great amount (from 42% to 56%) seems to be at odds with the amounts of bone loss (Ten Heggeler et al. 2011) recorded in the majority of socket preservation case studies.

Severe postextraction horizontal bone loss is observable, predominantly involving the buccal aspect (30%) rather than the lingual (10%) aspect (Cardaropoli et al. 2003; Araújo & Lindhe 2005). Several investigations were performed to establish the real dimension of the resorption. Ten Heggeler et al. 2011 reports ridge reductions of 2.6–4.5 mm in width and 0.4–3.9 mm in height in naturally healed sockets. These reductions complicate ideal implant positioning and predictable esthetic outcomes, particularly in anterior areas (Buser et al. 2008).

Postextractive socket grafts have been performed with several materials, including autologous bone marrow (Pelegrine et al. 2010), PLA-PGA sponge (Serino et al. 2008), and mineralized human bone allograft (Wang & Tsao 2008; Fotek et al. 2009), but normally, bovine bone mineral is the material most frequently reported in the literature (Carmagnola et al. 2003; Norton et al. 2003; Nevins et al. 2006; Cardaropoli & Cardaropoli 2008; Fickl et al. 2008a,b; Heberer et al. 2008, 2011; Araújo et al. 2009, 2010). Some of the above were criticized by Ten Heggeler et al. (2011) because they failed to compile clinical records thus introducing bias into the scientific and clinical literature. In addition and in particular, the above authors found very different bone volumes (from 14% to 45%) and residual graft amounts (11–25%) in studies with bovine bone mineral substitutes after 4–6 months. The result of the disappearance of bovine bone mineral particles after very short periods seemed to be in contrast with the results of other authors who documented persistence of the particles after longer periods (Oonishi et al. 2000; Proussaefs et al. 2002; Baldissera et al. 2007; Traini et al. 2007; Galindo-Moreno et al. 2012).

The aim of this work was to perform a clinical and histomorphometric study comparing natural socket healing with healing after grafting with mineralized human bone allograft and to consider the effects of buccal plate thickness.

Material and methods

The patients in this study were recruited, examined, and treated in three private dental offices, using strict inclusion and exclusion criteria.

The inclusion criteria were as follows: good oral hygiene and motivation defined as a full-mouth plaque score of 25% or lesser (O'Leary et al.1972); one single-rooted tooth with a minimum of 10-mm bony support requiring extraction and replacement with an implant; minimally atraumatic extraction; no flap elevation; and natural teeth next to the site.

The exclusion criteria were as follows: smoking habits, active infection, absence of keratinized mucosa, and lack of occlusal contacts with the opposing dentition. Additional exclusion criteria were the presence of diseases that affect bone metabolism or wound healing, a history of head and neck radiation therapy, regular medicinal consumption of steroids, tetracycline, bisphosphonate or other medication affecting bone turnover, and patient pregnancy at any time during the study.

All patients signed informed consent in which all study procedures were detailed, according to 2008 Helsinki protocols and ethical requirements (Puri et al. 2009).

Presurgical procedures

Full-arch alginate impressions of all to patients were taken to make study casts. These were used to fabricate occlusal templates that permit reproducible measurements of the clinical alveolar crest dimensions.

Surgery

A suitable software (Random Allocation Software – free software for research purpose, http://mahmoodsaghaei.tripod.com/Softwares/randalloc.html) was used to allocate teeth randomly to each study group, grafted group (test group) and nongrafted group (control group).

Under local anesthesia (Articain hydrochloride; Ultracain, Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany), the selected teeth were carefully extracted to maintain the alveolar socket walls and gingival architecture (Fig. 1). In accordance with the inclusion criteria, no flap was opened. All four bony walls of the socket should exist and remain intact, thus being a type I socket according to Elian et al. (2007). Granulation tissue was removed when found.

Using a periodontal probe (CP UNC15; Hu-Friedy, Chicago, IL, USA), the following measurements (Fig. 1) were recorded and rounded to the nearest millimeter:

- Depth of the socket, facial, and palatal measured from the most apical point of the socket to the most apical point on the corresponding alveolar crest;
- Height of the buccal and palatal cortical measured at the mesio-distal midpoint of the adjacent teeth using a horizontal reference line marked with a periodontal probe connecting the mid-facial cement-enamel junction of the adjacent teeth.

Using a sharp caliper (Boley gauge caliper; Hu-Friedy), measurements were taken (Fig. 1):

- Bucco-lingual ridge width at the mesio-distal midpoint between adjacent teeth at 3 mm apically from the ridge crest;
- Buccal plate thickness 3 mm apically from the ridge crest to the center of the mesio-distal aspect of the socket.

After a minimal detachment of the keratinized gingiva using the periodontal probe, the bone crest was located, and 3 mm below this point, the buccal bone width was measured with the bone caliper. Because the flap was not reflected, the points of the caliper were pierced through the soft tissue until they contacted the bone.

Totally, six measurements were taken at baseline (immediately after the extraction of the tooth), and consequently, three of which (height of the buccal and palatal cortices and bucco-lingual ridge width) were taken 4 months later at re-entry.

In grafted sites (test group – TG), after copious irrigation, particles of 0.25–1 mm of cancellous mineralized human bone allograft (MHBA – Puros; Zimmer Dental Inc, Carlsbad, CA, USA), a material exhibiting very satisfactory outcomes (Soardi et al. 2011), were grafted in the selected sites. The socket was gently filled to the crest of the ridge (Fig. 1), and a bioabsorbable collagen wound dressing (Collaplug; Zimmer Dental Inc) was placed on the top of the graft and secured

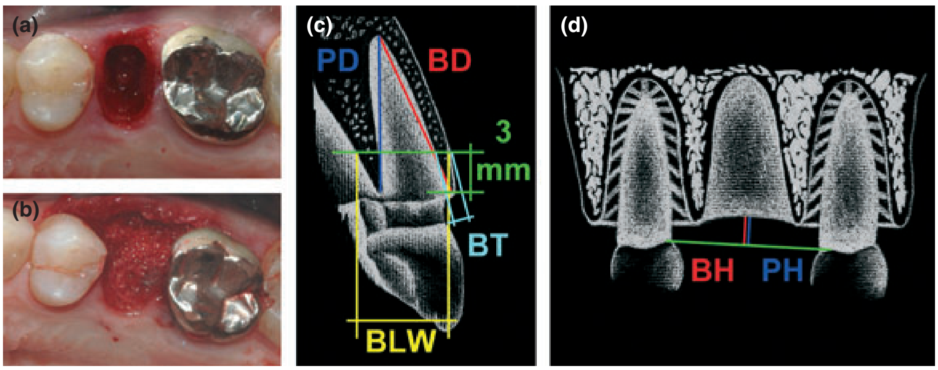

Fig. 1. Clinical photographs show socket after atraumatic extraction (a) and filled with mineralized human bone allograft (b). Schematic drawings (c and d) show the socket measurements. BD = buccal socket depth, measured from the most apical point of socket to the most apical point on the corresponding alveolar crest. BH = buccal cortical height, measured at mesio-distal midpoint of adjacent teeth. BLW = bucco-lingual width at mesio-distal midpoint between the adjacent teeth at 3mm apically from ridge crest. BT = buccal plate thickness at 3 mm apically from ridge crest. PD = palatal socket depth, measured as BD. PH = palatal cortical height, measured as BH.

with 4–0 absorbable sutures (Vicryl; Ethicon, Johnson & Johnson, New Brunswick, NJ, USA) using a cross-mattress technique. No primary wound closure was performed according to Wang et al. (2004, 2007, 2008).

In nongrafted sites (control group – CG), after the clinician ensured that a blood clot had formed, a cross-mattress suture (Vicryl; Ethicon) was performed, and the socket was allowed to heal without primary wound closure. An adhesive provisional restoration fixed to the adjacent teeth was utilized for all patients. The provisional restoration did not push the gingival mucosa to avoid compression of the soft and hard tissues.

Amoxicillin (Ratiopharm GmbH, Ulm, Germany), 1 g, twice a day for 7 days and, if needed, Synflex forte 550 mg (Recordati SpA, Milano, Italy) as analgesic were recommended after surgery. Patients were directed to use a chlorhexidine mouthwash (0.12%), twice a day, and to not brush the surgical sites for 2 weeks. Sutures were removed 10 days after the surgery. Monthly follow-up was scheduled to check possible wound dehiscence at the extraction site.

After four months, at implant placement appointment, a flap was elevated, three clinical measurements (height of the buccal and palatal cortices and bucco-lingual ridge width) were repeated, and a bone biopsy was taken from the control and test sites using a trephine. A 3-mm (external)-diameter trephine, under a saline irrigation was utilized at 600 rpm (up to 10 mm) to collect bone core specimens before implant, insertion (Tapered Screw Vent; Zimmer Dental Inc). Bone core biopsies were harvested from the area matching the center of the previous extraction socket, in the indicated area to place the implant, according to prosthetic requirements and using surgical guides designed for that purpose. Implant sites were accurately planned to exactly place implants in purely augmented skeletal segments, possibly including the residual crest. The retrieved bone core samples were immediately washed in sterile saline, gently wiped, labeled on the coronal side by China ink-pad blotting (17 Black; Pelikan Italia S.p.A., Milano, Italy) and then fixed for the histological study.

Histology

The cylindrical bone samples were fixed in 4% paraformaldehyde (all reagents from Fluka, Sigma-Aldrich Schweiz, Buchs SG, Switzerland) in 0.1 M phosphate buffer pH 7.2 for 4 h at room temperature. Specimens were dehydrated through ethanol series at 4°C and subsequently embedded in methyl methacrylate (PMMA) using a water bath at 4°C as described elsewhere (Bertoldi et al. 2008).

The PMMA blocks were serially sectioned to obtain two sets of sections: thick (100-μ thick) and thin (5-μ thick) sections.

First, each PMMA block was serially sectioned along the longitudinal axis of the cylindrical bone sample to its center using a diamond saw microtome (SP1600; Leica Microsystem, Nußloch, Germany).

A thick section (200 μm) was obtained from the center of cylindrical sample using the diamond saw microtome. This section was reduced to 100 μm by grinding, perfectly polished with emery paper and alumina and then X-ray microradiographed (3K5; Italstructures, Riva del Garda TN, Italy) at 15 kV and 10 mA on high-resolution film (SO 343; Eastman Kodak Co, Rochester, NY, USA).

A set of 5-μ thick sections was obtained starting from the level at which the thick section was performed, using a tungsten carbide knife (Profile D) on a bone microtome (Autocut 1150; Reichert-Jung GmbH, Nußloch, Germany). Thin sections were stained with toluidine blue or trichrome Gomori stain. Some slides were stained with total alkaline phosphatase (TAP) histochemical method, a marker of osteogenesis (azo dye = fast blue BB), or tartrate resistant acid phosphatase (TRAP) histochemical method, a marker of osteoclasts (azo dye = Fast Red RC) (Zaffe & D'Avenia 2007).

The microradiographs and the sections were analyzed and photographed using a microscope (Axiophot; Carl Zeiss AG, Oberkochen, Germany) under ordinary light. The amount of bone tissue (bone volume–BV), graft (graft volume–GV) per tissue volume (BV/TV and GV/TV, respectively), were evaluated on microradiographs using a suitable program for the image analyzer and software (AnalySIS®; Soft Imaging System GmbH, Münster, Germany).

Statistical analysis

Comparisons were performed using the nonparametric Mann–Whitney rank-sum test, Kruskal–Wallis followed by the Dunn multiple comparison test (Glantz 2003). The null hypothesis H0 was rejected for a critical significance level of $P < 0.05$.

Results

Surgery

Thirty-one selected patients, 10 men and 21 women, aged between 27 and 74, mean = 48.5 years, were enrolled in this study. A total of 31 hopeless anterior maxilla teeth (10 incisors, 3 canines, 18 bicuspids) were extracted, one per patient. Reasons for extraction were endodontic problems (n = 2), root fractures (n = 8), untreatable caries (n = 12) and advanced periodontitis (n = 9). Teeth with apical lesions were excluded.

Nineteen sockets, test group (TG), received mineralized human bone allograft (MHBA), whereas 12 sockets, control group (CG), healed naturally. Patients of the two groups were categorized in relation to the thickness of the buccal bone plate (Tomasi et al. 2010): (a) width ≤ 1 mm (thin), 17 patients, and (b) width >1 mm (thick), 14 patients. In the TG, 11 grafted sockets had a thin buccal plate (TG-a), whereas eight had a thick buccal plate (TG-b). In the CG, six ungrafted sockets had a thin buccal plate (CG-a), whereas six presented a thick buccal wall (CG-b).

All extraction sockets healed uneventfully. No signs of graft material loss were observed in any socket during healing.

At implant placement time of 4 months after extraction, after osteotomy preparation, all 31 sockets achieved primary (mechanical) implant stability and the implants were placed successfully.

A total of 31 core biopsies, one per patient, and a total of 31 implants were placed in those sites according to individual implant-prosthetic treatment plans.

Histology

Four months after the surgery, the biopsies of sockets of the control group showed presence of newly formed bony trabeculae surrounded by fibrous tissue (Fig. 2). The trabeculae formed a network of greatly anastomosed narrow trabeculae, which partially filled the socket linked to the alveolar wall. The common structure of the newly formed bony trabeculae, all containing irregularly shaped osteocytes, was the woven structure (Fig. 3). As a rule, the soft tissue resulted was rich in collagen fibers in the coronal half, but looser and with few fibers in the apical half (Fig. 3). Active osteoblasts were rarely found on the surface of the bone trabeculae, which resulted into mostly covered by lining bone cells. TAP resulted unexpressed in CG biopsies. Very few TRAP–positive osteoclasts were observed in CG biopsies, but some mononucleated cells (probably pre-osteoclasts) were revealed using the TRAP in a few medullary spaces.

Four months after surgery, the biopsies of grafted sockets (TG) showed the disappearance of MHBA particles in the apical half

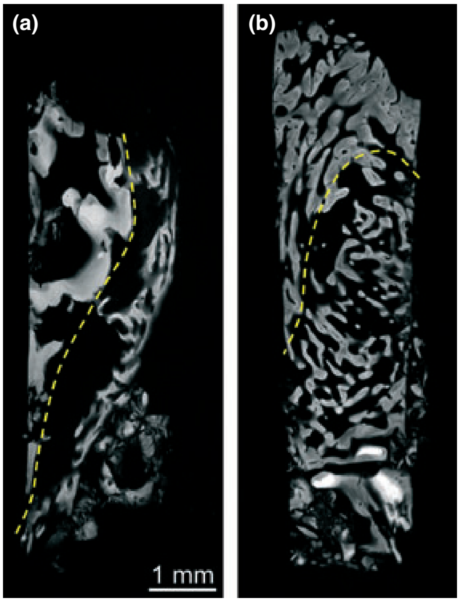

Fig. 2. Representative image showing two microradiographs of thick section of bone sample of a naturally healed socket (a) control group – CG, and a socket augmented by mineralized human bone allograft – MHBA (b) four months after surgery. The superimposed broken line marks the alveolar bone wall. Note in (b) how the graft (lighter than the bone) is noticeable only in the coronal half. The newly formed trabeculae have the typical aspect of the tissue with woven structure.

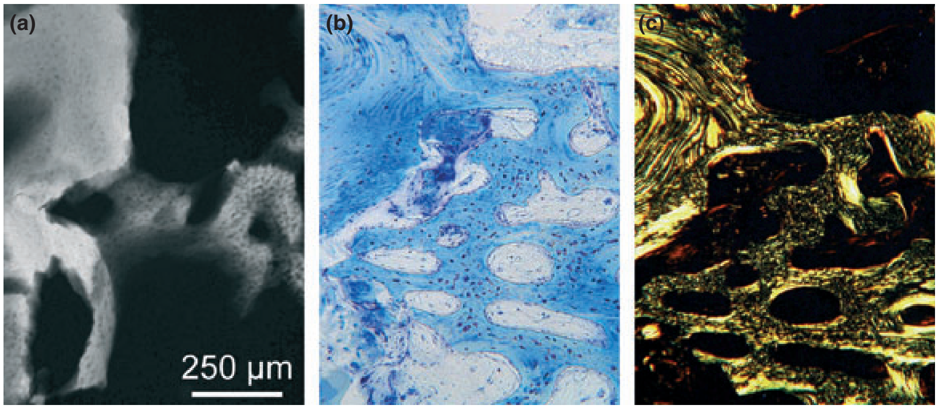

Fig. 3. Images show the biopsy of the same field of newly formed bone (alveolar wall on the left) of a naturally healed socket (control group), 4 months after surgery. Microradiograph of the thick section (a) and toluidine blue staining (b) under ordinary light, and trichrome Gomori stain, under polarized light (c) of the seriated thin section of the biopsy. Note in (b) the different cellularity of the basal bone, with few, ordered and ellipsoidal osteocytes, and the newly formed bone, with so many, disordered and irregularly shaped osteocytes. Note in (c) how the basal bone has a lamellar structure, whereas the newly formed has a woven one.

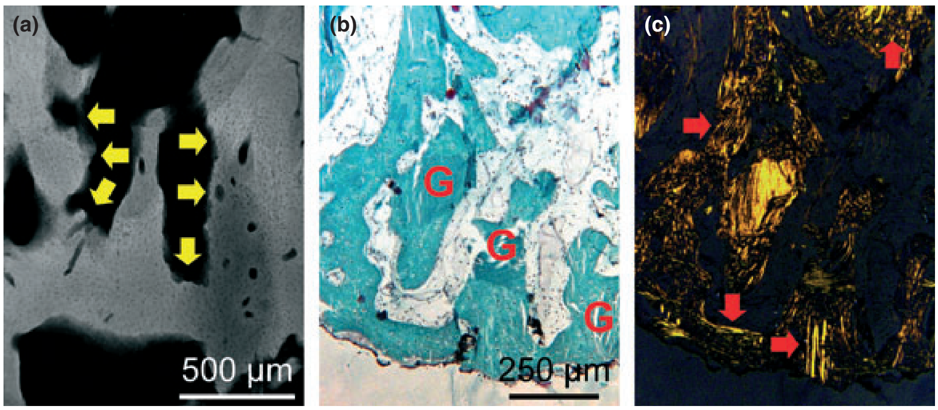

Fig. 4. Images show the coronal end (oral cavity on the bottom) of biopsies of a mineralized human bone allograft (MHBA) grafted socket (test group) 4 months after surgery. Microradiograph of a thick section (a) and trichrome Gomori stain (b, ordinary light – c, polarized light) of a thin section of the biopsy. The yellow arrows in (a) point to indented surfaces of the bone, eroded by osteoclasts. Note in (b) the black residues of the China ink labeling. The MHBA particles (G) appear almost completely surrounded by the newly formed bone (b) which has a prevailing woven structure (c) but some parts of the bone become lamellar (red arrows).

and a composite formed by MHBA particles and newly formed bone trabeculae in the coronal half (Fig. 2). The majority of MHBA particles connected directly with the newly formed bone but with very few particles being totally surrounded by fibrous tissue. A network of newly formed bony trabeculae without MHBA particles (Fig. 2) was the common finding in the apical half. The common structure of the newly formed bony trabeculae was the woven structure, but some parts of them displayed a more regular structure, parallel-fibered or lamellar, as in the coronal part. In the latter, woven bone commonly enveloped MHBA particles (Fig. 4) but parallel-fibered or lamellar bone portions, both with ellipsoidal osteocytes, also could be found to form parts of the trabeculae (Figs 4 and 5). Bone or particle surfaces covered by layered cuboidal osteoblasts, that is, active bone-forming cells, were not unusual, particularly in the tissue of the coronal end. Only the coronal end showed an appreciable TAP-positive expression. However, this decreased from the coronal to the apex (Fig . 5). Erosion surfaces were easily found on both MHBA particles and the enveloping bone (Fig. 4) because TRAP-positive (Fig. 5) osteoclasts preferably resorbed the newly formed bone, even if they do not disdain MHBA particles.

Quantitative evaluations and statistics

Clinical evaluations

No significant differences in height or width, between implant insertion/surgery were found within groups (Table 1), excluding the width reduction of CG-a (8.17–5.5 mm, $P = 0.025$). Naturally healed sockets lost slightly more palatal height than treated sockets in both buccal plate thicknesses (BPT) ≤ 1 and >1 mm, but without statistical significance (Table 1).

However, the differences of buccal height of BPT ≤ 1 mm (TG 0.27 and CG 1.17 mm), and width of both BPT (≤ 1 – TG 0.55 and CG 2.67 mm, >1 – TG 0.12 and CG 1.17 mm) were statistically significant (Table 2), excluding the buccal height in BPT >1 mm (TG -0.37 and CG -0.5 mm).

Histometry

The increase of the bone amount (BV/TV) of CG-b sockets (28.17%) respect that of CG-a (16.98%), and as a consequence, the comparison of the CG-b/CG-a soft tissue amount was statistically significant ($P = 0.004$) using Mann–Whitney test (Table 3). On the contrary, no significant BV/TV differences of TG-b (23.3%) and TG-a (18.84%) with the corresponding CG were found (Table 3).

The graft residual amount (GV/TV) in TG-b (21.96%) and TG-a (23.34), both similar (Table 3), gave rise to a soft tissue amount of TG (TG-b 54.21 and TG-a 58.52%) lower than that of the CG (CG-b 71.83 and CG-a 83.01%).

Ten of 31 biopsies contained the basal bone (Fig. 1), allowing us the evaluation of its

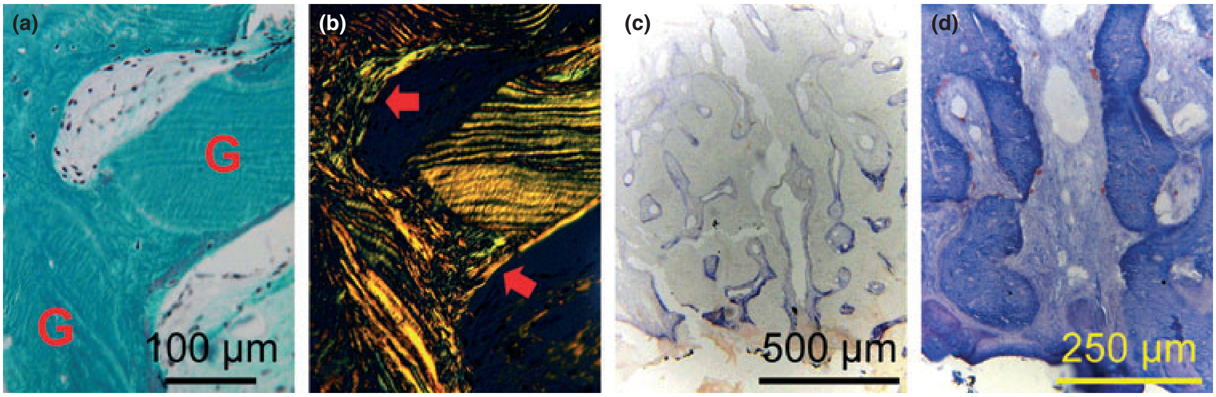

Fig. 5. Images show the internal (a and b) and the external end (c and d, oral cavity on the bottom) of the coronal half of biopsies of a mineralized human bone allograft (MHBA) grafted socket (test group) 4 months after surgery. Trichrome Gomori stain (a, ordinary light – b, polarized light), total alkaline phosphatase (c – TAP), and tartrate-resistant acid phosphatase (d – TRAP) treated section of a thin section of the biopsy. The red arrows in (b) point to the newly formed lamellar bone, containing ellipsoidal osteocytes, in apposition to the woven bone forming the network with the MHBA particles (G). Note in (c) the black residues of the China ink labeling, and how the TAP expression inward greatly decreases. TRAP-positive osteoclasts (red in d) are mostly found on both newly formed bone and MHBA surfaces.

***Table 1.* Height and width at surgery and implant insertion**

BPT			Surgery	Implant insertion	P
≤1 mm	CG-a	B-H	2.00 ± 0.89	3.5 ± 1.22	0.06
		P-H	1.83 ± 0.75	2.83 ± 0.75	0.07
		W	8.17 ± 1.72	5.50 ± 1.52	0.025*
	TG-a	B-H	2.27 ± 1.0	2.55 ± 1.29	0.67
		P-H	1.91 ± 0.70	2.46 ± 1.04	0.24
		W	7.18 ± 1.47	6.64 ± 1.63	0.41
>1 mm	CG-b	B-H	1.67 ± 0.52	2.17 ± 2.17	0.26
		P-H	1.50 ± 0.55	2.00 ± 0.89	0.34
		W	8.33 ± 1.36	7.17 ± 1.60	0.20
	TG-b	B-H	2.63 ± 0.7	3.00 ± 1.19	0.53
		P-H	2.38 ± 0.92	3.25 ± 1.39	0.17
		W	7.13 ± 0.83	7.00 ± 0.92	0.75

Data (mean ± SD) are expressed as mm.
BPT, buccal plate thickness; P, Probability using Mann–Whitney test; CG, control group; TG, test group; B-H, buccal height; P-H, palatal height; W, width.
*Statistically significant.

***Table 2.* Height and width differences between implant insertion and surgery**

BPT		CG	TG	P
≤1 mm	B-H	−1.17 ± 0.40	−0.27 ± 0.47	0.049*
	P-H	−1.00 ± 0.63	−0.55 ± 0.52	0.225
	W	2.67 ± 0.52	0.55 ± 0.68	0.002**
>1 mm	B-H	−0.50 ± 0.55	−0.38 ± 0.74	0.56
	P-H	−0.50 ± 0.55	−0.88 ± 0.83	0.44
	W	1.17 ± 0.41	0.125 ± 0.35	0.006**

Data (m ± SD) are expressed as mm.
BPT, buccal plate thickness; CG, control group; TG, test group; P, probability using Mann–Whitney test; B-H, buccal height; P-H, palatal height; W, width.
*Statistically significant.
**Statistically very significant.

***Table 3.* Bone and graft amount in control and test group**

		a(BPT < 1)	b(BPT < 1)	P
Bone	CG	16.98 ± 4.36	28.17 ± 3.91	0.004*
	TG	18.14 ± 16.91	23.82 ± 13.13	0.26
Graft	TG	23.34 ± 13.16	21.96 ± 14.44	0.71

Data (mean ± SD) are expressed as%.
P, probability using Mann–Whitney test; CG, control group; TG, test group.
*Statistically very significant.

***Table 4.* Soft tissue amount in control and test group, and basal bone**

			Dunn test
BPT ≤ 1 mm	CG-a	83.01 ± 4.36	*
	TG-a	58.52 ± 12.88	–
BPT > 1 mm	CG_b	71.83 ± 3.91	*
	TG-b	54.21 ± 8.25	–
	BB	44.73 ± 10.54	

Data (mean ± SD) are expressed as%.
Kruskal–Wallis test: $P < 0.001$.
CG, control group; TG, test group; BB, basal bone.
*Statistically significant using Kruskal–Wallis test followed by Dunn multiple comparison test ($P < 0.05$, BB = reference group).

mean BV/TV (55.27%) and soft tissue amount (44.73%), the latter statistically different from that of CG-a and CG-b but not from that of TG-a and TG-b (Table 4), using the Kruskal–Wallis, followed by the Dunn test.

Discussion

Many studies on ridge alterations showed that buccal plate resorption may have been devastating aesthetic outcomes for implants (Cardaropoli et al. 2003; Araújo & Lindhe 2005). Precisely, thickness of the buccal plate seems to have a significant influence on the amount of horizontal and vertical crest resorption in human sockets (Ferrus et al. 2010; Tomasi et al. 2010).

The results of this comparative study demonstrated that both crestal height and width bone loss after extraction of maxillary anterior teeth was significantly reduced by the placement of MHBA. In the CG with thin buccal plate (≤1 mm), naturally healed and treated sockets lost comparable palatal height over the 4-month period. This could be a consequence of a negligible impact on the vertical resorption at the palatal site by a thin buccal plate. On the contrary, sockets of TG (MHBA grafted) showed less buccal height and width bone loss than CG sockets, both with statistically significant differences.

In agreement with Nevins et al. (2006), it would seem appropriate to place an osteoconductive substance into the extraction socket of teeth with a thin buccal plate to avoid bone loss and the resulting compromises in implant placement. Within thick buccal plate groups (>1 mm), our results showed unaltered crestal height loss after MHBA grafting of sockets. On the contrary, grafted sockets lost less width than naturally healed sockets in those groups, and the difference was statistically significant over the 4-month period. These findings seemed to suggest that the placement of a graft into the extraction socket of teeth with a thick buccal plate significantly decreased the horizontal resorption of hard tissue ridge but did not reduce the vertical change.

Histomorphometric results highlight that the 28% of newly formed (woven) bone recorded in this study in nongrafted sockets with thick buccal plate is comparable to that reported by Trombelli et al. (2008). This amount was greatly different from that reported by others such as Carmagnola et al. (2003), Serino et al. (2008), Pelegrine et al. (2010), or Heberer et al. (2011), but these authors did not use polarized light to discriminate new (woven) bone from basal (lamellar) bone. Nevertheless, in nongrafted sockets, the bone amount of the "thick" group was statistically greater than that of the "thin" group, indicating that the thickness of the buccal bone plate markedly influenced new bone regeneration in human sockets 4 months after extraction.

The amount of residual graft (22–23%) was significantly lower than the limit (40%) set to obtain a successful implant outcome (Carmagnola et al. 2003). The amount of newly formed bone in grafted sockets was similar to that of CG-b sockets (in accordance with Heberer et al. (2011) and Norton et al. (2003)) probably indicating an upper limit for the formation of composite bone-MHBA in sockets grafted for 4 months. Nevertheless, the amount of mineralized material in grafted sockets was greater than 40% and similar to that of the basal bone, which has the same amount of nonmineralized tissue. The decreased horizontal and vertical resorption clinically recorded in sockets with thin buccal plate was probably because of the bone-MHBA network. The same effect, that is, the reduced amount of nonmineralized tissue, would be the reason of the limited horizontal resorption found in grafted sockets also in sites with thick buccal plate.

Conclusion

The results clearly demonstrated that a thin buccal bone plate showed a worse outcome on bony socket healing, and the formation of a bone-MBHA network not only predisposed a successful implant insertion outcome, but also acted as crestal size keeper.

Disclaimers

The authors do not have conflict of interests related to this study.

The study was self-funded.

References

Araújo, M.G., Liljenberg, B. & Lindhe, J. (2010) Dynamics of bio-oss collagen incorporation in fresh extraction wounds: an experimental study in the dog. *Clinical Oral Implants Research* **21**: 55–64.

Araújo, M.G., Linder, E. & Lindhe, J. (2009) Effect of a xenograft on early bone formation in extraction sockets: an experimental study in dog. *Journal of Clinical Oral Implants Research* **20**: 1–6.

Araújo, M.G. & Lindhe, J. (2005) Dimensional ridge alterations following tooth extraction an experimental study in the dog. *Journal of Clinical Periodontology* **32**: 212–218.

Baldissera, E.Z., Fontanella, V.R., Ito, W. & Pomar, F. (2007) Use of hydroxyapatite in tooth replantation radiographically followed up for 14 years: a case report. *Dental Traumatology* **23**: 47–50.

Bertoldi, C., Zaffe, D. & Consolo, U. (2008) Polylactide/polyglycolide copolymer in bone defect healing in humans. *Biomaterials* **29**: 1817–1823.

Buser, D., Chen, S.T., Weber, H.P. & Belser, U.C. (2008) Early implant placement following single-tooth extraction in the esthetic zone: biologic rationale and surgical procedures. *International Journal of Periodontics and Restorative Dentistry* **28**: 441–451.

Cardaropoli, G., Araújo, M., Hayacibara, R., Sukekava, F. & Lindhe, J. (2005) Healing of extraction sockets and surgically produced – augmented and non-augmented -defects in the alveolar ridge an experimental study in the dog. *Journal of Clinical Periodontology* **32**: 435–440.

Cardaropoli, G., Araújo, M. & Lindhe, J. (2003) Dynamics of bone tissue formation in tooth extraction sites an experimental study in dogs. *Journal of Clinical Periodontology* **30**: 809–818.

Cardaropoli, D. & Cardaropoli, G. (2008) Preservation of the postextraction alveolar ridge: a clinical and histologic study. *International Journal of Periodontics and Restorative Dentistry* **28**: 469–477.

Carmagnola, D., Adriaens, P. & Berglundh, T. (2003) Healing of human extraction sockets filled with bio-oss. *Clinical Oral Implants Research* **14**: 137–143.

Elian, N., Cho, S., Froum, S., Smith, R. & Tarnow, D. (2007) A simplified socket classification and repair techinque. *Practical Procedures & Aesthetic Dentistry* **19**: 99–104.

Ferrus, J., Cecchinato, D., Pietursson, E., Lang, N. P., Sanz, M. & Lindhe, J. (2010) Factors influencing ridge alterations following immediate implant placement into extraction socket. *Clinical Oral Implants Research* **21**: 22–29.

Fickl, S., Schneider, D., Zuhr, O., Hinze, M., Ender, A., Jung, R. & Hürzeler, M. (2009a) Dimensional changes of the ridge contour after socket preservation and buccal overbuilding: an animal study. *Journal of Clinical Periodontology* **36**: 442–448.

Fickl, S., Zuhr, O., Wachtel, H., Bolz, W. & Hürzeler, M. (2008a) Hard tissue alterations after socket preservation: an experimental study in the beagle dog. *Clinical Oral Implants Research* **19**: 1111–1118.

Fickl, S., Zuhr, O., Wachtel, H., Kebschull, M. & Hürzeler, M. (2009b) Hard tissue alterations after socket preservation with additional buccal overbuilding: a study in the beagle dog. *Journal of Clinical Periodontology* **36**: 898–904.

Fickl, S., Zuhr, O., Wachtel, H., Stappert, C., Stein, J. & Hürzeler, M. (2008b) Dimensional changes of the alveolar ridge contour after different socket preservation techniques. *Journal of Clinical Periodontology* **35**: 906–913.

Fotek, P.D., Neiva, R.F. & Wang, H.L. (2009) Comparison of dermal matrix and polytetrafluoroethylene membrane for socket bone augmentation: a clinical and histologic study. *Journal of Periodontology* **80**: 776–785.

Galindo-Moreno, P., Hernández-Cortes, P., Mesa, F., Carranza, N., Joudzbalys, G., Aguilar, M. & O'Valle, F. (2012) Slow resorption of anorganic bovine bone by osteoclast in maxillary sinus augmentation. *Clinical implant dentistry and related research*: DOI 10.1111/j.1708-8208.2012.00445.x.

Glantz, S.A. (ed.) (2003) Primer of Biostatistics. 5th edition. New York: Mc-Graw Hill.

Heberer, S., Al-Chawaf, B., Hildebrand, D., Nelson, J.J. & Nelson, K. (2008) Histomorphometric analysis of extraction sockets augmented with bio-oss collagen after a 6-week healing period: a prospective study. *Clinical Oral Implants Research* **19**: 1219–1225.

Heberer, S., Al-Chawaf, B., Jablonski, C., Nelson, J., Lage, H. & Nelson, K. (2011) Healing of ungrafted and grafted extraction sockets after 12 weeks: a prospective clinical study. *The International Journal of Oral & Maxillofacial Implants* **26**: 385–392.

Neiva, R., Pagni, G., Duarte, F., Park, C., Yi, E., Holman, L. & Giannobile, W. (2011) Analysis of tissue neogenesis in extraction sockets treated with guided bone regeneration: clinical, histologic, and micro-ct results. *International Journal of Periodontics and Restorative Dentistry* **31**: 457–469.

Nevins, M., Camelo, M., De Paoli, S., Friedland, B., Schenk, R., Parma-Benfenati, S., Simion, M., Tinti, C. & Wagenberg, B. (2006) A study of the fate of the buccal wall of extraction sockets of teeth with prominent roots. *International Journal of Periodontics and Restorative Dentistry* **26**: 19–29.

Norton, M., Odell, E., Thompson, I. & Cook, R.J. (2003) Efficacy of bovine bone mineral for alveolar augmentation: a human histologic study. *Clinical Oral Implants Research* **14**: 75–783.

O'Leary, T., Drake, R.B. & Naylor, J.E. (1972) The plaque control record. *Journal of Periodontology* **43**: 38.

Oonishi, H., Kadoya, Y., Iwaki, H. & Kin, N. (2000) Hydroxyapatite granules interposed at bone-cement interface in total hip replacements: histological study of retrieved specimens. *Journal of Biomedical Materials Research* **53**: 174–180.

Pelegrine, A., da Costa, C.E., Correa, M. & Marques, J.J. (2010) Clinical and histomorphometric evaluation of extraction sockets treated with an autologous bone marrow graft. *Clinical Oral Implants Research* **21**: 535–542.

Proussaefs, P., Lozada, J., Valencia, G. & Rohrer, M. (2002) Histologic evaluation of a hydroxyapatite onlay bone graft retrieved after 9 years: a clinical report. *Journal of Prosthetic Dentistry* **87**: 481–484.

Puri, K., Suresh, K., Gogtay, N. & Thatte, U. (2009) Declaration of helsinki, 2008: implications for stakeholders in research. *Journal of Postgraduate Medicine* **55**: 131–134.

Serino, G., Rao, W., Iezzi, G. & Piattelli, A. (2008) Polylactide and polyglycolide sponge used in human extraction sockets: bone formation following 3 months after its application. *Clinical Oral Implants Research* **19**: 26–31.

Soardi, C.M., Spinato, S., Zaffe, D. & Wang, H.L. (2011) Atrophic maxillary floor augmentation by mineralized human bone allograft in sinuses of different size: an histologic and histomorphometric analysis. *Clinical Oral Implants Research* **22**: 560–566.

Ten Heggeler, J.M., Slot, D.E. & Van derWeijden, G.A. (2011) Effect of socket preservation therapies following tooth extraction in non-molar regions in humans: a systematic review. *Clinical Oral Implants Research* **22**: 779–788.

Tomasi, C., Sanz, M., Cecchionato, D., Pjetursson, B., Ferrus, J., Lang, N.P. & Lindhe, J. (2010) Bone dimensional variations at implants placed in fresh extraction sockets: a multilevel multivariate analysis. *Clinical Oral Implants Research* **21**: 30–36.

Traini, T., Valentini, P., Iezzi, G. & Piattelli, A. (2007) A histologic and histomorphometric evaluation of anorganic bovine bone retrieved 9 years after a sinus augmentation procedure. *Journal of Periodontology* **78**: 955–961.

Trombelli, L., Farina, R., Marzola, A., Bozzi, L., Liljenberg, B. & Lindhe, J. (2008) Modeling and remodeling of human extraction sockets. *Journal of Clinical Periodontology* **35**: 630–639.

Vignoletti, F., Discepoli, N., Müller, A., de Sanctis, M., Muñoz, F. & Sanz, M. (2012) Bone modelling at fresh extraction sockets: Immediate implant placement versus spontaneous healing: an experimental study in the beagle dog. *Journal of Clinical Periodontology* **39**: 91–97.

Wang, H.L., Kiyonobu, K. & Neiva, R.F. (2004) Socket augmentation: rationale and technique. *Implant Dentistry* **13**: 286–296.

Wang, H.L. & Tsao, Y.P. (2007) Mineralized bone allograft-plug socket augmentation: rationale and technique. *Implant Dentistry* **16**: 33–41.

Wang, H. L. & Tsao, Y.P. (2008) Histologic evaluation of socket augmentation with mineralized human allograft. *International Journal of Periodontics and Restorative Dentistry* **28**: 231–237.

Zaffe, D. & D'Avenia, F. (2007) A novel bone scraper for intraoral harvesting: a device for filling small bone defects. *Clinical Oral Implants Research* **18**: 525–533.