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Hard tissue alterations after socket preservation with additional buccal overbuilding: a study in the beagle dog

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Abstract

Objectives: The aim of this study was to histometrically assess alterations of the ridge following socket preservation alone and socket preservation with additional buccal overbuilding.

Material and Methods: In five beagle dogs four extraction sites were randomly subjected to one of the following treatments:

Tx 1: The socket was filled with BioOss Collagen® and covered with a free gingival graft from the palate.

Tx 2: The buccal bone plate was augmented using the GBR-technique, the socket was filled with BioOss Collagen® and covered with a free gingival graft.

Tx 3: The buccal bone plate was forced into a buccal direction using a manual bone spreader. The socket was filled with BioOss Collagen® and covered with a free gingival graft from the palate.

Tx 4: The socket was filled with BioOss Collagen® and a combined free gingival/connective tissue graft was used to cover the socket and for buccal tissue augmentation.

For each experimental site, two histological sections were subjected to histometric analysis and evaluated for (i) vertical bone dimensions and (ii) horizontal bone dimensions.

Results: All treatment groups showed horizontal and vertical bone loss. The mean vertical bone loss of the buccal bone plate was significantly lower in Tx 4 than in the other groups, while no statistical significant differences could be detected among the groups in the horizontal dimension.

Conclusion: Overbuilding the buccal aspect in combination with socket preservation does not seem to be a suitable technique to compensate for the alterations after tooth extraction.

Key words: bone substitute; extraction socket; GBR; socket preservation

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Conflict of interest and source of funding

The authors declare that they have no conflicts of interests.

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Tooth extraction is followed by dimensional changes of the alveolar ridge contour (Amler et al. 1960, Pietrokovski & Massler 1967, Schropp et al. 2003, Araújo & Lindhe 2005, Fickl et al. 2008c). The resorption of the ridge is more pronounced on the buccal than on the lingual aspect of the extraction socket (Pietrokovski & Massler 1967, Araújo & Lindhe 2005).

Socket preservation at time of tooth extraction has been advocated to minimize horizontal ridge resorption and facilitate ideal implant placement and thus an aesthetic site reconstruction. Different approaches have been developed to preserve or improve the ridge contour following tooth extraction, including the use of immediate implants (Paolantonio et al. 2001, Botticelli et al. 2004, Araújo et al. 2005), or occlusive membranes with or without graft materials (Lekovic et al. 1997, 1998, Iasella et al. 2003). However, using these techniques with a reported loss of horizontal ridge dimensions between −1.17 and −1.73 mm, the original ridge contours cannot be preserved (Lekovic et al. 1997, 1998, Iasella et al. 2003).

Other techniques, such as grafting bone substitute materials have also been used for ridge preservation (Artzi & Nemcovsky 1998, Becker et al. 1998, Artzi et al. 2000, Carmagnola et al. 2003, Jung et al. 2004, Nevins et al. 2006, Araújo et al. 2008, Wang & Tsao 2008). Nevins et al. (2006) demonstrated an advantage of augmenting extraction sockets with deproteinized bovine bone material (DBBM) in a clinical study, as compared with untreated controls. However, the authors reported a mean reduction of the buccal bone plate of DBBM-treated extraction sockets of 2.42 mm, resulting in a failure to preserve the alveolar ridge. Recent reports from animal studies showed that the placement of DBBM into extraction sockets is a suitable technique for socket augmentation with the ability to maintain the ridge dimension to a certain amount (Araújo et al. 2008, 2009, Fickl et al. 2008a). Yet a preservation of the buccal bone plate and complete ridge stabilization could not be shown.

Herein, we aimed to evaluate whether an additional hard or soft-tissue overaugmentation of the buccal bone plate at time of socket preservation is able to achieve superior ridge dimensions when compared with socket preservation alone.

Material and Methods

The research protocol of this investigation was approved by the ethical committee of Biomatech (Namsa Company, Lyon, France). Five beagle dogs (approximately 1 year old, 10–11.3 kg) were used for this experiment. Animals were housed under laboratory conditions. The recommended temperature range for the room was 15–21°C. The recommended humidity for the room was >30%. The light cycle was controlled using an automatic timer (12 h light, 12 h dark).

Surgical procedure

Supragingival scaling was performed on all dogs 5 days before tooth extraction. Anaesthesia was induced by injecting atropine (0.05 mg/kg intra-muscular; Atropine®, Aguettant, Lyon, France) and tiletamine-zolazepam (5–10 mg/kg intra-muscular; Zoletil®100, Virbac, Carros, France). Subsequently, an injection of thiopenthal sodium was given (10–15 mg/kg/intravenous; Nesdonal®, Merial, Lyon, France) and the animals were placed on an O_2–N_2O isoflurane (1–4%) mixture. Local anaesthesia was induced by subcutaneous injection of articain in 4% solution with epinephrine 1:100,000 (Ultracain®, Hoechst, Frankfurt, Germany).

In both quadrants of the mandible the sockets harbouring the distal root of the third and fourth pre-molars (P_3, P_4) served as experimental sites. In order to mimic extraction sites of single rooted teeth, the mandibular pre-molars were hemisected with the use of a fissure bur. The distal roots were removed using a forceps without elevation of a muco-periosteal flap or compromising the marginal gingiva. The pulp tissues of the mesial roots were extirpated and engaged with a Gates-Glidden bur. After filling the root canals with gutta-percha, the coronal part of the pulp chamber was sealed with an auto-polymerizing resin material (Clearfil Core®, Kuraray, Tokyo, Japan). Consecutively the extraction sites were randomly assigned to one of the following experimental treatments:

Tx 1 (n = 5): The extraction socket was filled with DBBM integrated in a 10% collagen matrix (BioOss Collagen®, Geistlich Biomaterials, Wolhusen, Switzerland) and a free soft tissue graft was sutured to the orifice of the extraction socket. The free soft tissue punch according to the technique of Jung et al. (2004) and Landsberg & Bichacho (1994) was harvested with a scalpel from the palate with a thickness of approximately 3 mm. Several interrupted sutures (Seralene 7-0 ®, Serag Wiesner, Naila, Germany) were applied to fix the transplant to the marginal gingiva of the extraction socket (Fig. 1a and b). This treatment group served as a control group.

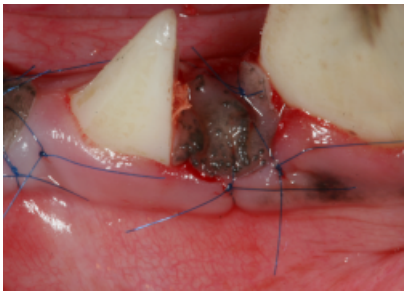

Fig. 1. After incorporation of BioOss Collagen®, the socket is superficially closed with a free gingival autograft, harvested from the palate.

Tx 2 (n = 5): After an intra-sulcular incision a full-thickness elevation of the marginal gingival tissue was performed over the distal root without any vertical releasing incisions. An absorbable collagen membrane (BioGide®, Geistlich Biomaterials, Wolhusen, Switzerland) was placed and the buccal bone plate was augmented with BioOss Collagen® using conventional guided bone regeneration techniques. Subsequently, the extraction socket was filled with BioOss Collagen® and closed with a free gingival autograft (Fig. 2).

Tx 3 (n = 5): The buccal bone plate was forced into a buccal direction with a specially designed bone spreading instrument mobilizing only the buccal bone plate approximately 5 mm. Care was taken not to break the buccal bone plate, but to achieve a bone spreading effect. BioOss Collagen® was packed into the socket to prevent the buccal bone plate from re-collapsing, and the extraction socket was closed with a free gingival autograft (Fig. 3).

Tx 4 (n = 5): An undermining split thickness preparation of the buccal aspect was performed. The socket was filled with BioOss Collagen® and a combined free gingival/connective tissue graft was obtained from the palate.

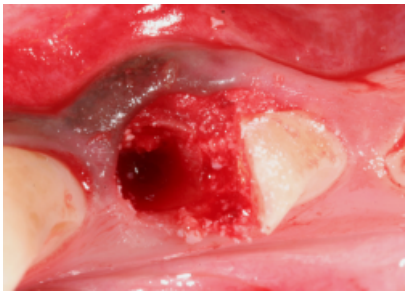

Fig. 2. After augmenting the buccal bone plate using the guided bone regeneration technique (BioGide®/BioOss Collagen®), socket preservation is performed.

Fig. 3. The buccal bone plate is forced into a buccal direction using a specially constructed instrument.

Fig. 4. After filling the extraction socket with BioOss Collagen® a modified connective/free gingival autograft is inserted into a buccal pouch and sutured to the orifice of the extraction socket.

The connective tissue portion of the graft was inserted into the undermined buccal pouch and sutured with several interrupted sutures (Seralene 7-0 ®) (Fig. 4).

After surgery, the following regimen was administered:

- The animals were observed once daily for any clinical abnormality.
- Antimicrobial prophylaxis: spiramycine 750,000 IU and metronidazole 125 mg/day per os for 13 days (Stomorgyl®, Merial, Lyon, France).
- Anti-inflammatory drug: carprofene 50 mg/day per os for 13 days (Rimadyl®, Pfizer Santé Animale, Orsay, France).
- Each animal received an injection of butorphanol (0.3 mg/kg) (Torbugesic®, Fort Dodge Animal Health, Southampton, UK) post-surgically and on the following day.
- The dogs were placed on a soft diet throughout the entire observation period.
- Tooth cleaning with toothbrush and dentifrice and administration of 0.2% chlorhexidine-solution was performed three times per week for 4 weeks.
- The sutures were removed 2 weeks post-surgery. Healing presented uneventful. The soft tissue grafts were fully integrated without any sign of necrosis.

Termination procedure

The animals were sacrificed 4 months after tooth extraction. The animals were weighed and anesthetized by an intra-muscular injection of Zoletil® (5–10 mg/kg intra-muscular; Virbac, Carros, France). An injection of heparin 25,000 IU (100 IU/kg; Leo Pharmaceutical, Saint Quentin Fallavier, France) was administered to each animal. The animals were sacrificed by a lethal injection of a barbiturate (Dolethal®, Vetoquinol, Paris, France). For each experimental site, the width of the remaining mesial root was calculated and initiating from the distal border of the root, half of the measurement was calculated to the distal. In this spot, assuming to be centre of the former distal root, a tattoo mark was performed to facilitate the location of the histological section. The head of each animal was exsanguinated and then fixed by arterial perfusion with approximately 300 ml of 10% formaldehyde in phosphate buffer pH 7 through the carotid artery. The mandibles were block resected and each hemi-mandible was identified and fixed in 10% buffered formalin solution.

Histologic analysis

Segments containing the experimental units and the mesial roots were dissected using a diamond saw (Exakt Apparatebau, Norderstedt, Germany). The biopsies were processed for ground sectioning according to the methods described by Donath & Breuner (1982) and Donath (1993). In brief, the samples were dehydrated in increasing grades of ethanol and infiltrated with Technovit® 720 VLC-resin (Kulzer, Friedrichsdorf, Germany). For each site, three buccolingual sections were performed in the area of the tattoo mark using a microcutting and grinding technique. By microgrinding and polishing, the three sections were reduced to 20 μm and marked with a modified Paragon staining for qualitative and semi-quantitative light microscopy analysis.

The three sections of each site were observed using a stereomicroscope (Leica Stereomikroskop MZ 16, Leica, Wetzlar, Germany) fitted with $\times 5$, $\times 10$, $\times 20$ and $\times 40$ objectives and equipped with a colour image analysing system SAMBA® (Samba Technologies, Grenoble, France). One blinded co-worker conducted all measurements.

Vertical measurements

The vertical distance between the margins of the buccal and lingual bone walls was determined as follows: a line (HL) was placed on top of the lingual crest (LBC) perpendicular to the long axis of the tooth. This could be detected by carefully evaluating the staining difference between newly depositioned bone and the former bone surrounding the alveolus. Subsequently, a perpendicular vertical line was drawn reaching to the top of the buccal bone crest (BBC). The vertical distance between HL and the BBC was measured and expressed in millimetres. Mean values and standard deviations were calculated for each experimental unit (Fig. 5).

Horizontal measurement

The bucco-lingual width of the alveolar ridge was measured according to (Araújo & Lindhe 2005). In brief, parallel lines to the horizontal plane (LBC) were placed at 1 mm (value 1), 3 mm (value 3) and 5 mm (value 5) below the lingual crest, representing three different levels of the alveolar ridge. These parallel lines were perpendicular to the long axis of the tooth, which was identified by the colour difference between newly created bone and former surroundings of the alveolus. The horizontal distance between the borders of the alveolar ridge were measured and expressed in millimetres. Mean values and standard deviations were calculated for each experimental unit (Fig. 6).

Statistical analysis of mean values of the histometric measurements was performed to analyse the difference between the groups using a parametric analysis of variance test at a 5% risk (SPSS® 15.0, SPSS Inc., Chicago, IL, USA).

Results

Histological observations

During the healing period of 4 months, there was no clinical evidence of infec-

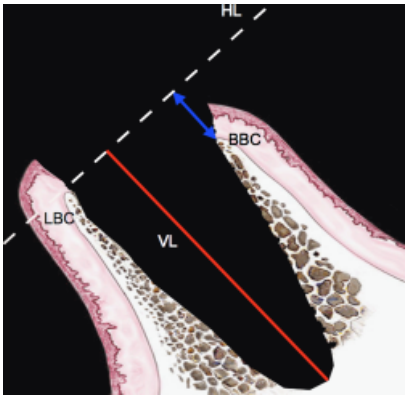

Fig. 5. Vertical measurements: HL, horizontal line; VL, vertical line; BBC, buccal bone crest; LBC, lingual bone crest.

tion, necrosis or significant osteolysis in any of the treatment groups. Histologically, a slight to moderate infiltrate of neutrophils and macrophages was present in a proportion of sites, often associated with gingival hyperplasia. However, this mucosal inflammatory response could be considered to be of minor extent and not attributable to the grafting procedure. In all treatment groups, no remnants of the free gingival graft could be identified at the top of the defect after 4 months. There was a moderate to marked grade of bone regeneration in the socket, characterized by osteoblastic activity and by a moderate grade of osseointegration of the BioOss Collagen® graft, with individual variations for osseointegration ranging from a null to marked grade. A hard tissue bridge sealing the former extraction socket could be detected with BioOss Collagen® particles being incorporated. Most of the BioOss Collagen® particles in the coronal and apical portion were in direct contact with woven and lamellar bone. A small part of the biomaterial, in particular at the coronal aspect, was incorporated in connective tissue (Figs 7–10).

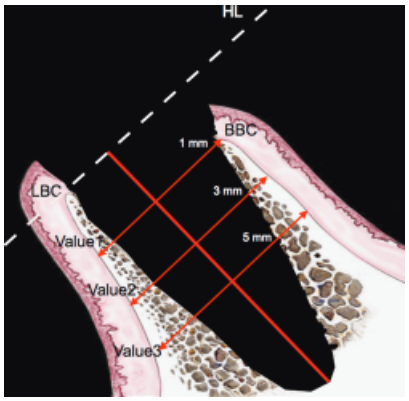

Fig. 6. Horizontal measurements: HL, horizontal line; BBC, buccal bone crest; LBC, lingual bone crest; value1, bucco-oral measurement 1 mm below the LBC; value2, bucco-oral measurement 3 mm below the LBC; value3, bucco-oral measurement 5 mm below the LBC.

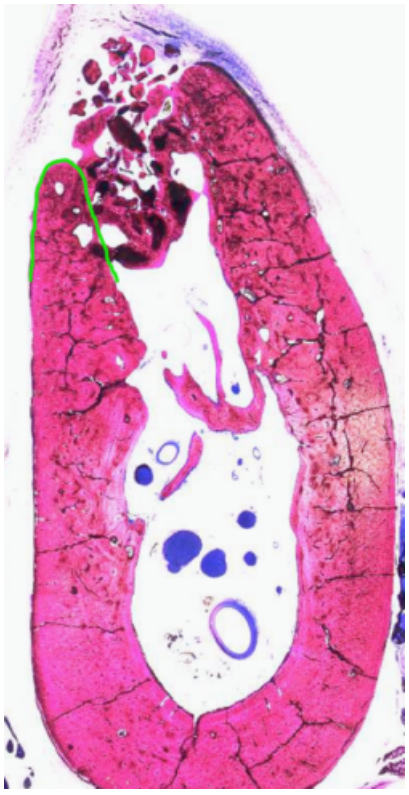

Fig. 7. Bucco-lingual section of Tx 1. Note the pronounced loss of the buccal bone plate (marked with green line).

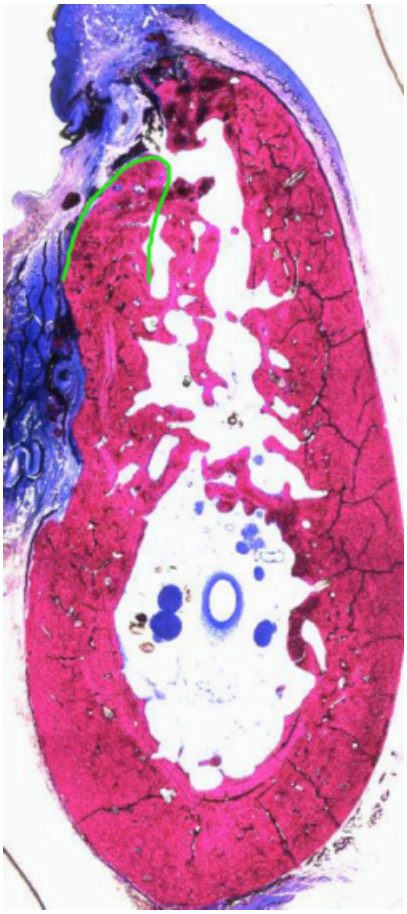

Fig. 8. Bucco-lingual section of Tx 2. The resorption of the alveolar bone can be seen on the buccal aspect, where the muco-periosteal flap was raised.

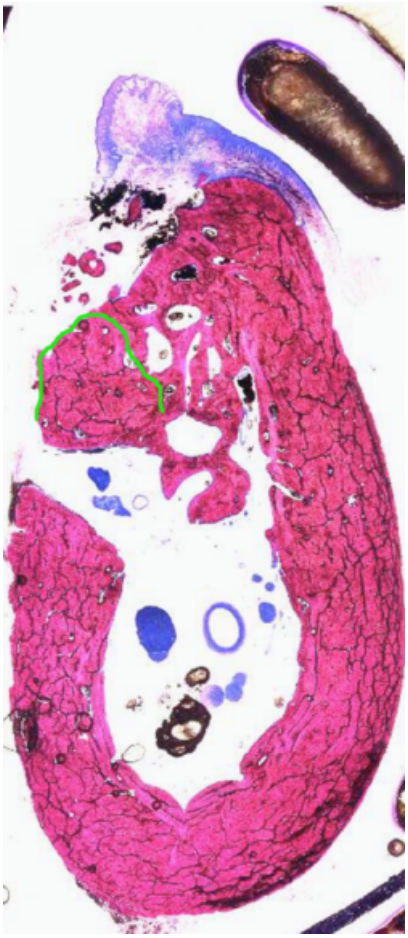

Fig. 9. Bucco-lingual section of Tx 3. Note the marked resorption of the entire buccal bone aspect.

Histometric evaluation

Vertical measurements (Table 1, Fig. 11)

As displayed in Table 1, the vertical distance between the buccal and the lingual bone crest showed considerable inter-individual variability. The comparison of the control treatment Tx 1 (socket preservation) and the test procedures Tx 2, Tx 3 and Tx 4 showed no significant differences except for Tx 4, which demonstrated significantly less relative bone loss of the buccal bone plate than the control and the other test groups.

Horizontal measurements (Table 1, Fig. 12)

The horizontal measurements of the bone dimension characterizing the width of the buccal to lingual ridge at 1, 3 and 5 mm are shown in Table 1. No statistical significant difference could be observed

between the four groups at the three levels of measurement. However, the values at the ''1 mm level'' showed a higher heterogeneity compared with the ''3 and 5 mm levels'' demonstrating a low inter-individual variability.

Discussion

The present study evaluated the use of a DBBM integrated in a 10% collagen matrix (BioOss Collagen®) with and without additional hard or soft tissue augmentation of the buccal bone plate using histometrical measurements. It could be demonstrated that the supplementary extra-socket grafting is unable to compensate for the resorptional alterations of the buccal compacta.

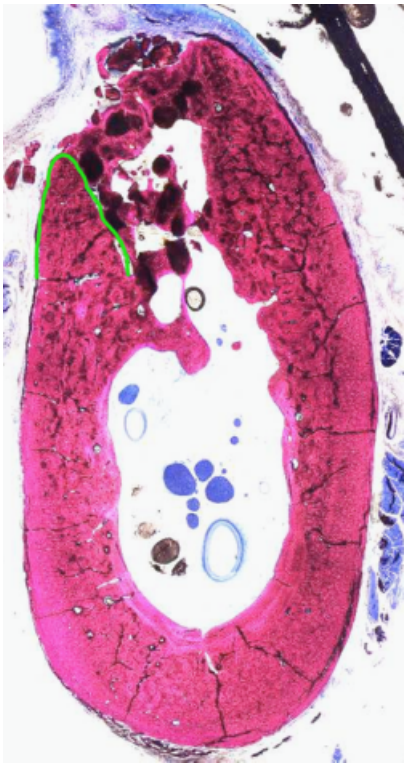

Fig. 10. Bucco-lingual section of Tx 4. The buccal bone plate is situated apically to the lingual bone plate.

Previous animal studies reported that bone substitutes placed into the extraction socket could not alter the biologic principles occurring after tooth extraction (Araújo et al. 2008, 2009, Fickl et al. 2008a). Araújo et al. (2008, 2009) concluded that incorporation of Bio-Oss Collagen® fell short in inhibiting the process of modelling and remodelling of the extraction socket. In a recent publication, it was shown that after socket preservation a mean vertical resorption of the buccal bone plate of up to 3.2 mm can be expected (Fickl et al. 2008a). In the present study, we found mean vertical alterations between 1.6 and 3.5 mm for the test and control groups, indicating that no treatment was able to maintain the buccal bone plate in its original height. Thus, the data presented herein corroborate the aforementioned studies. Neither immediate implants (Araújo et al. 2005), nor grafting the socket with bone substitutes (Araújo et al. 2008, Fickl et al. 2008a) nor augmentation procedures of the buccal bone plate are able to alter the biologic process of extraction socket remodelling with particular respect to the resorption of the buccal bone plate.

Furthermore, when regarding the horizontal dimension of the post-extraction ridge, no significant differences between the control group (BioOss Collagen®/Free gingival graft) and the experimental groups were detected 4 months after socket preservation. These findings seem to be somewhat surprising as extra-socket grafts were utilized in the experimental groups to compensate for the expected bone resorption. However, the findings corroborate data by Simon and colleagues, who raised a full-thickness flap, placed demineralized freeze-dried cortical osseous graft material into and over the extraction socket and covered it with a non-resorbable barrier membrane. A large percentage of the placed bone graft was lost during the 4-month healing period. The loss of augmented width ranged from 39.2% to 67.4% (Simon et al. 2000). These findings emphasize the negative impact of any additional trauma on the buccal bone plate at the time of tooth extraction. Our group recently demonstrated that supplementary surgical trauma during tooth extraction – i.e. incisions, flap elevation, suturing – is followed by approximately 0.5–0.7 mm more volumetric alteration in particular at the buccal aspect compared with a ''flapless'' extraction procedure (Fickl et al. 2008b). After elevation of a muco-periosteal flap for tooth extraction, osteoclasts were present in the exposed area of the outer alveolar ridge 1 and 2 weeks after tooth extraction (Araújo & Lindhe 2005). Most recently Araújo and Lindhe reported on hard tissue healing 6 months following tooth extraction with or without the prior elevation of mucosal full-thickness flaps (Araújo & Lindhe 2009). The authors reported no difference with respect to hard tissue loss between the two treatment groups. These results may be seen somewhat controversial to the

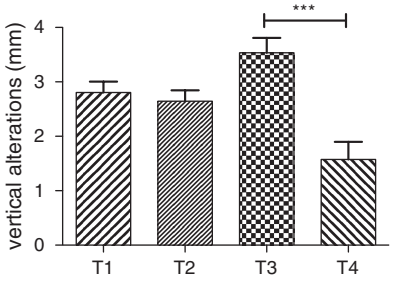

Fig. 11. Histogram describing the mean vertical distance between lingual bone crest and buccal bone crest.

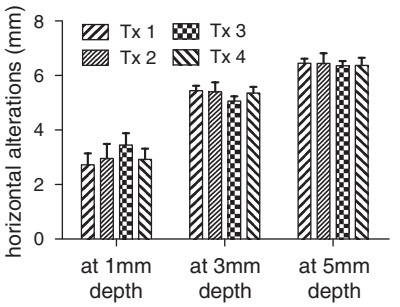

Fig. 12. Histogram describing the mean horizontal width at values 1, 2 and 3 for the different treatment groups.

Table 1. Mean vertical distance between the buccal and lingual bone crest and horizontal width at the different levels below the lingual bone crest

Treatment	Vertical distance between the margins of original buccal and lingual crests (mm)	Horizontal measurement (mm)		
		at 1 mm in depth	at 3 mm in depth	at 5 mm in depth
T1	2.8	2.7	5.4	6.5
SD	0.7	0.9	0.4	0.3
T2	2.6	2.6	5.2	6.1
SD	0.8	0.7	0.6	0.6
T3	3.5	3.5	5.1	6.4
SD	1	0.9	0.3	0.4
T4	1.6	3.3	5.6	6.7
SD	1.2	1	0.5	0.6

above-described data, however, Araújo & Lindhe (2009) also stated that ''the buccal aspect of the mesial tooth portion in the flap group had undergone more attachment and bone loss than was the case in the corresponding site of the flapless group'' . It may be concluded that the effect of flap elevation following tooth extraction remains controversial. However, in this study it seems that the effect of invasive over-augmentation procedures was nullified by an additional resorption of the buccal bone plate induced by supplementary trauma applied to the buccal tissue during the extra intervention. Yet the distinctive biologic mechanisms and reasons remain unclear.

However, the integration of a connective tissue graft into a supraperiosteal buccal pouch demonstrated significantly less bone loss of the buccal bone plate compared with the other groups. As a high standard deviation was assessed for that group, these results should be analysed with caution and may be explained by the limited amount of experimental sites. Nevertheless a loss of buccal bone plate was reported for that treatment group indicating that the biologic procedure after tooth extraction could not be prevented. This may be seen in concordance with the clinical study of Costich & Ramfjord (1968), who found signs of resorption in histological sections up to 6 weeks after gingivectomy and also up to 4 weeks after split-thickness flaps (Costich & Ramfjord 1968). Furthermore Pfeifer (1965) observed histologically an increased osteoclastic activity 7, 14 and 21 days after apically repositioned flaps and split thickness flaps (Pfeifer 1965). It must be assumed that both the preparation of a muco-periosteal and a mucosa flap induce bone re-modelling, which may be seen as an additional trauma supplementary to tooth extraction.

In conclusion within the limitations of this animal study, surgical techniques to overbuild the extraction socket at time of tooth extraction failed to preserve the width of the alveolar ridge. It may be speculated that additional trauma during tooth extraction may aggravate the resorption process of the extraction socket. However, with respect to vertical alterations, the integration of a connective tissue portion seemed to be somewhat resorption protective. Hence further studies evaluating the progress of modelling and re-modelling following socket preservation and additional grafting procedures could clarify the distinctive reasons for the observed volumetric alterations.

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Clinical Relevance

Scientific rational for this study: The goal of the present study was to determine the effect of an additional extra-socket graft on the buccal bone plate during socket preservation techniques.

Principal findings: No difference could be found between the experimental treatment groups (buccal hard or soft tissue augmentation and socket preservation) and the control group (socket preservation alone) concerning horizontal bone dimensions 4 months after the surgical intervention. The use of an additional soft tissue graft inserted into a buccal split-thickness punch yielded less vertical resorption of the buccal bone plate compared with the other groups.

Practical implications: Within the limits of this study an additional extra-socket graft seems to be ineffective at time of tooth extraction. The additional surgical trauma seems to aggravate the bone resorption thus nullifying the augmentative effect.