Flap versus flapless procedure for ridgepreserva alveolar extraction sockets: a histological evalu randomized clinical trial

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Abstract

Objective: The aim of this study was to evaluate and compare the histological and histomorphometric features of two different procedures carried out in extraction socket grafting; namely, the flapped and flapless technique.

Materials and methods: Patients considered eligible for the study were randomized to receive tooth extraction and ridge preservation with the porcine bone and collagen membrane, with a fullthickness mucoperiosteal flap and primary soft tissue closure (control group), or, with a flapless procedure and a secondary soft tissue closure (test group). After 3 months of healing, the surgical re-entry procedure was performed and implants were inserted in the test as well as in the control sites. Bone core samples were harvested from both groups and processed to be observed under light microscopy. Outcome variables were percentages of newly formed bone, residual graft particles and marrow spaces.

Results: Thirty-four patients were enrolled in the study. All of the scheduled implants were placed.Histological and histomorphometrical analyses did not report significant differences between the two groups (with *P*-values ranging from 0.690 to 0.917). The mean percentages of newly formedbone, soft tissues and residual grafted particles were 22.5 and 22.5%, 59.3 and 59.4%, and 18.6 and 18.2% respectively for flap and flapless approach.

Conclusion: No histological and histomorphometrical differences were observed when comparing the flap and the flapless technique for tooth extraction and socket grafting procedures.

The treatment of extraction sockets is a daily challenge for the clinical practice. Several bone dimensional changes occur after tooth extraction given that the alveolar process is a tooth-dependent tissue (Barone et al. 2008). The preservation of the alveolar ridge is rec- ommended to maintain the existing soft and hard tissues, to preserve a stable ridge volumeand to simplify the subsequent rehabilitation treatments, either for implant placement or for the traditional prosthetic restorations (Darby et al. 2009; Hämmerle et al. 2012; Novaes et al. 2012; Vignoletti et al. 2012). Bone modelling and remodelling are unavoidable during the healing of an extrac- tion socket (Darby et al. 2009; Barone et al. 2012, 2013; Vignoletti et al. 2012); many authors have pointed out that most of the resorption occurs during the first 3 months, although dimensional changes have beenobserved up to 1 year after a tooth extraction (Schropp et al. 2003; Araujo & Lindhe 2005; Vignoletti et al. 2012). The resorption of the alveolar ridges showed the greatest amountof bone loss in the horizontal dimension anda concomitant loss of vertical ridge height, which has been reported to be more evidentat the buccal level (Fickl et al. 2008a; Covani et al. 2011; Vignoletti et al. 2012). Morpho- logical changes of the extraction sites resulted in a narrower and shorter ridge; moreover, the alveolar crest shifted lingually/ palatally according to a specific pattern.Some clinical data indicated that the alveolar crest tends to move two-thirds lingually/pala- tally from the original buccal edge, thus the re-absorption at the mid-facial point repre-sented the double of bone loss at the distaland the mesial points (Covani et al. 2011). As, over the last years, aesthetic outcomes have received more emphasis in implant treatment planning, the resorption of the alveolar ridge has become a clinically rele- vant problem and may cause failing aesthetic outcomes with an implant-supported crownand/or bridge. Indeed, adequate architectureof the alveolar bone and soft tissues are required to obtain a functional and aesthetic prosthetic rehabilitation (Buser et al. 1993, 2008; Darby et al. 2009; Barone et al. 2011; Kan et al. 2011). The ridge preservation procedure is recom- mended in the following conditions: when the implant placement is not possible at the time of tooth extraction; when the patient is not available for an immediate implant place- ment; when primary stability of the implant cannot be obtained; and when adolescent patients should be treated (Hämmerle et al. 2012). A recent consensus report (Hämmerle et al. 2012) assessed that it is important to distinguish between the various procedures used to preserve the alveolar ridge. The "ridge preservation" techniques include all the procedures that preserve the ridge volume within the envelope existing at the time of extraction (Hämmerle et al. 2012). Several studies (Fiorellini & Nevins 2003; Barone et al. 2011) demonstrated that implants placed in grafted bone had a survival rate similar to implants placed in native bone. The ridge preservation technique allowed wider and longer implant placement when compared to non-augmented sockets, and, therefore, reduced the need for simultaneous augmentation procedures at the time of implant placement (Darby et al. 2009; Baroneet al. 2011). The use of various techniques and bioma- terials has been proposed over the years; however, no significant differences have beenshown between the different biomaterials, although collagen alone did not prove to be suitable to counteract tissue changes after tooth extraction (Farina et al. 2009; Oghli & Steveling 2010; Barone et al. 2011, 2012,2013; Vignoletti et al. 2012). A muco-periosteal flap reflection – with its interruption of vascular supply to underlying bone — during tooth extraction may have accounted for the slightly more pronounced bone remodelling of the alveolar ridge, when compared to a flapless extraction (Fickl et al. 2008b; Engler-Hamm et al. 2011; Novaeset al. 2011; Canullo et al. 2012). However, nofirm conclusions could be drawn on the advantages of flapless versus flap elevation during tooth extraction. Moreover, it shouldbe taken into

consideration that soft tissueprimary closure was originally believed to be necessary for proper incorporation of the graft(Lekovic et al. 1997, 1998; Fickl et al. 2008a;Darby et al. 2009). The early exposure of the membrane to the oral cavity was thought to be a complication which could jeopardise the effectiveness of tissue augmentation (Simion et al. 1997; Engler-Hamm et al. 2011); these findings pointed out the importance of achieving full closure and primary healing when the socket is grafted and covered witha membrane (Darby et al. 2009).In actual fact, the effect of flapless/flapped surgery on the healing process is still contro- versial, with results from experimental mod- els reporting less pronounced bone remodelling of the alveolar ridge after socket preservation using a flapless approach (Fickl et al. 2008b). However, other authors did not report any significant difference between the flapless and flapped approach (Araujo & Lind-he 2009). The objective of this study was to investi- gate and to compare the effect of soft tissue primary closure on the bone healing ofextraction sockets grafted with a xenograftand a collagen membrane. The histologic and histomorphometric examinations of grafted extraction sockets, where a mucoperiosteal flap was coronally moved to obtain a soft tissue primary closure, were compared to those of extraction sockets where no flap was raised and the collagen membrane was left intentionally exposed to the oral cavity. This study reported histological outcomes up to 3 months after grafting. The clinical outcome of this trial has been reported in a recent arti- cle (Barone et al. 2014). Furthermore, to eval-uate the success of the procedure over time, the patients were to receive a follow-up until the fifth year. This study was reported according to the CONSORT guidelines (Appendix S1) (Moher et al. 2010).

Material and methods

Study population and design

Patients requiring at least one single premo- lar or molar tooth extraction and subse- quently an implant-supported restoration, and who were 18 years old or older and ableto sign an informed consent form, were eligi- ble for inclusion in this trial. The criteria for exclusion were as follows:

- History of systemic diseases that wouldcontraindicate oral surgical treatment.
- Long-term non-steroidal anti-inflamma- tory drug therapy.
- Lack of opposite occluding dentition inthe area intended for extraction and subsequent implant placement.
- Intravenous and oral biphosphonate ther-apy.
- The absence of adjacent teeth.
- Sockets with a complete loss of a bonewall.
- Presence of severe untreated periodontal disease.
- Unwillingness to return for the follow-upexamination.
- Use of more than 10 cigarettes per day.
  Subjects smoking <10 cigarettes per day were requested to stop smoking before and after surgery; however, their compli- ance could not be monitored.

Patients were recruited from the consulta- tion clinic at the Dentistry Department of Versilia General Hospital, University of Pisa, from January 2010 to September 2011. The study was approved by the Ethics Committee of the Versilia General Hospital, Lido di Camaiore, Italy. The principles outlined in the declaration of Helsinki on clinical research involving human subjects were adhered to. All patients received thorough explanations and had to complete a written informed consent form prior to being enrolledin the trial. Patients who were included inthe study were accurately evaluated by exam- ining clinical aspects and periapical/pano- ramic radiographs; moreover, data were collected for each patient such as age, gender,smoking habits, and indications for tooth extraction based on both clinical and radio- graphic examination, tooth location and presence/absence of adjacent teeth. After the consent form had been signed, all patients underwent at least one session of oral hygiene prior to the extraction procedures to provide a more favourable oral environment for wound healing. All patients received tooth extraction and a ridge preservation pro- cedure at baseline; 3 months after tooth extraction, all sites were re-entered, bone biopsies were taken and implants were inserted. Extraction sockets were randomly allocated to either a test (no flap with a sec- ondary soft tissue healing) or control (flapelevation and primary soft tissue closure) group using a computerized random alloca- tion process. The randomized codes were enclosed in sequentially sealed envelopes. Immediately after tooth extraction, the enve- lopes were opened and indicated to the sur- geon to include the extraction socket as a test or a control site according to the ran-domization list. The treatment allocation was concealed to the clinician who was involved in enrolling and treating the patients included in this trial. The clinician (GI) involved in the histologic and histomor- phometric examination was blinded to group allocation.

Surgical treatment

All patients received antibiotic therapy (2 g of amoxicillin or 600 mg clindamycin — if allergic to penicillins) 1 h before the extrac- tion procedure and continued to take the antibiotic postoperatively (1 g amoxicillin or 300 mg clindamycin) twice a day for 4 days. All patients rinsed for 1 min with chlorhexi- dine mouthwash 0.2% prior to the surgery(and twice a day for the following 3 weeks), and were treated under local anaesthesia using lidocaine with adrenaline 1 : 50.000. All surgical procedures were undertaken by one surgeon (AB). All the patients were trea- ted with the same surgical technique and periotomes were used around each single selected tooth. Moreover, ultrasound bone surgery (Piezosurgery, Mectron, Italy) was performed where necessary to avoid bucco- lingual movements, thus preventing damage or full fracture to the facial bone wall. The extraction socket was thoroughly curetted and irrigated with sterile saline solution. Extraction sockets allocated in the test group were filled and slightly condensed with corticocancellous porcine bone (MP3, Osteobi- ol, Coazze, Italy), and a trimmed collagen membrane (Evolution; Osteobiol, Coazze,Italy) was used to completely cover the socket;the soft tissues were only undermined and no releasing incisions were performed. The colla-gen membrane was intentionally left exposed to the oral cavity and sutures were used to sta-bilize the membrane. Extraction sockets allocated in the control group had a full thickness mucoperiosteal flap with two releasing incisions, and cortico-cancellous porcine bone and collagen mem- brane were applied; subsequently, the buccal flap was advanced coronally to allow a soft tissue primary closure. All patients were

instructed to continue with prophylactic antibiotic therapy, and naproxen sodium 550 mg tablets were prescribed as an anti-inflammatory to be taken two times a day for as long as required. Removable prostheses, if present, were not permitted for use until they had been adjusted and refitted no sooner than 3 weeks after surgery..After 3 months of healing, the surgical re-entry procedure was performed andimplants (Intralock®, Boca-Raton, FL, USA) were inserted in test as well as in control sites. Surgical trephine burs were used to har- vest bone core samples from the augmented socket sites. After harvesting the bone samples, the osteotomy site was prepared according to the implant system manufacturer's recommenda-tions. Patients received the same drug prescription as that prescribed after the initial surgery. The bone cores were coded and sent for analysis to the Department of Medical,Oral and Biotechnological Sciences, Univer- sity of Chieti-Pescara, Italy. After 4 months, implants were manually tested for stability and impressions were taken using polyvinyl-siloxane impressionmaterial (Flexitime; Heraeus/Kulzer, Hanu, Germany) and customized resin impressiontrays. Final prosthetic restorations were cemented and patients were enrolled in anoral hygiene programme, with a recall visit every 3 months.

Histological analysis

The bone cores were retrieved, immediately stored in 10% buffered formalin and then processed to obtain thin ground sections. The specimens were processed using the Precise 1Automated System (Assing, Rome, Italy) (Pi-attelli et al. 1997). The specimens were dehy- drated in a graded series of ethanol rinses and embedded in a glycolmethacrylate resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). After polymerization the speci-mens were sectioned, along their longitudinal axis, with a high-precision diamond disc at about 150 lm and ground down to about 30 lm with a specially designed grinding machine. Three slides were obtained from each specimen. These slides were stained with acid fuchsin and toluidine blue and examined in transmitted and polarized light using a Leitz Laborlux® microscope (Leitz, Wetzlar, Germany). Histomorphometry of the percentages of newly formed bone, residual grafted material and marrow spaces was carried out using a light microscope (Laborlux S; Leitz) con-nected to a high resolution video camera (3CCD, JVC KY-F55B; JVC®, Yokohama, Japan) and interfaced to a monitor and PC (Intel Pentium III 1200 MMX; Intel®, Santa Clara, CA, USA). This optical system was associated with a digitizing pad Matrix (Vision GmbH, Oppenweiler, Germany) and a histometric software package with image capturing capa- bilities (Image-Pro Plus 4.5; Media Cybernet-ics Inc., Immagini& Computer Snc Milano, Italy). One single well-trained examiner (GI), who was not involved in the surgical treat- ment, evaluated the histological results. The histomorphometric data were obtained from three sections for each specimen.This study aimed to ascertain any signifi- cant differences in the histological outcomes between the two procedures. Outcome mea- sures were as follows: percentages of newly formed bone, residual graft particles and mar-row spaces.

Statistical analysis

To obtain an effective size of the samples, a power analysis was performed; mean and stan-dard deviation reported by preliminary histo-morphometric analysis for similar procedures (without xenograft employment) in animal models, and using a power of 0.9 and a signifi-cance of .05 (Statistics Toolbox, MatLab 7.0.1;The MathWorks, Natick, MA, USA). The sample size calculation was performed using the data related to the percentage ofmineralization of the tissue at 4 months in animal models subjected to flap and flapless procedures (Caneva et al. 2010). The results of the power analysis suggested that a sample size of 68 might be necessary. Normal distribution for each histomorpho- metric variable was carried out, but not con- firmed by the Shapiro–Wilk test with a significance of .05. The possible influence of gender was assessed by Friedman's nonpara-metric two-way Analysis of Variance (ANO- VA) for each of the outcome variables. Pair- wise comparisons were performed by the Wilcoxon rank sum test for unmatched data and *P*-values were obtained; the statistical significance was set at $P = 0.05$. All measurements in the text and tables are described as median and interquartile ranges, m (IQR: the difference between the 75th and 25th percentiles). The data distribution was plotted by a whiskers graph in Fig. 1.

Results

Forty-three patients were initially considered eligible, even though nine patients were not included in the trial for the following rea-sons: two patients were affected by uncon- trolled diabetes, one patient was under treatment with oral bisphosphonates, three patients did not comply with the oral hygiene instructions, one patient refused toattend follow-up visits for the following5 years and two patients had a complete loss of buccal bone wall after tooth extraction. Thirty-four patients, who were 18 years old or older, underwent a tooth extraction with a ridge preservation procedure and a further implant treatment. All the 34 patients were enrolled in the trial and randomized as fol- lows: 17 to the flapless group (test group) and17 to the flap group (control group). The test group received a ridge preservation procedurewithout a flap and a secondary soft tissue healing was left; while, the control groupreceived a ridge preservation procedure witha mucoperiosteal flap to achieve a primary wound closure (Fig. 2). Corticocancellous por-cine bone and a collagen membrane were used to completely cover the extraction socket as grafting material in both experi- mental groups. All the ridge preservation pro-cedures had a successful outcome and implants were inserted in all the experimen- tal sites. The clinical outcome of this trialcan be found in a recent article (Barone et al. 2014). The main baseline patient characteristics were reported in Table 1; the two groups did not show any imbalances.

Histological findings

In all biopsies, trabecular bone was formed over the entire grafted area; grafted material particles were present in all specimens.

Control group

In the control group specimens pre-existing bone was found, which was characterized by remodelling areas, showing cement lines and newly formed bone in close contact with the biomaterial particles (Fig. 3). At higher magnification, most of the bio- material particles were connected by newly formed bone characterized by large osteocyticlacunae. A few biomaterial granules had beenpartially reabsorbed and replaced by newlyformed bone. The newly formed bone wasobserved inside some partially reabsorbed particles. The newly formed bone had a high affinity for dyes and was acid

fuchsine posi- tive, and, therefore, a highly stained line was observed at the grafting material and new bone interface. At an even higher magnification, large osteocytic lacunae were observed (Fig. 4). Collagen fibres with a parallel orien- tation, as occurs in lamellar bone, were seen in the marginal portion of the bone cores close to the pre-existing bone (Fig. 5). No gaps were observed at the bone particle interface and the newly formed bone was always in strict contact with the grafting material. Marrow stromal cells and blood vessels were found inside the marrow spaces. A vascular growth was also observed next to the newly formed bone (Fig. 6). No inflammatory cell or foreign body reaction was noted around the grafted particles.

Test group

In the test group, trabecular bone and resid-ual biomaterial particles were observed. Atlow power magnification, no pre-existingmature bone was found in contact with thegrafted biomaterial particles (Fig. 7). At higher magnification, biomaterial residualparticles of different sizes could be detected.Small and large particles were partially sur-rounded by newly formed bone. Few particlespresented irregularly shaped margins, proba-bly due to a resorption process. There wereno gaps at the bone-particle interface and thenew bone was in strict contact with the gran-ules. Newly formed bone was characterizedby large osteocytic lacunae and bridged upmost part of the biomaterial particles (Fig. 8).Collagen fibres with a parallel orientation, as occurs in lamellar bone, can be observed in the remodelling areas of pre-existing bone (Fig. 9). The marrow spaces of the newly formed bone contained a small number of marrow stromal cells and a vascular network. Some blood vessels were also seen close to the grafted particles (Fig. 10). No inflamma- tory cells or foreign body reaction cells were seen on the biomaterial surface.

Nonparametric two-way analysis of variance showed no statistically significant influence, of gender on the histomorphomet- ric results. This data were verified by nonparametric pair-comparison tests, show- ing no significant differences between the control and the test group, in terms of newly formed bone, marrow spaces and residual graft particles (Table 2). In detail, the analysisshowed that the median of the new bone per- centage in the control group was 21(3), while in the test group it was 21(2); the marrow spaces percentage in the control group was 61(8) while in the test group it was 59(8); andthe percentage of residual grafted particles in the control group was 18(5), while in the test group it was 19(5).

Discussion

Tooth extraction generally results in a loss of bone volume and remodelling of soft tissues (Schropp et al. 2003; Araujo & Lindhe 2005; Barone et al. 2008, 2011; Cardaropoli & Card-aropoli 2008). The socket bone walls will be markedly reduced in height and width; thedimensional changes have been seen to bemore pronounced at the buccal than at thepalatal/lingual bone plates. The ridge preserva-tion procedure allows to counteract the bone loss after tooth extraction (Barone et al. 2008,2012, 2013), even though the bone modelling and remodelling after a tooth extraction is notcompletely avoidable (Fickl et al. 2008a). Thedimensional bone changes occurring after flapand flapless procedures for tooth extraction were reported to be very similar (Araujo & Lindhe 2009), even though contradictory out- comes were observed by other authors (Fickl et al. 2008b) who reported differences in the remodelling of the alveolar process after flap or flapless approaches.

The present randomized clinical trial was performed to evaluate clinical and histologi- cal differences between flap versus flapless tooth extraction and ridge preservation proce-dures. While the clinical findings were reported in a previous publication (Barone et al. 2014) and showed that the flapless technique could preserve the horizontal hard tissues dimension and increase the kerati- nized gingiva more successfully than the flapped technique; this study analyzed the histological differences of the augmented bone.The collagen membrane was covered with an advanced flap in the control sites, whereas no flap was raised and the collagen membrane was left exposed in the test sites. The main finding of this study was that –3 months after ridge preservation – no signifi-cant differences could be found in the histological and histomorphometrical analy-sis when comparing a flap with a flapless approach for ridge preservation. Some authors (Christgau et al. 1997; Piat- telli et al. 1997; Engler-Hamm et al. 2011) have demonstrated that the membrane expo- sure to the oral cavity might cause bacterial penetration and also lower the quantity and quality of bone augmentation (Simion et al. 1997; Oh et al. 2003). On the contrary, some more recent studies have shown that the sec- ondary wound healing with membrane expo- sure did not seem to jeopardise bone regeneration (Cardaropoli & Cardaropoli 2008) in the ridge preservation procedures.Moreover, it should be taken into consider- ation that flap advancement, which is used to obtain a soft tissue primary closure, has been associated with marginal recession at adjacent teeth, defective interdental papilla,loss of keratinized mucosa and a shift of the muco-gingival junction in the coronal direc- tion. The histomorphometric data from thepresent randomized controlled study failed to show differences in the amount of newly formed bone and residual graft particlesbetween the test and the control sites.Therefore, the similarity between the flap and flapless approach supports the hypothesisthat the secondary soft tissue closure and membrane exposure did not affect the quality of bone regeneration. Based on this study,collagen membrane exposure to the oral cav- ity can be recommended, thus allowing a bet- ter preservation of the keratinized mucosa on the facial aspect. This might facilitate hygiene therapy and the aesthetic outcome of implant-supported restoration as well as reduce the risk for bleeding on probing, gingi-val recession and plaque-induced peri-implan-titis. Tissue regeneration, during the ridge preservation procedures, had similarly devel- oped in the control (flap approach) as well as in the test groups (flapless approach). Thecollagenated porcine bone supported new hard tissue formation in the extraction sock- ets and the graft particles seemed to become integrated with the newly formed bone. Por- cine bone has been shown to be osteoconduc- tive, with no adverse reactions and no inflammatory infiltrate (Barone et al. 2005,2008, 2011; Orsini et al. 2006; Nannmark &Sennerby 2008; Figueiredo et al. 2010; Iezziet al. 2012). This biomaterial has been reported to be reabsorbable, with clear active resorption signs of the porcine bone particle (Nannmark & Sennerby 2008) and presenceof scalloped lacunae (Pagliani et al. 2012).

The histomorphometrical analysis in the present study revealed that 22.5% of the total bone area was filled with new bone in the siteswhere a flap was raised and 22.5% in the flap-less sites. Some other authors using a differentexperimental model (beagle dogs) and a differ-ent graft biomaterial (anorganic bovine bone) found out that the newly formed bone occu- pied between 15.6% and 18.1% of the total tis-sue volume (Suaid et al. 2013). In the present randomized controlled study, the percentage of the residual graft material was 18.2% of thetotal bone area in the control as well as in the test group. On the contrary, the use of a differ-ent biomaterial with different healing time showed 32.8% of residual graft particles (Degidi et al. 2012). The slow resorption rate of some biomate rials could be considered a clinical advantage in that it helps in stabilizing the contour, contrary to what has been reported with autogenous bone where a high resorption rate of the original volume was measured (Sbor- done

et al. 2011). In conclusion, no differences in the histo- logic and histomorphometric analysis were found in this randomized clinical trial when comparing the flap with flapless approach for ridge preservation procedure. This study sup- ported the hypothesis of the non-detrimental effect of collagen membrane exposure on bone regeneration during the ridge preserva- tion procedures with a flapless approach. This was a short-term follow-up study and the definitive outcomes will be published after 5 years of evaluation of implant restorations.

References

Araujo, M.G. & Lindhe, J. (2005) Dimensional ridge alterations following tooth extraction: an experi- mental study in the dog. *Journal of Clinical Peri- odontology* 32: 212–218.

Araujo, M.G. & Lindhe, J. (2009) Ridge alterations after tooth extraction with and without flap ele- vation. An experimental study in the dog. *Clini-cal Oral Implants Research* 20: 545–549.

Barone, A., Aldini, N.N., Fini, M., Giardino, R., Calvo Guirado, J.L. & Covani, U. (2008) Xenograft versus extraction alone for ridge preservation after tooth removal: a clinical and histomorpho-metric study. *Journal of Periodontology* 79: 1370–1377.

Barone, A., Crespi, R., Aldini, N.N., Fini, M., Giar- dino, R. & Covani, U. (2005) Maxillary sinus aug-mentation: histologic and histomorphometric analysis. *International Journal of Oral and Maxil- lofacial Implants* 20: 519–525.

Barone, A., Ricci, M., Calvo-Guirado, J.L. & Covani, U. (2011) Bone remodelling after regen- erative procedures around implants placed in fresh extraction sockets: an experimental study in Beagle dogs. *Clinical Oral Implants Research* 22: 1131–1137.

Barone, A., Orlando, B., Cingano, L., Marconcini, S., Derchi, G. & Covani, U. (2012) A randomised clinical trial to evaluate and compare implants placed in augmented vs. non-augmented extrac- tion sockets: a 3-year evaluation. *Journal of Peri- odontology* 83: 836–846.

Barone, A., Ricci, M., Tonelli, P., Santini, S. & Covani, U. (2013) Tissue changes of extraction sockets in humans. A comparison of spontaneous healing vs. ridge preservation with secondary soft tissue healing. *Clinical Oral Implants Research* 24: 1231–1237.

Barone, A., Toti, P., Piattelli, A., Iezzi, G., Derchi, G. & Covani, U. (2014) Extraction socket healing in humans after ridge preservation techniques: a comparison between flapless and flapped proce- dure in a randomised clinical trial. *Journal of Periodontology* 85: 14–23.

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. *The Interna- tional Journal of Periodontics & Restorative Den- tistry* 28: 441–451.

Buser, D., Dula, K., Belser, U., Hirt, H.P. & Bert- hold, H. (1993) Localized ridge augmentation using guided bone regeneration. Surgical proce- dure in the maxilla. *The International Journal of Periodontics & Restorative Dentistry* 13: 29– 45.

Caneva, M., Botticelli, D., Salata, L.A., Souza, S.L.S., Bressan, E. & Lang, N.P. (2010) Flap vs. "flapless" surgical approach at immediate implants: a histomorphometric study in dogs. *Clinical Oral Implants Research* 21: 1314–1319.

Canullo, L., Cocchetto, R. & Loi, I. (2012). Rimod- ellamento Osseo Perimplantare: Background Sci- entifico e Implicazioni Cliniche, 7–8. Passirana di Rho (MI): Quintessenza edizioni.

Cardaropoli, D. & Cardaropoli, G. (2008) Preserva- tion of the postextraction alveolar ridge. A clini- cal and histologic study. *The International Journal of Periodontics & Restorative Dentistry* 28: 469–477.

Christgau, M., Bader, N., Schmalz, G., Hiller, K.A. & Wenzel, A. (1997) Postoperative exposure of bi-oresorbable GTR membranes: effect on healing results. *Clinical Oral Investigations* 1: 109–118.

Covani, U., Ricci, M., Bozzolo, G., Mangano, F., Zini, A. & Barone, A. (2011) Analysis of the pat- tern of the alveolar ridge remodelling following single tooth extraction. *Clinical Oral Implants Research* 22: 820–825.

Darby, I., Chen, S.T. & Buser, D. (2009) Ridge preservation techniques for implant therapy. *International Journal of Oral and Maxillofacial Implants* 24: 260–271.

Degidi, M., Piattelli, A., Perrotti, V. & Iezzi, G. (2012) Histologic and histomorphometric evalua- tion of an implant retrieved 8 years after inser- tion in a sinus augmented with anorganic bovine bone and anorganic bovine matrix associated with a cell-binding peptide: a case report. *The Interna- tional Journal of Periodontics & Restorative Den- tistry* 32: 451–457.

Engler-Hamm, D., Cheung, W.S., Yen, A., Stark, P.C. & Griffin, T. (2011) Ridge preservation using a composite bone graft and a bioabsorbable mem- brane with and without primary wound closure: a comparative clinical trial. *Journal of Periodontol- ogy* 82: 377–387.

Farina, E., Menditti, D., De Maria, S., Mezzogiorno, A., Esposito, V., Laino, L. & Carinci, F. (2009) A model of human bone regeneration: morphologi- cal, cellular and molecular aspects. *Journal of Clinical Periodontology* 1: 42–53.

Fickl, S., Zuhr, O., Wachtel Bolz, W. & Hürzeler, M.B. (2008b) Tissue alteration after tooth extrac- tion with and without surgical trauma: a volu- metric study in beagle dog. *Journal of Clinical Periodontology* 35: 356–363.

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

Figueiredo, M., Henriques, J., Martins, G., Guerra, F., Judas, F. & Figueiredo, H. (2010) Physicochem- ical characterization of biomaterials commonly used in dentistry as bone substitutes–comparison with human bone. *Journal of Biomedical Materi- als Research. Part B, Applied Biomaterials* 92: 409–419.

Fiorellini, J.P. & Nevins, M.L. (2003) Localized ridge augmentation/preservation. A systematic review. *Annals of Periodontology/The American Academy of Periodontology* 8: 321–327.

Hämmerle, C.H., Araújo, M.G. & Simion, M. (2012) Osteology Consensus Group 2011. Evidence-based knowledge on the biology and treatment of extraction sockets. *Clinical Oral Implants Research*, 23(Suppl. 5): 80–82.

Iezzi, G., Degidi, M., Piattelli, A., Mangano, C., Scarano, A., Shibli, J.A. & Perrotti, V. (2012) Comparative histological results of different bi- omaterials used in sinus augmentation proce- dures: a human study at 6 months. *Clinical Oral Implants Research* 23: 1369–1376.

Kan, J.Y., Rungcharassaeng, K., Lozada, J.L. & Zimmerman, G. (2011) Facial gingival tissue sta- bility following immediate placement and provi- sionalization of maxillary anterior single implants: a 2- to 8-year follow-up. *Internatinal Journal of Oral and Maxillofacial Implants* 26: 179–187.

Lekovic, V., Camargo, P.M., Klokkevold, P.R., Weinlaender, M., Kenney, E.B., Dimitrijevic, B. & Nedic, M. (1998) Preservation of alveolar bone in extraction sockets using bioabsorbable membranes. *Journal of Periodontology* 69: 1044–1049.

Lekovic, V., Kenney, E.B., Weinlander, M., Han, T., Klokkevold, P., Nedic, M. & Orsini, M.A. (1997) Bone regenerative approach to alveolar ridge maintenance following tooth ectraction. Report of 10 cases. *Journal of Periodontology* 68: 563–567.

Moher, D., Hopewell, S., Schultz, K.F., Montori, V., Gotzsche, P.C., Devereaux, P.J., Elbourne, D., Egger, M. & Altman, D.G. (2010) Consort

2010 Explanation and Elaboration: updated guidelines for reporting parallel group rando- mised trials. *Journal of Clinical Epidemiology* 63: 1e–37e.

Nannmark, U. & Sennerby, L. (2008) The bone tis- sue responses to prehydrated and collagenated cortico-cancellous porcine bone grafts: a study in rabbit maxillary defects. *Clinical Implant Den- tistry and Related Research* 10: 264–270.

Novaes, A.B., Jr, Fernandes, P.G., Suaid, F.A., de Moraes Grisi, M.F., Scombatti de Souza, S.L., Taba, M., Jr, Palioto, D.B. & Muglia, V.A. (2012) Ridge preservation with acellular dermal matrix and anorganic bone matrix cell-binding peptideP-15 after tooth extraction in humans. A histo- morphometric study. *Journal of Osseointegration* 4: 23–29.

Novaes, A.B., Jr, Martins de Barros, R.R., Papal- exiou, V. & Gonçalves de Almeida, A.L. (2011) Buccal bone loss after immediate implantation can be reduced by the flapless approach. *Journalof Osseointegration* 3: 45–55.

Oghli, A.A. & Steveling, H. (2010) Ridge preserva- tion following tooth extraction: a comparison between atraumatic extraction and socket seal surgery. *Quintessence International* 41: 605–609.

Oh, T.J., Meraw, S.J., Lee, E.J., Giannobile, W.V. &Wang, H.L. (2003) Comparative analysis of colla-gen membranes for the treatment of implant dehiscences defects. *Clinical Oral Implants Research* 14: 80–90.

Orsini, G., Scarano, A., Piattelli, M., Piccirilli, M., Caputi, S. & Piattelli, A. (2006) Histologic and ultrastructural analysis of regenerated bone in maxillary sinus augmentation using a porcine bone-derived biomaterial. *Journal of Periodontol- ogy* 77: 1984–1990.

Pagliani, L., Andersson, P., Lanza, M., Nappo, A., Verrocchi, D., Volpe, S. & Sennerby, L. (2012) A collagenated porcine bone substitute for augmen- tation at Neoss implant sites: a prospective 1-year multicenter case series study with histology.*Clinical Implant Dentistry and Related Research* 14: 746–758.

Piattelli, A., Scarano, A. & Quaranta, M. (1997)High-precision, cost-effective system for produc- ing thin sections of oral tissues containing dental implants. *Biomaterials* 18: 577–579.

Sbordone, L., Levin, L., Guidetti, F., Sbordone, C., Glikman, A. & Schwartz-Arad, D. (2011) Apicaland marginal bone alterations around implants in maxillary sinus augmentation grafted with autogenous bone or bovine bone material and simultaneous or delayed dental implant posi- tioning. *Clinical Oral Implants Research* 22: 485–491.

Schropp, L., Wenzel, A., Kostopoulos, L. & Karring, T. (2003) Bone healing and soft tissue contour changes following single-tooth extraction: a clini- cal and radiographic 12-month prospective study. *The International Journal of Periodontics & Restorative Dentistry* 23: 313–323.Simion, M., Maglione, M., Iamoni, F., Scarano, A., Piattelli, A. & Salvato, A. (1997) Bacterial pene- tration through Resolut resorbable membrane in vitro. A histological and scanning electron micro- scopic study. *Clinical Oral Implants Research* 8: 23–31.

Suaid, F., Grisi, M.F.M., Souza, S.L.S., Palioto, D.B., Taba, M. & Novaes, A.B. (2013) Buccal bone remodelling after tooth extraction using the flapless approach with and without syn- thetic bone grafting. A histomorphometric studyin dogs. *Clinical Oral Implants Research* 24: 407–413.

Vignoletti, F., Matesanz, P., Rodrigo, D., Figuero, E., Martin, C. & Sanz, M. (2012) Surgical proto- cols for ridge preservation after tooth extraction.A systematic review. *Clinical Oral Implants Research* 23(Suppl. 5): 22–38.

Figures

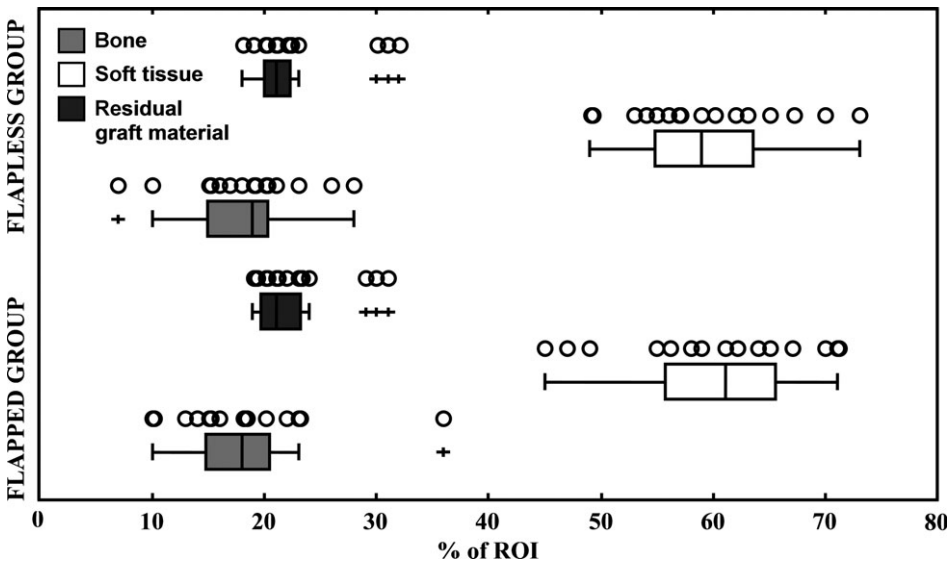

Fig. 1. Summary of the histomorphometric data with percentage of bone, soft tissue and residual graft material depicted for the two surgical techniques considered both as scatter data and box and whiskers plot, in which thebox line represents the lower quartile, median and upper quartile values, while the whisker lines include the rest ofthe data. Outliers were data with values beyond the ends of the whiskers.

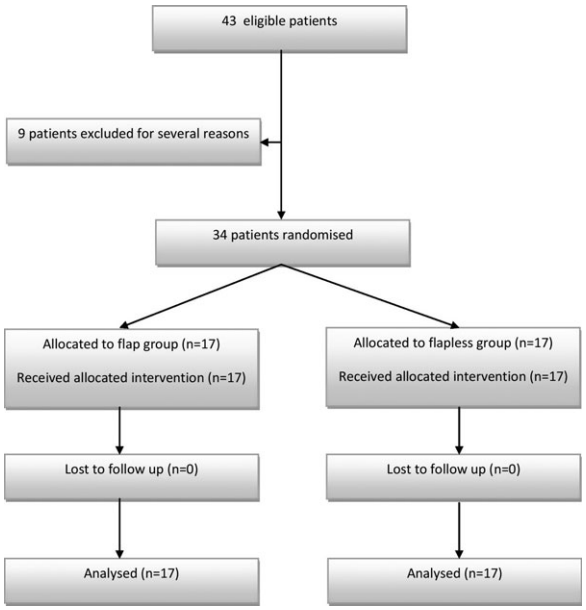

Fig. 2. Flow diagram of the progress through the phases of the two experimental groups.

Variable	Flap group (control)	Flapless group (test)
Male	9 (53%)	5 (29%)
Female	8 (47%)	12 (71%)
Mean age (range)	47 (35–71) years	43.5 (21–67) years
Number of maxillary pre-molars	3 (17.6%)	3 (17.6%)
Number of mandibular pre-molars	2 (11.7%)	1 (5.9%)
Number of maxillary molars	2 (11.7%)	4 (23.5%)
Number of mandibular molars	10 (58.8%)	9 (52.9%)
Reason for extraction: tooth fracture	7 (41%)	7 (41%)
Reason for extraction: tooth decay	8 (47%)	8 (47%)
Reason for extraction: endodontic failure	1 (5.9%)	1 (5.9%)
Reason for extraction: perio disease	1 (5.9%)	1 (5.9%)
Total number of inserted implants	17	17
Number of 5 mm diameter implants	9 (53%)	11 (64.7%)
Number of 4 mm diameter implants	8 (47%)	6 (35.3%)
Mean implant length (SD) (mm)	12.1 0.99 mm	12.1 0.77 mm

Table 1. Patient and implant characteristics in the two experimental groups

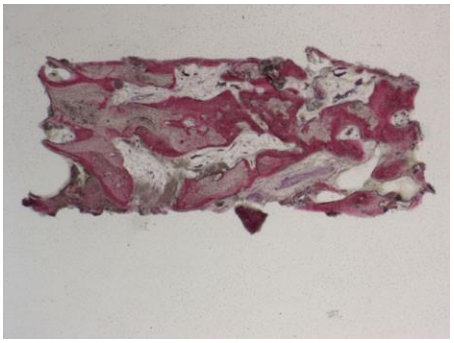

Fig. 3. Control group. Pre-existing bone, newly formed bone and biomaterial residual particles. Acid fuchsin- toluidine blue. Original magnification 912.

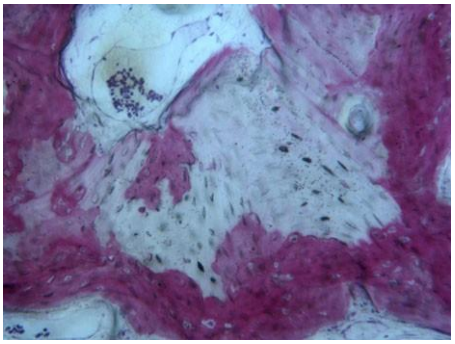

Fig. 4. Control group. New bone inside the residual grafted particles. Large osteocytic lacunae inside the bone tissue. Acid fuchsin-toluidine blue. Original mag- nification 9100

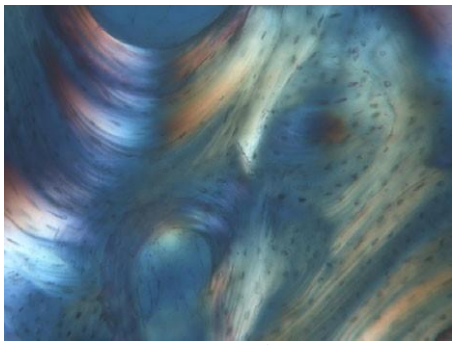

Fig. 5. Control group. Collagen fibres with a parallel orientation were seen close to the pre-existing bone. Acid fuchsin-toluidine blue. Polarized light 9100.

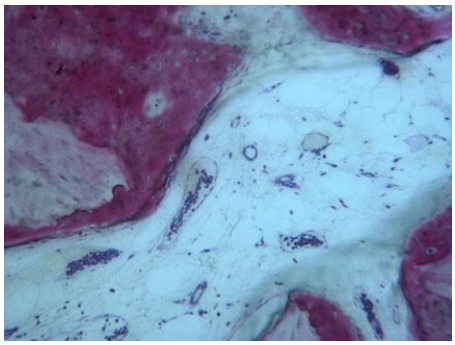

Fig. 6. Control group. Blood vessels at the newly formed bone-old bone interface. Acid fuchsin-toluidine blue. Original magnification 9100.

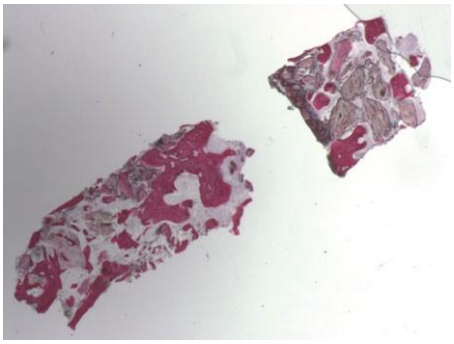

Fig. 7. Test group. Biomaterial residual particles and newly formed bone. Acid fuchsin-toluidine blue. Origi- nal magnification 912.

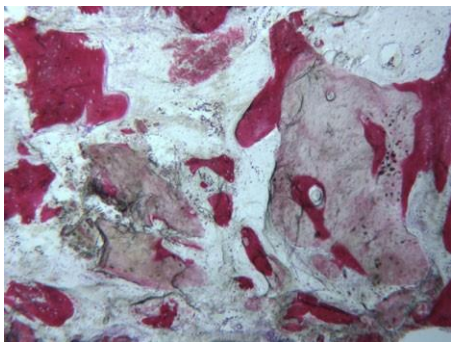

Fig. 8. Test group. Newly formed bone inside and out- side the grafted particles. Acid fuchsin-toluidine blue. Original magnification 940.

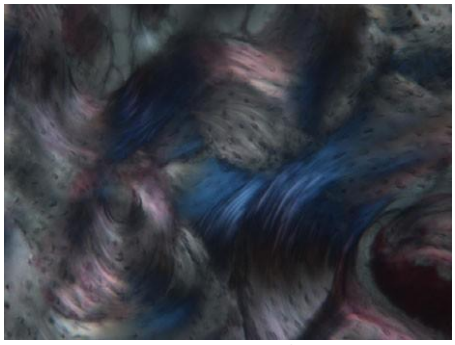

Fig. 9. Test group. Collagen fibres with a parallel orien- tation in the remodelling areas of pre-existing bone can be observed. Acid fuchsin-toluidine blue. Polarized light9100.

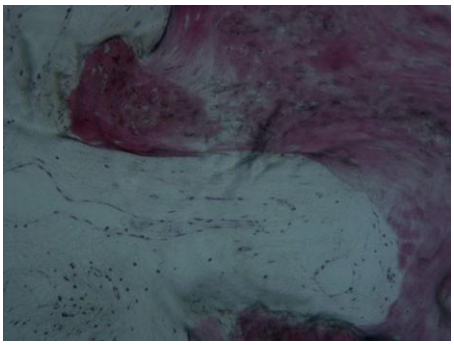

Fig. 10. Test group. Newly formed bone with blood vessels of various dimensions inside the marrow spaces. Acid fuchsin-toluidine blue. Original magnification9100

	Measure of dispersion	Newly formed bone (%)	Marrow spaces (%)	Residual graft material (%)
Flapless (test group)	Mean SD	22.5 4.3	59.4 6.8	18.2 5.2
	Median (iqr)	21 (2)	59 (8)	19 (5)
Flapped (control group)	Mean SD	22.5 3.9	59.3 7.5	18.2 6.1
	Median (iqr)	21 (3)	61 (8)	18 (5)
Nonparametric two-way ANOVA *P*-value *(gender & group)*		0.8808	0.3341	0.4547
Wilcoxon test *P*-value (test vs. control)		0.917	0.850	0.690

SD: standard deviation; iqr: interquartile range.

Table 2. Mean standard deviation and median (interquartile range) percentages for the two surgical procedures employed, and related *P*-valueobtained by Wilcoxon rank sum test for procedures' comparison. No statistical differences were found

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. CONSORT 2010 checklist of information to include when reporting a ran- domized trial.