CLINICAL ORAL IMPLANTS RESEARCH

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Bio-Oss® Collagen in the buccal gap at immediate implants: a 6-month study in the dog

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Key words: biomaterial, dogs, extraction socket, grafting, implants

Abstract

Background: Following tooth extraction and immediate implant installation, the edentulous site of the alveolar process undergoes substantial bone modeling and the ridge dimensions are reduced.

Objective: The objective of the present experiment was to determine whether the process of bone modeling following tooth extraction and immediate implant placement was influenced by the placement of a xenogenic graft in the void that occurred between the implant and the walls of the fresh extraction socket.

Material and methods: Five beagle dogs about 1 year old were used. The 4th premolar in both quadrants of the mandible ($_4P_4$) were selected and used as experimental sites. The premolars were hemi-sected and the distal roots removed and, subsequently, implants were inserted in the distal sockets. In one side of the jaw, the marginal buccal-approximal void that consistently occurred between the implant and the socket walls was grafted with Bio-Oss® Collagen while no grafting was performed in the contra-lateral sites. After 6 months of healing, biopsies from each experimental site were obtained and prepared for histological analyses.

Results: The outline of the marginal hard tissue of the control sites was markedly different from that of the grafted sites. Thus, while the buccal bone crest in the grafted sites was comparatively thick and located at or close to the SLA border, the corresponding crest at the control sites was thinner and located a varying distance below SLA border.

Conclusions: It was demonstrated that the placement of Bio-Oss® Collagen in the void between the implant and the buccal-approximal bone walls of fresh extraction sockets modified the process of hard tissue healing, provided additional amounts of hard tissue at the entrance of the previous socket and improved the level of marginal bone-to-implant contact.

Following tooth extraction, the edentulous site of the alveolar process will undergo both quantitative and qualitative changes (e.g., Pietrokovski & Massler 1967; Amler 1969; Schropp et al. 2003). Thus, during healing, the bundle bone will gradually disappear, the socket will be filled with granulation tissue, provisional matrix and woven bone that eventually will be replaced with trabecular bone and marrow (e.g., Amler 1969; Evian et al. 1982; Kuboki et al. 1988; Cardaropoli et al. 2003; Araújo & Lindhe 2005). Moreover, (i) the walls of the socket will be markedly reduced both with respect to height and width (Araújo et al. 2005, 2006) and (ii) the dimensional changes will be more pronounced in the buccal than in the lingual/

palatal compartments of the extraction site (Pietrokovski & Massler 1967; Pietrokovski et al. 2007). Although early case reports indicated that implants placed in fresh extraction sites may counteract post-extractive bone modeling, later studies in humans and experimental animals documented that such treatment would, in fact, not influence the tissue alterations described above (Botticelli et al. 2004; Araújo et al. 2005, 2006; Blanco et al. 2008; Evans & Chen 2008).

Different approaches have been advocated to preserve or improve the dimension and contour of the ridge following tooth extraction including the use of various graft or filler materials such as autografts, allografts, synthetic graft, etc. and/or barrier membranes (for a review, see Botticelli 2006). One particular graft material, comprised of deproteinized bovine bone mineral has been widely used in attempts to preserve the dimension of the alveolar ridge following tooth extraction (ridge preservation) as well as in angular bone defects at teeth and in sinus augmentation procedures (e.g., Hürzeler et al. 1997; Berglundh & Lindhe 1997; Piattelli et al. 1999; Artzi et al. 2000, 2002; Yildirim et al. 2000; Froum et al. 2002; Carmagnola et al. 2003; Norton et al. 2003; Nevins et al. 2006). In some of the studies referred to, grafting apparently had a successful outcome, while in other reports the benefits of such therapy were less obvious.

In one recent animal experiment, it was demonstrated that the placement of a xenogenic graft comprised of bovine bone combined with collagen from porcine origin in fresh extraction sockets of dogs promoted *de novo* hard tissue formation, in particular in the marginal portion of the extraction site (Araújo et al. 2008). Here, the dimension of hard tissue walls was maintained and the profile of the ridge was preserved. In a subsequent 2-week study (Araújo et al. 2009), it was shown that the early phases of hard tissue formation were altered in extraction sockets that were grafted with Bio-Oss® Collagen immediately after tooth removal. It appeared that this modified wound healing and bone modeling may have been influenced by the presence of multinucleated cells that occurred in tissues harboring the xenogenic graft. Thus, in the grafted sites, substantial amounts of newly formed bone could only be detected in the apical portion of the socket where the graft material was absent. In the remaining portions of the grafted sockets a mildly inflamed provisional matrix surrounded the majority of the Bio-Oss® particles, whose surface was frequently, but not always, coated with multinucleated cells. In isolated areas of the grafted sites, multinucleated cells were absent and the foreign material was surrounded by newly formed woven bone that bridged adjacent granules of the xenogenic particles.

The objective of the present experiment was to determine whether the process of bone modeling following tooth extraction and immediate implant placement could be influenced by the placement of Bio-Oss® Collagen in the void that occurred between the implant and the walls of the fresh extraction socket.

Material and methods

The ethical committee of the State University of Maringá approved the research protocol. Five beagle dogs about 1 year old and weighing between 10 and 12 kg each were used. During surgical procedures, the animals were anesthetized with intravenously administered Ketamin (10%, 8 mg/kg, Agener União, São Paulo, Brazil).

The fourth premolars in both quadrants of the mandible ($_4P_4$) were selected and used as experimental sites. The mesial root canals were reamed and filled with gutta-percha.

The fourth premolars were hemi-sected and the distal roots were carefully removed with the use of forceps (Fig. 1). The recipient sites were prepared for implant installation according to the guidelines provided by the manufacturer. Implants (Straumann® Dental Implant System;

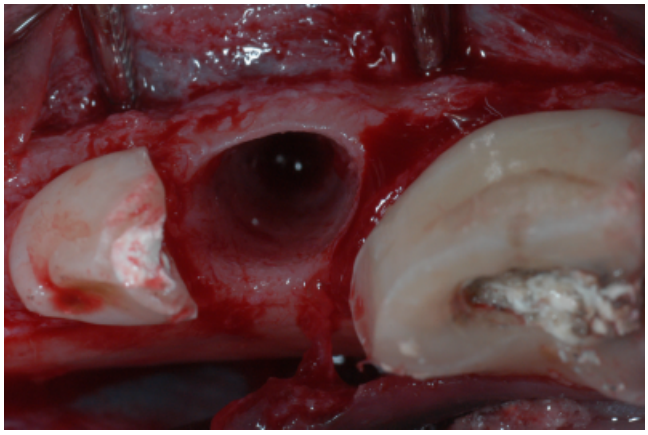

Fig. 1. Clinical photograph illustrating the alveolar socket immediately after the extraction of the distal root of the mandibular fourth premolar.

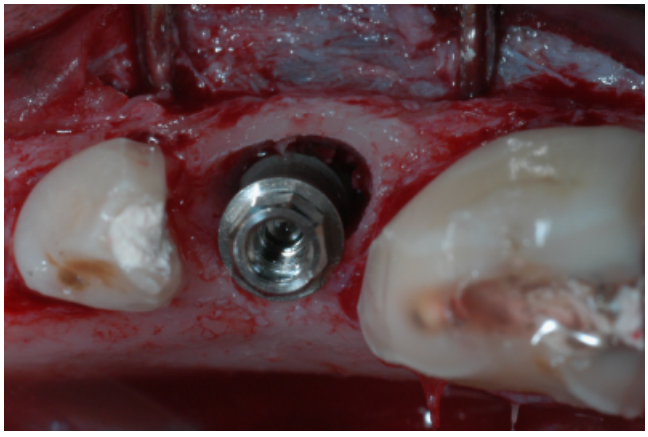

Fig. 2. Clinical photograph illustrating the position of the implants placed in the distal extraction socket of the mandibular fourth premolar. Note that a void (gap) had been established between the implant and the buccal and approximal bone walls.

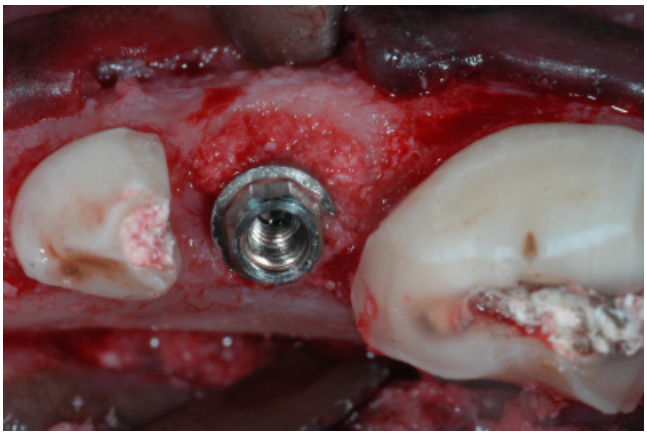

Fig. 3. Clinical photograph illustrating that the void between the implant and the bone walls had been filled with the xenogenic graft.

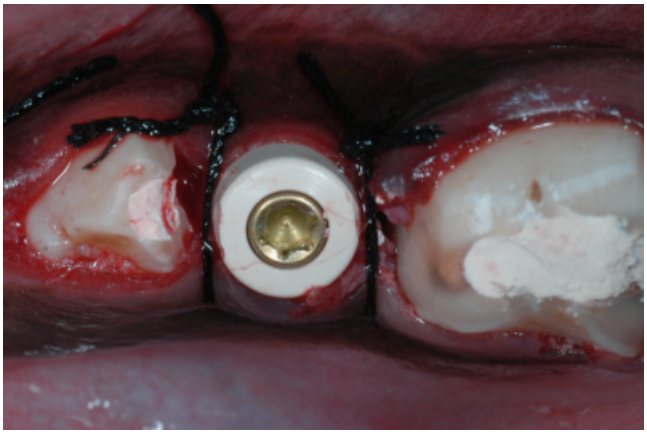

Fig. 4. Clinical photograph of the occlusal aspect of the mandibular fourth premolar illustrating the mucosal flaps that had been secured with interrupted sutures.

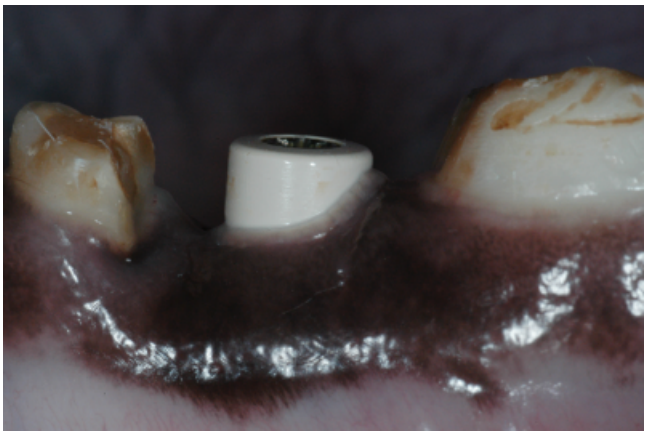

Fig. 6. Clinical photograph illustrating the implant sites after 6 months of healing. The peri-implant mucosa at both the test and the control sites had normal texture and color and was free of signs of inflammation.

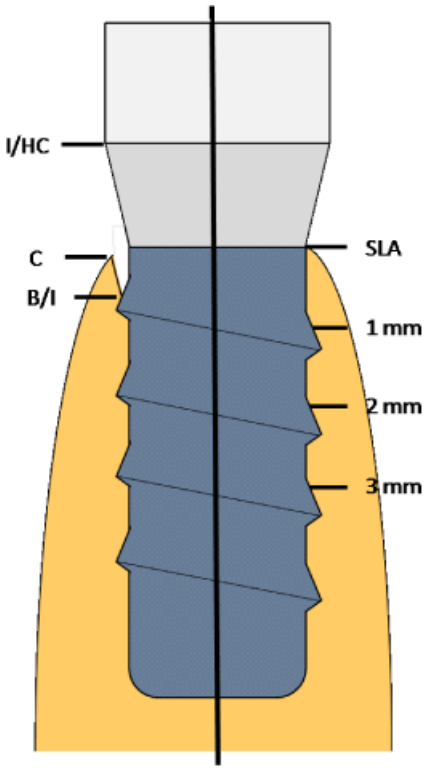

Fig. 5. Schematic drawing describing different landmarks from which histometric measurements were performed. IHC, level of the implant – healing cap connection; SLA, marginal border of the rough implant surface; C, marginal level of the bone crest; B/I, marginal level of bone to implant contact; 1, 2 and 3 mm represent the levels 'apical of SLA' at which the width of the buccal and lingual walls was determined.

Standard Implant, 3.3 mm wide and 6 or 8 mm long; Straumann, Basel, Switzerland) were installed. Each implant was placed in a lingual position in the socket. Hence, a 1–2 mm wide and 3 mm deep buccal-approximal void similar to a three-wall bone defect was established between the titanium rod and the inner bone walls (Fig. 2). The marginal level of the SLA-coated surface of all implants was, following placement, located flush with or slightly apical of the adjacent buccal bone crest. On one side of the jaw, the marginal buccal-approximal void that consistently occurred between the implant and the socket walls was grafted with Bio-Oss® Collagen (Geistlich Pharma AB, Wolhusen, Switzerland), while no grafting was performed in the contra-lateral sites (Fig. 3).

Healing caps (Straumann® Dental Implant System) were adjusted to the implants. The flaps and the wound margins were replaced and secured to allow a semi-submerged healing of the experimental sites (Fig. 4). The sutures were removed after 2 weeks. Every second day, the animals were exposed to mechanical plaque removal. In addition, a clorhexidine (0.2%) gel was placed every second day placed at healing caps and adjacent teeth.

After 6 months of healing, the dogs were euthanized with an overdose of Ketamin and perfused with a fixative containing a mixture of 5% glutaraldehyde

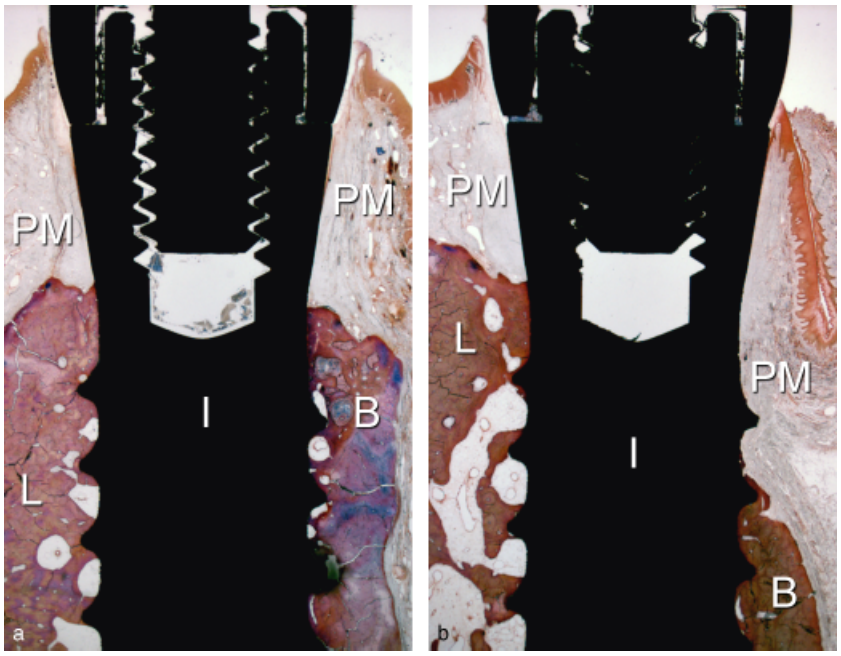

Fig. 7. Buccal–lingual section representing the test (a) and control (b) sites. B, buccal bone wall; I, implant; L, lingual bone wall; PM, peri-implant mucosa. Ladewig's fibrin staining; original magnifications × 16.

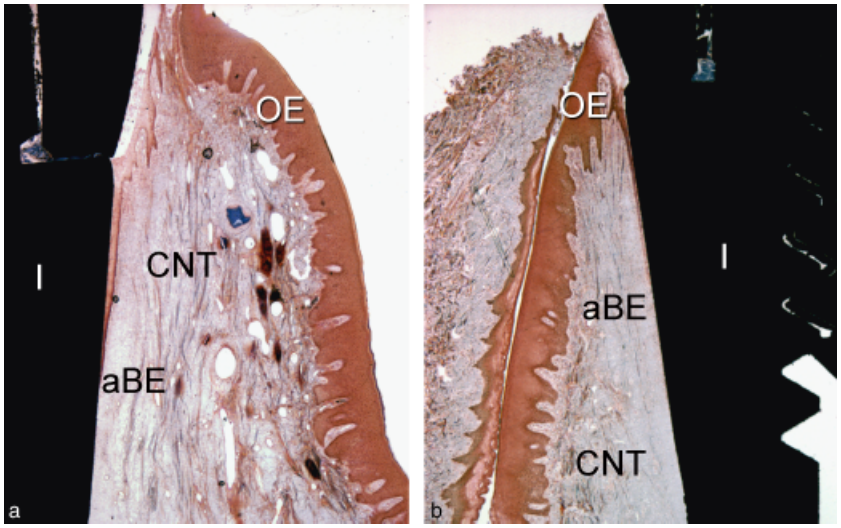

Fig. 8. Higher magnification of the buccal aspect of the peri-implant mucosa in Fig. 7. Test site (a), control site (b). Note that the mucosa is rich in collagen fibers and contains few inflammatory cells. In addition, in the test site (a) note the presence of a Bio-Oss® particle in the connective tissue lateral below the oral epithelium. CNT, connective tissue; aBE, apical portion of the barrier epithelium; I, implant; OE, oral epithelium. Ladewig's fibrin staining; original magnifications × 50.

and 4% formaldehyde (Karnovsky 1965). The mandibles were removed and placed in the fixative. Radiographs were obtained from the implant locations and the position of the 'interproxmial' bone was estimated.

Each experimental site, including the mesial root and the distal socket area, was dissected using a diamond saw (Exact® Apparatebeau, Norderstedt, Hamburg, Germany). The tissues were processed for ground sectioning according to the methods described by Donath & Breuner (1982) and Donath (1988). The samples were dehydrated in increasing grades of ethanol and infiltrated with Technovit® 7200 VLC-resin (Kulzer, Friedrichrsdorf, Germany), polymerized and sectioned using a cutting – grinding unit (Exact® Apparatebeau).

From each biopsy unit, one buccal–lingual section representing the central area of the site was prepared. The sections were reduced to a thickness of about 25 μm by micro-grinding and polishing. The sections were stained in Ladewig's fibrin stain (Donath 1993). The histological examinations were performed in a Leitz DM-RBE® microscope (Leica, Wetzlar, Germany) equipped with an image system (Q-500 MC®, Leica).

Histological examination

In the sections, linear measurements (magnification × 16–50) were made between the following landmarks (Fig. 5):

- (PM): margin of the peri-implant mucosa.
- (aBE): apical cells of the barrier epithelium.
- (SLA): the marginal termination of the rough surface.
- (C): the crest of the buccal or the lingual bone wall.
- (B/I): the most coronal point of contact between bone and implant.
- (I/HC): the contact between the implant and the healing cap.

The widths of the buccal and lingual bone walls were determined by measuring the distance between the buccal or the lingual surface of the implant body and the outer surface of the hard tissue wall. The assessments were performed at the SLA level as well as 1, 2 and 3 mm apical of SLA.

Results

Healing following tooth extraction, implant installation and grafting was uneventful in all dogs. At the 6-month examination interval, the mucosa surrounding all implants was virtually free of clinical signs of inflammation (Fig. 6). The margin of the peri-implant mucosa resided at all 10 experimental sites on the healing caps.

In the radiographs, it was observed that the crest of the 'interproximal' bone at the experimental sites was located at the level of the cut distal furcation fornix of the mesial root. Thus, there was no apparent loss of bone at the 'interproximal' region during the study period.

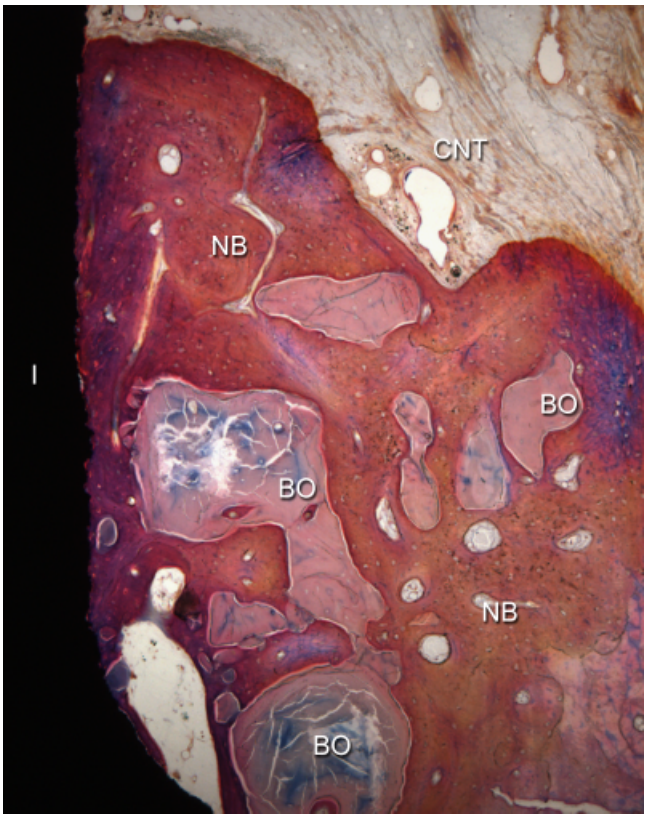

Fig. 9. Higher magnification of the buccal bone crest in Fig. 7a. Note the presence of a number of Bio-Oss® particles that were included in the newly formed bone in the previous gap. BO, Bio-Oss® particle; CNT, connective tissue; I, implant; NB, new bone. Ladewig's fibrin staining; original magnification × 100.

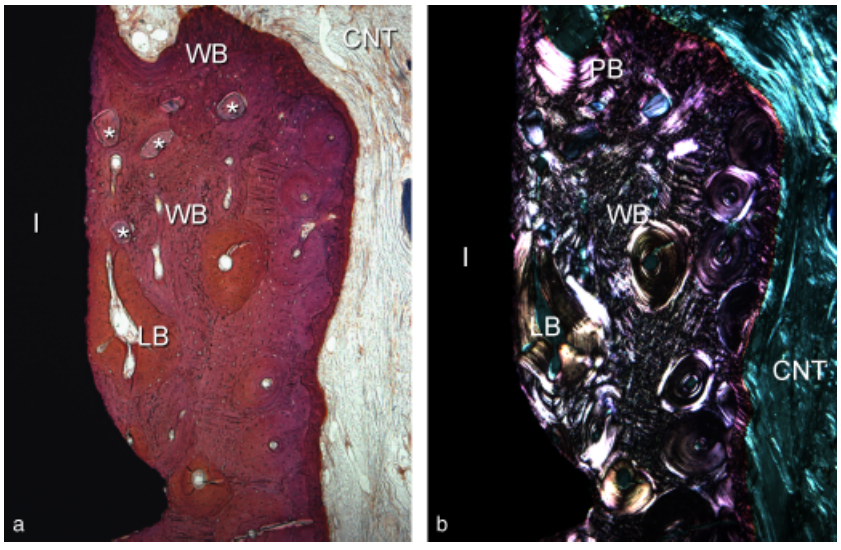

Fig. 10. Buccal section of a test site that illustrates the newly formed bone inside the walls of the previous gap that had been grafted with bovine bone (a). In polarized light (b) various Bio-Oss® particles can be identified within the new bone that was comprised mainly of woven bone and parallel-fibered bone. *, Bio-Oss® particle; CNT, connective tissue; I, implant, LB, lamellar bone; PB, parallel-fibered bone; WB, new bone. Transmitted light (a) and polarized light (b). Ladewig's fibrin staining; original magnification × 50.

The microscopic examination of the ground sections revealed that all implants were well-integrated with the surrounding bone tissue that was comprised of mineralized bone and marrow (Fig. 7). Furthermore, the supracrestal soft tissue in all 10 implant sites (i) was rich in well-oriented bundles of collagen fibers and (ii) harbored only small inflammatory cell infiltrations (Fig. 8a and b). The attachment between the mucosa and the implant was made up of a barrier epithelium that was continuous in the apical direction, with a zone of connective tissue attachment that was in apparent direct contact with the titanium cylinder. In the test sites, Bio-Oss® particles were frequently observed in the peri-implant mucosa (Fig. 8a).

The outline of the marginal hard tissue of the control sites was markedly different from that of the test sites. Thus, while the buccal bone crest in the test sites was comparatively thick and located at or close to the SLA level (SLA), the corresponding crest at the control sites was thinner and located at a varying distance below SLA. Furthermore, in the test sites, the marginal bone-to-implant contact (B/I) was at the same level at the buccal and lingual aspects, whereas in the control, the lingual B/I was located more close to the upper rim of the implant than its buccal counterpart.

In the test sites, the buccal void that had been grafted with Bio-Oss® Collagen following implant placement was, at sacrifice, filled with newly formed bone in which a varying number of Bio-Oss® particles had been trapped (Fig. 9). This newly formed hard tissue that was mainly comprised of woven and parallel-fibered bone was, in four out of five test sites, continuous with the old lamellar bone of the buccal bone wall (Fig. 10).

In the control sites (Fig. 11), the buccal bone wall was thin and tapered in the 'coronal' direction. Also, this marginal bone tissue was comprised of woven bone and small amounts of parallel fibered bone (Fig. 12). Immediately above the most coronal portions of the thin buccal bone wall, isolated remnants of woven bone could be identified.

Histometric measurements

The distance between PM and the I/HC on the buccal aspect was 1.8 ± 0.8 mm (test) and 0.8 ± 0.6 mm (control). The corresponding dimension at the lingual aspect was 2 ± 0.8 mm (test) and 1.8 ± 0.4 mm (control). In other words, the soft tissue margin at the buccal aspect of control sites

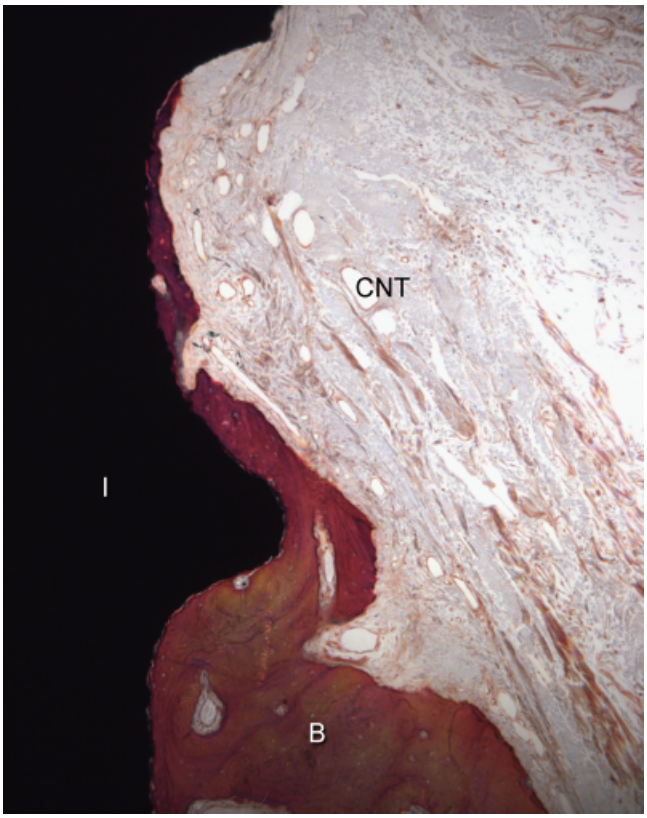

Fig. 11. Higher magnification of the buccal bone crest in Fig. 7b. Note that the buccal bone wall is thin and tapered and in the coronal direction. CNT, connective tissue; B, bone; I, implant. Ladewig's fibrin staining; original magnification × 100.

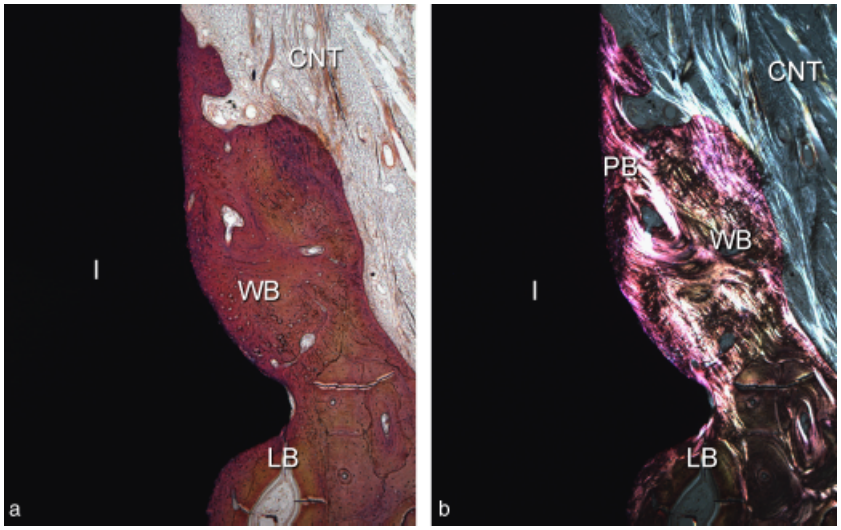

Fig. 12. The buccal crest in a control site (a). In polarized light (b), it is observed that the newly formed bone is comprised of woven bone, parallel-fibered bone and some lamellar bone. CNT, connective tissue; I, implant; LB, lamellar bone; PB, parallel-fibered bone; WB, new bone. Transmitted light (a) and polarized light (b). Ladewig's fibrin staining; original magnification × 50.

was located about 1 mm apical of that of the test sites.

The length of the barrier epithelium (PM–aBE; Table 1) varied between 2 ± 0.5 and 2.9 ± 0.7 mm, while the zone of connective tissue attachment (aBE–B/I) varied between 0.7 ± 0.4 and 1.9 ± 0.9 mm. The width of the buccal soft tissue at the implant/healing cap level (PM width; Table 1) was 1 ± 0.3 mm in the test and 0.4 ± 0.4 mm in the controls.

In both groups, the B/I level at the buccal aspect was apical of the SLA border (test: 0.1 ± 0.5 mm, control 1.3 ± 0.7 mm). At the lingual aspects, the corresponding bone level was at SLA (Table 2). In both experimental groups, the distances between the bone crest and the B/I level at the buccal and lingual aspects were, respectively, about 0.1 and 0.8 mm.

In both test and control sites (Table 3), the thickness of the lingual bone wall increased from 1.9 mm at the level of SLA to about 3.6 mm at 3 mm apical of SLA. The thickness of the marginal portion of the buccal bone wall varied considerably between the test and the control sites. Thus, at SLA and at 1 mm apical of SLA, there was virtually no bone at the control sites, while at the corresponding levels at the test sites the bone was 0.4 ± 0.6 and 1.1 ± 0.5 mm thick. Also, at more apical levels, the buccal bone of the test sites was markedly wider than that of the controls (Table 3; Fig. 12).

Discussion

The present experiment demonstrated that the placement of Bio-Oss® Collagen in the void between an implant and the buccal-approximal bone walls of a fresh extraction socket (i) modified the process of hard tissue healing, (ii) provided additional amounts of hard tissue at the entrance of the previous socket, (iii) improved the level of marginal bone-to-implant contact and (iv) prevented soft tissue recession.

The observation that following tooth extraction and immediate implant placement healing resulted in a substantial reduction of the dimension of the buccal bone wall is in agreement with the findings reported previously (Botticelli et al. 2004, 2006; Araújo et al. 2005, 2006; Blanco et al. 2008). It was suggested (Araújo & Lindhe 2005) that the reduction of the buccal bone wall was in part related to (i) the loss of bundle bone and (ii) the pre-surgical thickness of the buccal bone tissue (i.e. biotype). In the current experiment, the reduction of the height of the buccal bone wall was expressed as the distance between the SLA border and the marginal B/I and was estimated to be 1.3 ± 0.7 mm long. This is an agreement with the find-

Table 1. **Results of the histometric measurements describing some dimensions (mm) of the peri-implant mucosa at the buccal and lingual aspects of the implant sites (mean ± SD)**

	PM–I/HC		PM–aBE		aBE–B/I		PM width	
	Buccal	Lingual	Buccal	Lingual	Buccal	Lingual	Buccal	Lingual
Test	1.8 ± 0.8	2 ± 0.8	2.6 ± 0.7	2.2 ± 0.4	1.1 ± 0.4	1.4 ± 0.4	1 ± 0.3	1.5 ± 0.5
Control	0.8 ± 0.6	1.8 ± 0.4	2 ± 0.5	2.9 ± 0.7	1.9 ± 0.9	0.7 ± 0.4	0.4 ± 0.4	1.4 ± 0.8

For abbreviations, see text.

Table 2. **Results of histometric measurements describing the distance (mm) between B/I to the various landmarks (mean ± SD)**

	SLA–B/I		C–B/I	
	Buccal	Lingual	Buccal	Lingual
Test	−0.1 ± 0.5	0.2 ± 0.2	0.2 ± 0.2	−0.9 ± 0.6
Control	−1.3 ± 0.7	0.1 ± 0.1	0 ± 0	−0.7 ± 0.2

Negative values indicate that C or B/I was apical to SLA.
For abbreviations, see text.

Table 3. **Results of the histometric measurements describing the width (mm) of the buccal and lingual bone walls in the implant sites (mean ± SD)**

	At SLA		At 1 mm		At 2 mm		At 3 mm	
	Buccal	Lingual	Buccal	Lingual	Buccal	Lingual	Buccal	Lingual
Test	0.4 ± 0.6	1.9 ± 1.4	1.1 ± 0.5	2.7 ± 1	1.1 ± 0.5	3.1 ± 0.8	1.1 ± 0.3	3.5 ± 0.6
Control	0 ± 0	1.9 ± 0.4	0.1 ± 0.2	2.7 ± 0.2	0.4 ± 0.3	3.3 ± 0.3	0.7 ± 0.5	3.7 ± 0.4

For abbreviations, see text.

ings of Blanco et al. (2008) from a comparable study in the beagle dog. In previous similar experiments from this laboratory (i.e. tooth extraction and immediate implant placement) (Araújo et al. 2005, 2006), the buccal bone wall of premolar sites during healing lost on average between 2 and 2.5 mm. In this context, it should be realized that in the present study, implants with a smaller diameter were used than in the earlier experiments (3.3 vs. 4.1 mm), and hence a larger void occurred between the buccal bone wall and the endosseous implant. In this buccal gap, healing resulted in new bone formation coronal to the receding buccal bone wall. This conclusion is substantiated by the observation (Araújo et al. 2006) that the presence of a large gap between the buccal wall and the implant apparently promoted new bone formation and enhanced the level of bone-to-implant contact.

In the current experiment, single teeth (roots) were gently removed to allow the preservation of the attachment apparatus of neighboring teeth. Hence, the level of the 'interproximal' bone between the implant and the tooth underwent only minor changes during the 6-month interval. This is in agreement with data from a clinical study by Schropp et al. (2003). They studied soft and hard tissue changes that occurred during a 12-month period following single tooth extraction (premolars and molars) in 46 subjects. The authors reported that, while the width of the edentulous socket site was markedly reduced, only a minor change occurred at the mesial and distal aspects of the extraction site. The fact that in the current study almost no bone loss occurred in the 'interproximal' region may also have reduced the bone-level change that occurred at the buccal and lingual aspects of the implant as suggested by Botticelli et al. (2006)

The present study, also demonstrated that the placement of a xenogenic graft in the gap between the implant and the buccal bone wall evidently modified the pattern of hard tissue modeling. The new tissue that formed in the gap region to a large extent compensated for the hard tissue that was lost following tooth extraction in the buccal bone wall. This in agreement with data previously presented showing that the placement of Bio-Oss® Collagen in fresh extraction sockets may counteract post-extraction ridge reductions (Araújo & Lindhe 2009).

The newly formed hard tissue in the grafted sites was comprised of woven and parallel fibered bone that had become established in close contact with the biomaterial. Thus, it may be argued that the processes of modeling and remodeling at the 6-month interval was incomplete and that, hence further dimensional change may occur during later phases of healing.

Moreover, several bone multicellular units (BMUs; Frost 1964) were observed in the center as well as in the periphery of newly formed bone. Such BMUs were only occasionally in direct contact with the Bio-Oss® particles that seemed not to undergo resorption.

The marginal portion of the buccal bone was comprised of woven and parallel fibered bone. This kind of immature bone is, as a rule, replaced over time with mature lamellar bone and marrow. It is not known, however, whether such a remodeling will take place in the marginal portion of bone surrounding implants.

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