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Bone loss after full-thickness and partial-thickness flap elevation

Stefan Fickl[1], Moritz Kebschull[2], Peter Schupbach[3], Otto Zuhr[4], Ulrich Schlagenhauf[1], Markus B. Hürzeler[4,5]

[1]Department of Periodontology, Julius-Maximilians-University, Würzburg, Germany; [2]Department of Periodontology, Operative and Preventive Dentistry, University of Bonn, Bonn, Germany; [3]Schupbach Histology, Research Laboratory for Biomaterials, Horgen, Switzerland; [4]Private Office, Munich, Germany; [5]Department of Operative Dentistry and Periodontology, Albert Ludwigs University, Freiburg, Germany

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Abstract

Objectives: The aim of this study was to histologically assess whether elevation of partial-thickness flaps results in reduced bone alterations, as compared with full-thickness flap preparations.

Material and Methods: In five beagle dogs, both mandibular second premolars (split-mouth design) were subjected to one of the following treatments: Tx1: elevation of a partial-thickness flap over the mesial root of P_2 and performing a notch at the height of the bone. Tx2: elevation of a full-thickness flap over the mesial root of P_2 and performing a notch at the height of the bone. After 4 months, sections were evaluated for: (i) vertical bone loss and (ii) osteoclastic activity using histometry.

Results: Elevation of both full- and partial-thickness flaps results in bone loss and elevated osteoclastic activity. Partial-thickness flaps can result in less bone loss than full-thickness flaps, but are subject to some variability.

Conclusion: Use of partial-thickness flaps does not prevent from all bone loss. The procedure may result most of the times in less bone loss than the elevation of full-thickness flaps. Further research has to evaluate the determinants of effective outcomes of partial-thickness flap procedures.

Key words: bone loss; full-thickness flap; partial-thickness flap

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Muco-periosteal flaps are used to obtain access to the bone and root surface in periodontal, endodontic and oral surgical procedures. During the dissection procedure, the soft tissues are incised and the periosteum is usually separated from the alveolar bone proper. Experimental studies in animals and humans have demonstrated that the exposure of the alveolar bone by flap elevation induces osteoclastic activity and results in bone resorption (Donnenfeld et al. 1964, Pfeifer 1965, Tavtigian 1970, Wood et al. 1972, Staffileno 1974, Bragger et al. 1988). In two clinical re-entry studies, a mean crestal bone loss after full-thickness flap elevation of 0.6 mm (Donnenfeld et al. 1964) and 0.47 mm (Tavtigian 1970) was reported. Bragger et al. (1988) compared the bone density after surgical and non-surgical periodontal therapy. They concluded that in 69% of the surgical groups and 34% of the non-surgical groups a loss of density was evident, indicating bone-remodelling. Most recently, it was demonstrated that when a full-thickness flap is raised during tooth extraction, an additional 0.7 mm of tissue alteration has to be expected (Fickl et al. 2008).

Conflict of interest and source of funding statement:

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The authors declare that they have no conflicts of interest.

Flap elevation by means of a split-thickness flap has also been evaluated in literature (Pfeifer 1965, Wood et al. 1972, Staffileno 1974). Pfeiffer investigated the reaction of the alveolar bone to mucoperiosteal full-thickness and split-flap techniques in man (Pfeifer 1965). In both flap procedures, osteoclastic activity was observed. Although a considerable amount of osteoclastic activity was evident along the entire length of the alveolar bone when a muco-periosteal flap was raised, there was less osteoclastic activity with the split-flap technique (Pfeifer 1965). Wood et al. (1972) compared crestal radicular bone response to full- and partial-thickness flaps. Regardless of the flap procedure, crestal bone loss was demonstrated.

To date, it is unclear whether leaving the periosteum on the bone is effective to limit bone loss after flap elevation, and therefore, whether the use of partial-thickness flaps over full-thickness flaps results in a clinically relevant advantage. Thus, the aim of this experimental animal study is to assess whether partial-thickness flap elevation results in reduced bone alterations, as compared with full-thickness flap procedures.

Material and methods

The research protocol of this and a previously published investigation was approved by the ethical committee of Biomatech (Namsa Company, Lyon, France) (Fickl et al. 2009). The same five beagle dogs used for Fickl et al. (2009) were utilized 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).

A priori calculation of sample size and statistical power

Sample size for this study was based on measurements of the primary outcome, vertical bone loss, derived from our own published data (Fickl et al. 2008). In an identical setting (the same surgeon, identical instruments, the same animal strain), a mean bone loss after full-thickness flap elevation of 0.7 ± 0.3 mm was found (Fickl et al. 2008). We hypothesized that elevation of a partial-thickness flap would result in reduced bone loss of clinically relevant (>0.5 mm) extent. Using these data in a power calculation for a paired (split-mouth) comparison, a study of four pairs was determined to be sufficiently powered (80%) to detect a clinically relevant difference.

Fig. 1. An intra-sulcular incision is combined with a vertical releasing.

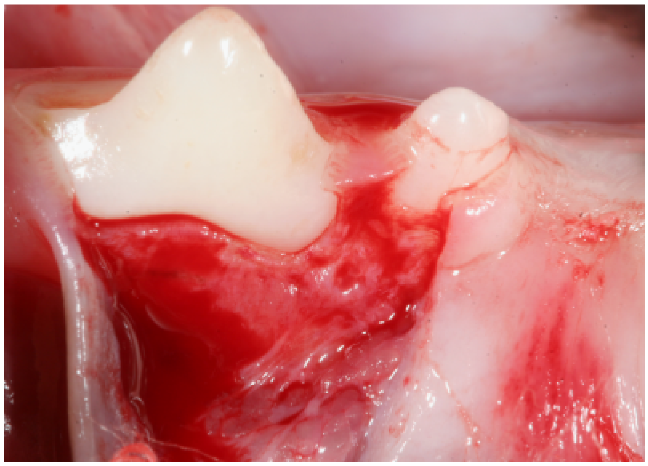

Fig. 2. Tx1: A partial thickness flap is elevated at the mesial root of the second premolar.

Surgical procedure

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

In both quadrants of the mandible, the mesial root of the second premolar (P_2) served as experimental sites. One dog presented with a missing second premolar.

Tx1 ($n = 5$): An intra-sulcular incision was performed initiating from the distal line angle of P_2 and extended to the facial aspect of P_1 (Fig. 1). Consecutively, a vertical releasing incision was performed and extended beyond the muco-gingival junction. A partial-thickness flap was raised using a 15c blade in an equal tissue thickness of 1.5 mm (Fig. 2). A notch was performed into the facial aspect of the mesial root of P_2 at the height of the buccal bone plate. The flap was repositioned using interrupted sutures (Gore-Tex® CV5, W.L. Gore & Associates, Putzbrunn, Germany).

Tx2 ($n = 4$): An intra-sulcular incision was traced starting from the distal line angle of P_2 and extended to the facial aspect of P_1. Consecutively, a vertical releasing incision was performed and extended beyond the muco-gingival junction. A full-thickness flap was raised using a periosteal elevator (Fig. 3). A notch was performed into the facial aspect of the mesial root of P_2 at the height of the buccal bone plate. The flap was repositioned using interrupted sutures (Gore-Tex® CV5, W.L. Gore & Associates).

Post-surgical procedure

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 per day per os for 13 days (Stomorgyl®, Merial).
- Anti-inflammatory drug: carprofene 50 mg per os and per day 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 months.

The sutures were removed 2 weeks post-surgery. Healing presented uneventful.

Termination procedure

The animals were sacrificed 4 months after surgery. The animals were weighed and anaesthetized by an intramuscular injection of Zoletil (Virbac – 5–10 mg/kg intramuscular). An injection of heparin 25,000 IU (Leo Pharmaceutical, Saint Quentin Fallavier, France – 100 IU/kg) was administered to each animal. The animals were sacrificed by a lethal injection of a barbiturate (Dolethal®, Vetoquinol, Paris, France).

Histological procedure

The fixed samples were dehydrated in a graded series of ethanol and infiltrated with a Technovit 7200VLC resin (Kulzer, Friedrichsdorf, Germany). Infiltrated specimens were placed into embedding moulds, and polymerization was performed under blue light. Polymerized blocks were initially cut buccolingually into two halves and glued to a Plexiglas slide using Technovit 4000 resin (Kulzer). After polishing, the specimens were cleaned with benzene and the surface was glued to a second glass slide in vacuum. Subsequently, a 100 μm thick section attached to the second slide was sawed by using a diamond blade saw and 50–100 g pressure. Grinding and final polishing steps with 1200-, 2400-, and 4000-grit sandpaper achieved a final thickness of 50 μm. Three sections from each block were used for toluidine blue/Pyronin G staining. The sections were evaluated and photographed using both a Leica stereomicrscope MZ16A and a Leica DM600B light microscope (Leica, Wetzlar, Germany).

Histometrical analysis

Histometrical evaluations were performed using ImageAccess software (Imagic, Glattbrug, Switzerland). The following measurements were conducted in all three sections (Fig. 4):

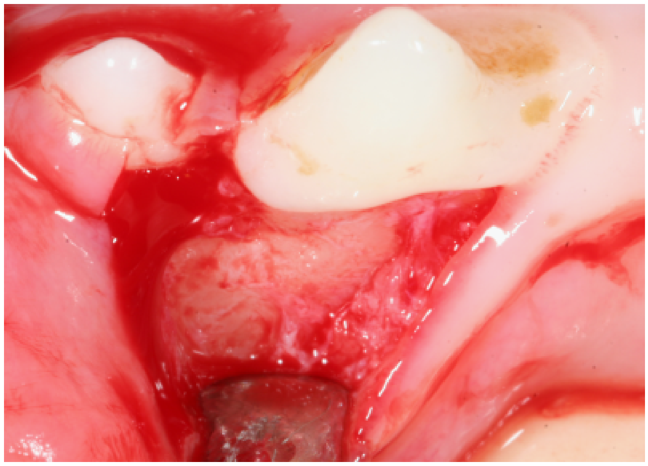

Fig. 3. Tx2: A full-thickness flap is elevated at the mesial root of the second mandibular premolar.

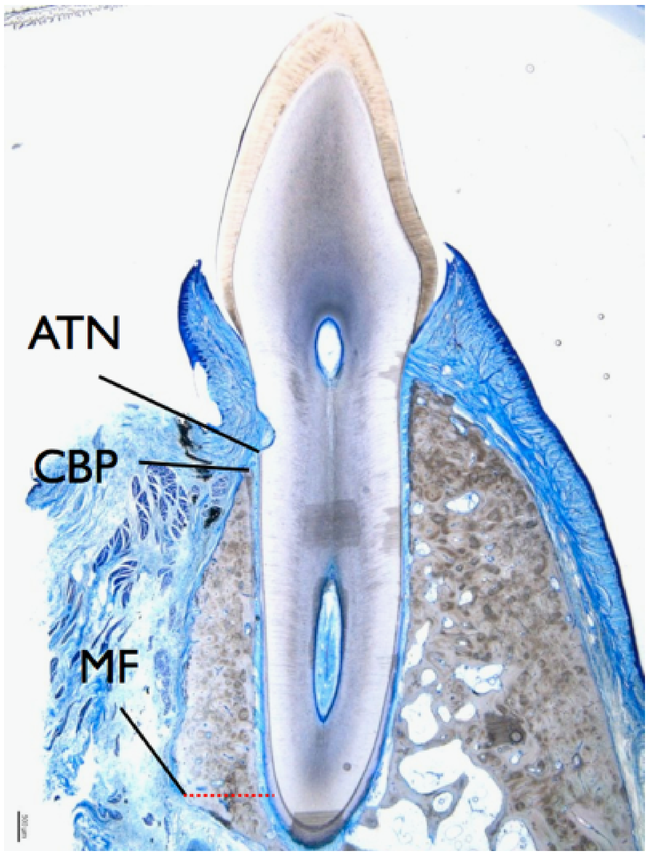

Fig. 4. The distance in mm between the apical termination of the notch (ATN) and the first peak of bone (CBP) was measured and expressed in mm. Areas of osteoclastic activity were measured in mm between ATN and the mental foramen (MF) and expressed in mm.

1. The distance in mm between the apical termination of the notch (ATN) and the first peak of bone (CBP) was measured and expressed in mm. Mean values were calculated per specimen.
2. Areas of osteoclastic activity were calculated between the apical termination of the notch (ATN) and coronal aspect of the mental foramen (MF). The cross-section dimension of each region of osteoclastic activity was assessed in its maximum circumference in mm. Measurements in all three sections were summarized and mean values were calculated.

Statistical analyses

After data collection, the raw data were subjected to a *post hoc* power calculation to determine whether a statistical analysis using a statistical test was appropriate. In appropriately powered comparisons, testing of our hypothesis (less bone loss and remodelling after partial- than full-thickness flaps) was performed using paired, one-tailed *t*-tests utilizing GraphPad Prism V (GraphPad Software, La Jolla, California, USA). In all comparisons, a *p*-value of <0.05 was considered statistically significant.

Results

Light microscopic observations

The light microscopic evaluation of the specimens showed the presence of typical resorption areas along the buccal aspect of the alveolar bone (Figs 5 and 6). These resorption areas were filled by newly formed bone. However, single osteoclastic lacunae still can be recognized (Fig. 7). The number of areas undergoing bone resorption was variable between the different specimens.

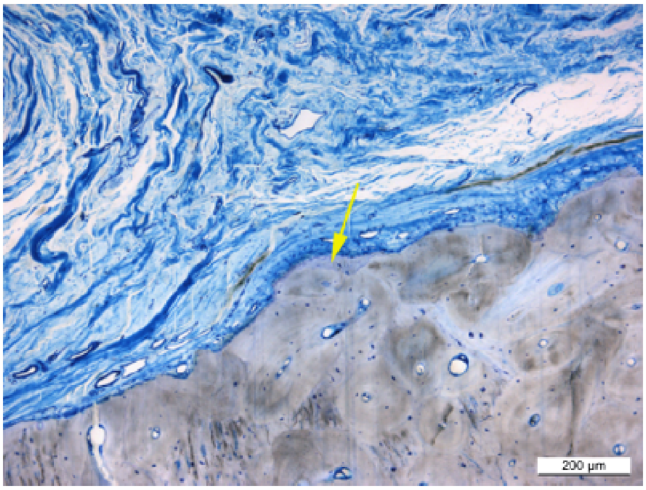

Fig. 5. Single area of bone resorption and remodelling with newly formed bone (yellow arrow) indicating a low osteoclastic activity.

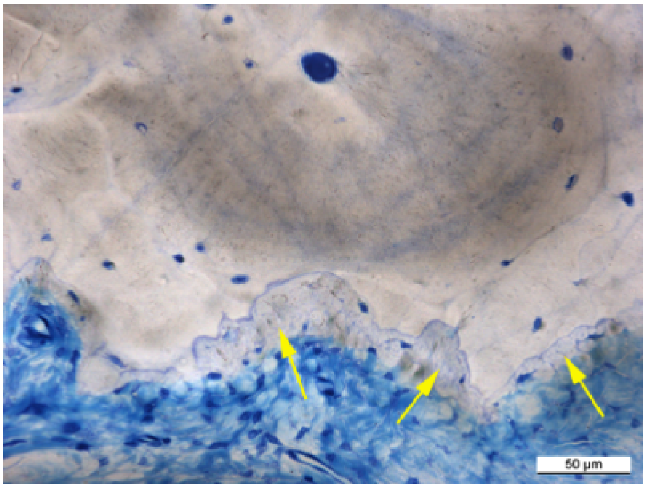

Fig. 6. Numerous areas of bone resorption filled with newly formed bone (yellow arrows) indicating a high osteoclastic activity.

Histometrical measurements

The vertical distance between the apical termination of the notch and the peak of the bone crest showed considerable inter-individual variability (Fig. 8a). The vertical distance was 0.16 ± 0.44 mm for Tx1 and 0.28 ± 0.50 mm for Tx2, respectively.

Osteoclastic activity occupied 2.27 ± 0.72 mm of the buccal bone surface for Tx1 and 3.52 ± 0.52 mm for Tx2, respectively (Fig. 8c).

However, analysis of the individual pairs revealed that the observed high variability is due to a single outlier, dog #3. In this animal, the elevation of a split-thickness flap resulted in considerably more bone loss and osteoclastic activity than the elevation of a full-thickness flap. The other three pairs showed an inverse behaviour, with more bone loss and remodelling in the full-thickness group. However, the amount of bone loss and osteoclastic activity in the partial-thickness group of dog #3 is comparable with the three other pairs. If dog #3 is excluded, the comparison was sufficiently powered (77% for vertical bone loss, 99.2% for osteoclastic activity) to perform statistical testing of our hypothesis – less bone loss and osteoclastic activity after partial-thickness than full-thickness flap elevation – using paired, one-tailed *t*-tests. Indeed, a both clinically and statistically significant difference could be observed for vertical bone loss (Fig. 8b, 0.12 ± 0.38 *versus* 0.64 ± 0.14 mm). Similarly, the observed osteoclastic activity was significantly lower in the partial-thickness than in the full-thickness group (Fig. 8d, 1.96 ± 0.47 *versus* 4.2 ± 0.95 μm).

Discussion

This study was conducted to assess the clinical relevance of two different flap elevation procedures for the post-operative resorption of the underlying alveolar bone. Based on observations by Pfeifer (1965), the use of partial-thickness flaps was recommended over full-thickness flaps to preserve alveolar bone from resorption. In this study, we hypothesized that a partial-thickness flap would result in a clinically significant improvement in post-operative bone loss, and designed our study to be adequately powered to detect such a difference.

The results of the present study demonstrate that the elevation of both a full-thickness and a partial-thickness flap induces vertical bone loss and resorptive alterations of the underlying bone structure. These findings could be seen in accordance with Pfeiffer and co-workers who demonstrated that both full- and partial-thickness procedures were followed by osteoclastic activity (Pfeifer 1965). Wood et al. (1972) showed that regardless of the flap procedure, crestal bone loss was evident. Costich and Ramfjord (1968) found signs of resorption in histological sections up to 6 weeks after gingivectomy and up to 4 weeks after split-thickness flaps (Costich & Ramfjord 1968).

The effects of the different surgical techniques for flap elevation on the primary outcome, vertical bone loss, and the secondary outcome, osteoclastic resorption were less clear at first. In contrast to our recent study (Fickl et al. 2008), we observed a pronounced variability, inherently reducing the power of our analyses and rendering statistical testing inappropriate. However, a more thorough investigation of our paired data sets revealed that a single pair was in fact responsible for a large proportion of the observed variability. Nevertheless, the exclusion of the outlier should be evaluated with caution, as the result of the partial-thickness flap group of this pair was coincident with results from another dog. Still, analyses performed after excluding the outlier support our hypothesis of a beneficial effect of partial-thickness flap elevation on vertical bone levels. Importantly, the observed difference in bone loss after removal of the outlier of 0.52 mm can be considered clinically relevant.

However, these findings must be considered with great caution. Even in the well-controlled experimental setting utilized in this study, the use of a partial-thickness flap resulted in more vertical bone loss and osteoclastic resorption in one of the four experimental pairs (25% of the study sample). After removal of the outlier from the experimental groups, a 2.5-fold higher standard deviation was still observed in the split-thickness group, as compared with data from the full-thickness group. Likely, this variability might be due to the technical difficulties of partial-thickness flap elevation. It is well established that the thickness of the connective tissue portion remaining on the bone and covering the periosteum can determine the successful outcome of the partial-thickness flap procedure (Pfeifer 1965, Wood et al. 1972), while the periosteum *per se* gives only a limited degree of protection to the underlying bone after removal of the epithelial covering. It was concluded that unless a definitive layer of connective tissue was left on top of the periosteum, the tendency was for the thin periosteum to undergo necrosis with

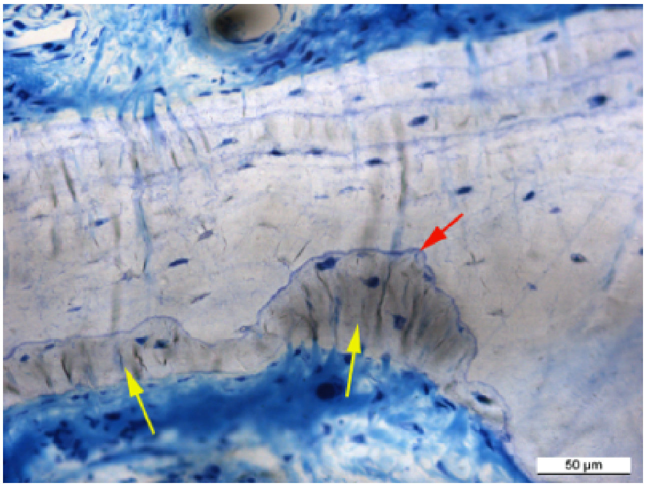

Fig. 7. Higher magnification of an area of bone resorption filled with newly formed bone (yellow arrows). Note the presence of a single osteoclastic lacuna (red arrow).

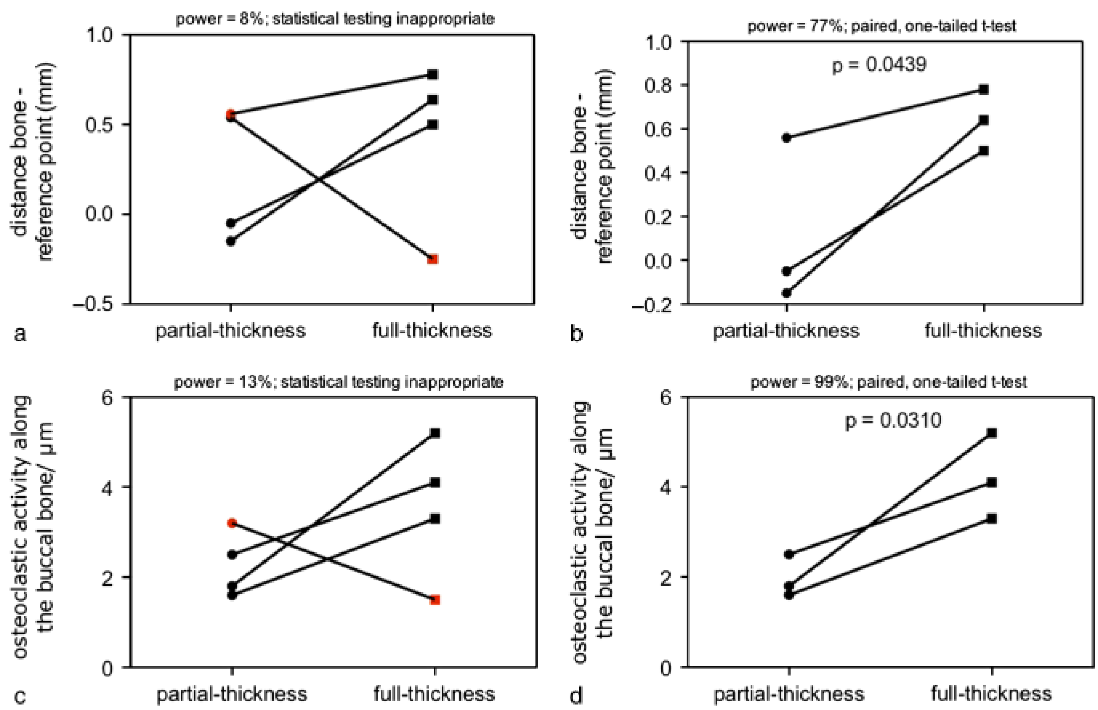

Fig. 8. Diagram indicating the vertical distance from reference point to the peak of bone (a) and the osteoclastic activity along the buccal aspect (c). Please note the high standard deviation and very low power that forbids statistical testing. Reduced dataset after removal of the outlier (b/d) and statistical testing using paired, one-tailed *t*-tests.

subsequent healing as occurred after full flap and bone denudation.

On the other hand, the data can also be interpreted in this way, that the excluded outlier (as seen in Fig. 8) might represent a true success of a full-thickness flap, as no bone loss could be assessed following full flap elevation and the partial-thickness elevation resulted in bone loss comparable with another specimen. Regarding the reasons for this inverse behaviour in one out of four groups, it might be speculated that there are more influencing factors than only the elevation or the thickness of the flaps that determine the amount of bone loss following flap preparation. For example, it was reported that the location of the incision might also be an important factor for inducing bone resorption (Binderman et al. 2001). It was shown that when the incision for flap elevation was performed apically to the periodontal ligament, no significant bone loss was observed. Yet, an incision in the marginal gingiva for flap elevation induced marked bone remodelling (Binderman et al. 2001). It was speculated that not the elevation of a mucoperiosteal flap per se induces bone remodelling, but rather the injury to the marginal gingiva that triggers bone loss after flap elevation (Binderman et al. 2001). Other investigations also report on significant bone loss initiated on the periodontal ligament aspect of the alveolar bone when the marginal gingiva was incised in a coronal approach (Yaffe et al. 1994). These observations cannot be explanative for the inverse behaviour of one of the experimental pairs in the present study, but might clarify that multiple factors play a modifying role, which are not entirely clear to date.

We therefore conclude that the present study was able to demonstrate that both full- and partial-thickness flaps induce bone remodelling. It might also be assumed that the partial-thickness flap technique can have a certain potential to be superior over full-thickness flaps concerning preservation of alveolar bone. On the other hand, the observed standard deviation indicates that the full-thickness flap technique is less subject to variability, but may also have the potential, as seen in one out of four pairs, to result in complete bone preservation. Thus, before any clinical recommendations can be drawn, further studies in animals and humans are needed (i) to replicate our findings, (ii) to determine the exact predictors for an effective partial-thickness flap procedure and (iii) to evaluate further influencing factors for bone following flap elevation.

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Address:
Dr. Stefan Fickl
Department of Periodontology
Julius-Maximilians-University Würzburg
Pleicherwall 2
97070 Würzburg
Germany
E-mail: fickl_s@klinik.uni-wuerzburg.de

Clinical Relevance

Scientific rational for this study: The goal of the present study was to determine whether partial-thickness flaps are advantageous over full-thickness flaps.

Principal findings: Both treatment groups induced bone remodelling. Partial-thickness flaps may have the potential to result in less bone loss than full-thickness flaps.

Practical implications: Partial-thickness flaps can result in less pronounced bone loss after flap elevation; however, a possible variability can be expected.