CASE REPORT

Ridge Preservation Using a Novel Freeze-dried Enzyme-deantigenic Bone Paste: A Histomorphometric-retrospective Pilot Case Series

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Abstract

Aim and objective: The aim of this study is to provide preliminary retrospective evidence concerning the histologic and histomorphometric outcome of a novel freeze-dried equine-derived bone paste (EDEBEX) for ridge preservation of sockets following tooth extraction.

Materials and methods: This pilot retrospective case series describes the histologic and histomorphometric outcome of three patients who received the equine-derived bone paste in post-extractive sockets to allow the preservation of the alveolar ridge. Patients were later rehabilitated with monolithic-zirconia, implant-supported prostheses.

Results: All patients healed uneventfully. The collected biopsies showed a prevalence of bone formation at 4 months, compact lamellar bone, with well-defined lamellae surrounding Haversian and Volkmann's canals at 6 months, and an intermediate degree of maturation in active anabolic phase at 7 months after grafting. The amount of mineralized matrix was 63.3–70.7%, whereas medullar spaces were 26.0–30.7%.

Conclusion: Histologic examination showed that the bone paste was fully biocompatible. Bone regeneration occurred within the first 4 months from grafting, with 63.3–70.7% mineralized bone matrix. The residual biomaterial, when present, did not exceed, on average, 2%.

Clinical significance: Ridge preservation using bone substitutes as an alternative to autogenous bone is known to be effective. However, available clinical evidence still does not indicate the biomaterial, if any, that should be preferred to carry it out. The equine bone paste used in the present study appears to be a good candidate for further investigation because it is easy to handle in the clinical setting and it displays a good bone formation rate.

Keywords: Bone formation, Equine bone substitutes, Freeze-dried bone paste, Post-extractive sockets, Ridge preservation, Three-dimensional collagen matrix, Xenograft.

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Introduction

Tooth extraction is usually followed by ridge remodeling and resorption, because of the lack of masticatory load, according to a well-known spatial and temporal pattern.[1–3] Atrophy may progress to such an extent that implant placement may become unfeasible; even when it can be carried out, any implant-supported rehabilitation may be at risk of functional and esthetic failure.[2] Ridge atrophy may be contrasted by grafting the post-extractive sockets with a bone graft according to the ridge preservation[4] technique. Autogenous bone is still considered as the gold standard concerning bone grafting, because of the cells' osteoinductive effect and the growth factors it contain.[5] Autogenous bone collection requires opening a second surgical site, either intraoral or extraoral, thus exposing the patient to additional risk and discomfort.[6] Several synthetic and natural biomaterials have been proposed as autogenous bone substitutes.[7] Xenografts, animal-derived bone grafts, may represent a feasible option as bones of all mammal species, including mankind, share a similar three-dimensional (3D) morphology and chemical composition of their mineral portion.[8] Hence, xenografts might have a biological advantage over other natural or synthetic grafts when used as bone grafts to regenerate the patient's bone.[9] So far, the process used to make non-antigenic animal bone seems to strongly affect the remodeling characteristics of the graft. Recent studies, in fact, have shown that the enzyme-deantigenic equine bone (EDEB) displays a different behavior than the anorganic bovine bone (ABB), the most used xenograft in oral and maxillofacial surgery, with EDEB undergoing significantly faster remodeling and producing, at a given time, a greater amount of the newly formed bone.[10,11] To manufacture ABB, bovine bone is subjected to high temperatures to eliminate bovine antigens;[12] EDEB, instead, is made non-antigenic subjecting equine bone to the action of hydrolytic enzymes.[9] These latter allow the preservation of the 3D structure of the mineralized bone and the conservation of the natural bone collagen in native conformation. EDEB is being used extensively in different fields, including oral, maxillofacial, and orthopedic surgery.[13–22] Biomaterials for ridge preservation should be easy-handling and should display a certain degree of space-keeping properties. Furthermore, they should favor

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soft tissue regeneration when they are grafted in post-extractive sockets, and gingival healing is achieved by second intention.[23] Bone pastes fulfill these requirements, as they are user-friendly formats that are easy to handle and capable of holding optimal space-keeping properties when they are hard enough (e.g., when they are moldable). Moldable pastes can adapt to the site they are grafted in, assuring complete socket filling and direct contact with the surrounding bone tissue, possibly facilitating bone repair.[24] Recently, a novel line of bone pastes has been placed on the market as the evolution of EDEB and can be indicated as EDEBEX. This product consists of a hydrogel (namely Exur®, from which the name EDEBEX: EDEB + Exur) made by polyethylene glycol/hydroxyl-propyl methyl cellulose (PEG/HPMC)-based that acts as a carrier, containing cancellous and cortical EDEB granules and/or equine demineralized bone matrix.[25] The gel is added with a subsidiary amount of vitamin C modulating its rheology. When human bone marrow stem cells were co-cultivated with EDEBEX, they were found to over-express some bone regeneration modulators, such as, the RUNT-related transcription factor 2 (RUNX2), the bone sialoprotein, and the osteocalcin.[25] When EDEBEX was grafted in artificially induced femoral defects in rabbits, histological assessment of the regenerated bone after 1 and 2 months showed that the bone tissue had undergone a significant re-organization within the lesion boundaries.[25] At present, clinical and histological data concerning the use of EDEBEX in humans are limited to a recent case report with respect to one patient who underwent socket preservation and was followed up for 36 months.[26] The patient underwent successful implant-supported bone rehabilitation; at 36 months, the peri-implant bone levels had been maintained with the implant being successful according to the Albrektsson and Zarb criteria;[27] histomorphometric analyzes showed that the amount of the newly formed bone at implant insertion, 3.5 months after the grafting surgery, was 60.12%. The authors of the present study have been using a freeze-dried version of EDEBEX for some months and have collected additional clinical and histomorphometric evidence concerning its use to achieve ridge preservation. This small case series' aim is to describe these additional results.

Materials and Methods

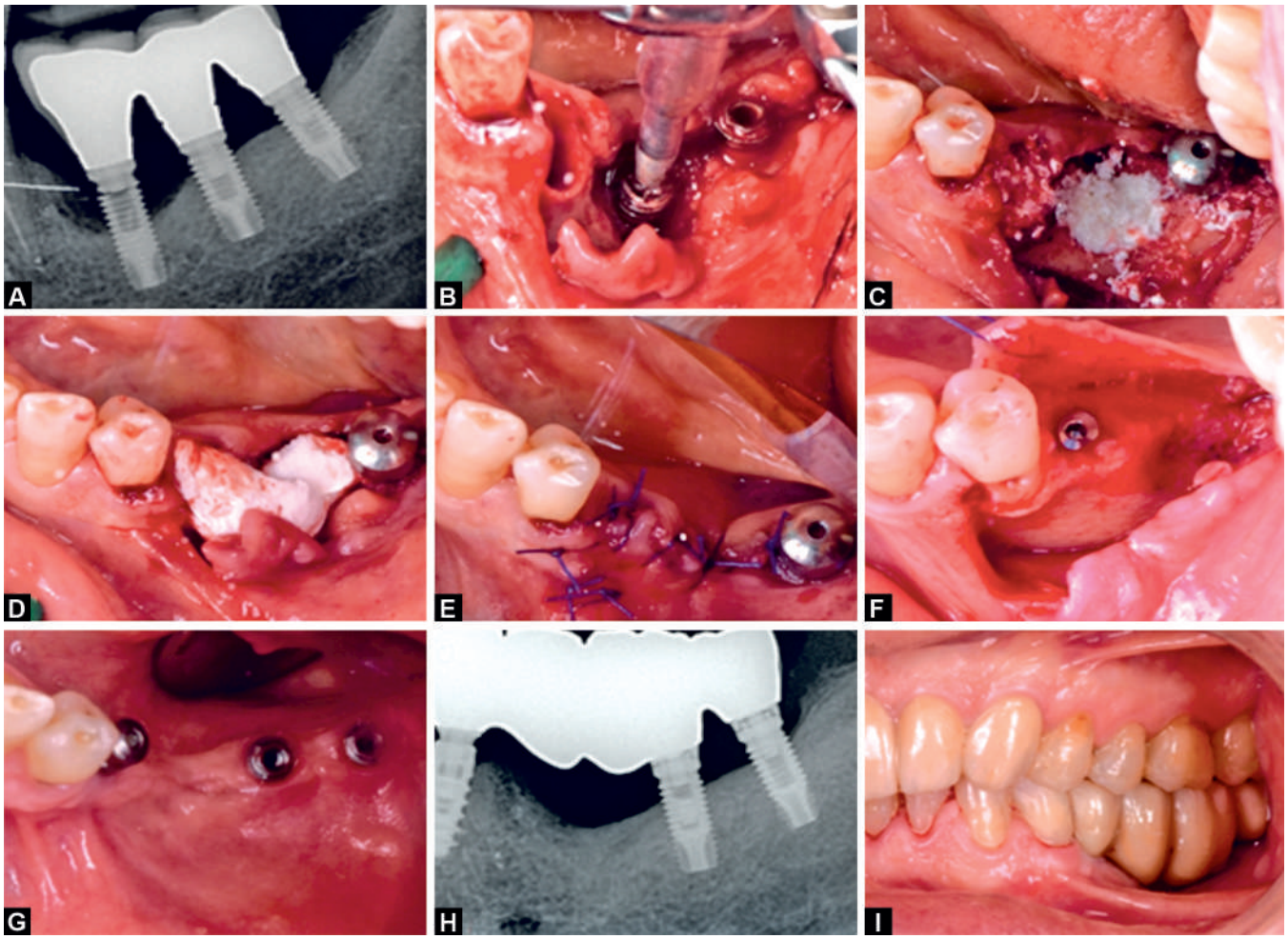
This case series concerns three patients who underwent socket preservation surgeries at the author's facility. The first patient (Patient A, 70 years old, male) was treated because of a fractured implant (3.6) and the adjacent irrecuperable tooth (3.5), both requiring removal; the second patient (Patient B, 58 years old, female) had one tooth (4.5), already devitalized and supporting a bridge, extracted because of an invasive caries; and the third patient (Patient C, 36 years old, female) had residual roots (1.6) from a destructing caries that required removal. All patients were treated according to the principles of ridge preservation, and all had the freeze-dried equine-derived bone paste EDEBEX (Activabone Putty, Bioteck S.p.A., Arcugnano, Italy) grafted in their post-extractive sockets. Radiographs and clinical pictures of the three patients and the surgeries they were subjected to are shown in Figures 1 to 3, respectively. In patients A and C, the grafted site was protected with a collagenic 3D matrix (Biocollagen Xenomatrix, Bioteck S.p.A., Arcugnano, Italy). In patient B, instead, neither a membrane nor a collagenic matrix was used to cover the graft. Healing by second intention was achieved in patients B and C. For all three patients, a two-step rehabilitating procedure was planned, involving grafting the sockets to preserve them from resorption followed by a delayed implant placement. Patients provided their informed consent to the treatment and to the collection of the biopsy for research publication purposes.

All patients underwent a thorough oral hygiene 2 days before the surgery. For antibiotic prophylaxis, 2 g of amoxicillin/clavulanic acid (Augmentin, GlaxoSmithKline, Verona, Italy) were administered 1 hour before the surgery and then every 12 hours for 8 days. The patients also rinsed their mouth for 2 minutes with chlorhexidine 0.20% mouth rinse (Corsodyl, GlaxoSmithKline) and received 100 mg of a non-steroidal anti-inflammatory drug (Aulin, Roche, Milano, Italy). Local anesthetic was injected into the oral mucosa with 1% articaine with epinephrine 1:100,000 (Molteni Dental, Milano, Italy).

The teeth were extracted atraumatically and the sockets debrided from any residual of the fibrous tissue. Subsequently, the bone paste — still dry — was placed into the sockets and gently pressed with a round instrument to fill them. A flap was elevated only for patient A. After grafting, a 3D collagenic matrix was used to cover the graft in patients A and C. In patient A, the flap was sutured with single stitches, while gingival margins of patients B and C were stabilized using cross stitches, and gingival rims were left open seeking for second intention healing. A 4–0 non-resorbable suture (Vicryl Plus, Ethicon, Johnson and Johnson, Pomezia, Italy) was used in all cases. A second surgery was performed after 4 months in patient A, after 6 months in patient B, and after 7 months in patient C. In all cases, the clinical appearance of soft tissues and the radiographic appearance of the grafted area were quite satisfying. Antibiotic prophylaxis, postsurgical treatment, anesthetic treatment, and pain management were carried out similar to the first surgery. After gaining access to the bone ridge, a bone core (patient A, position 3.5; patient B, position 4.5; patient C, position 1.6) was collected using a trephine and placed in a test tube containing buffered 10% formalin for subsequent histological analysis. The implant sites were then prepared following the drilling sequence suggested by the manufacturer and the implants were placed (patient A, position 3.5, 3.7 × 10 mm; Safe, Biotec S.r.l., Dueville, Italy; patient B, position 4.5, 3.7 × 8.0 mm; position 4.7, 3.7 × 8.0 mm, Safe, Biotec S.r.l.; patient C, position 1.6, 4.1 × 10 mm, Safe, Biotec S.r.l.). Patient A was later rehabilitated with a four-element monolithic-zirconia bridge supported by the implant placed in position 3.5 and the two implants still in place in positions 3.7 and 3.8. Patient B was rehabilitated with a three-element monolithic-zirconia bridge supported by implants in positions 4.5 and 4.7. Owing to personal reasons, patient C decided to postpone the rehabilitation with a single monolithic crown supported by the implant in position 1.6. Patients were followed up every month for the next 6 months, and then every 6 months after implant surgery.

Bone Paste EDEBEX

The equine-derived bone paste used in the present study (Activabone Putty, Bioteck S.p.A.) contains equine bone, type I collagen extracted from equine tendons, and a hydrogel as a carrier. The paste is provided freeze-dried and recovers its moldable consistency when rehydrated (either with saline or when in contact with blood). Equine bone is obtained through the Zymo-Teck procedure, a Bioteck proprietary enzymatic antigen-elimination process, which guarantees grafts with preserved biological and biomechanical properties. This treatment is performed at controlled temperature (<60°C) and completely removes the antigens from the bone tissue, without affecting the native quaternary structure



Figs 1A to I: Patient A: (A) The patient presented with a fractured implant at position 3.6; (B) He had the implant removed and tooth at position 3.5 extracted; (C) Post-extractive sockets were filled with EDEBEX; (D) EDEBEX graft was protected with a three-dimensional collagenic matrix; (E) Gingival rims were stabilized with single stitches and soft tissues left to heal by first intention. Healing occurred uneventfully; (F) After 4 months, a biopsy was collected and at the same time an implant was placed; (G) Soft tissues at 4 months from implant placement and (H) radiographic results at 22 months from grafting showing mature bone; (I) The patient was rehabilitated with a 4-element monolithic-zirconia implant-supported bridge

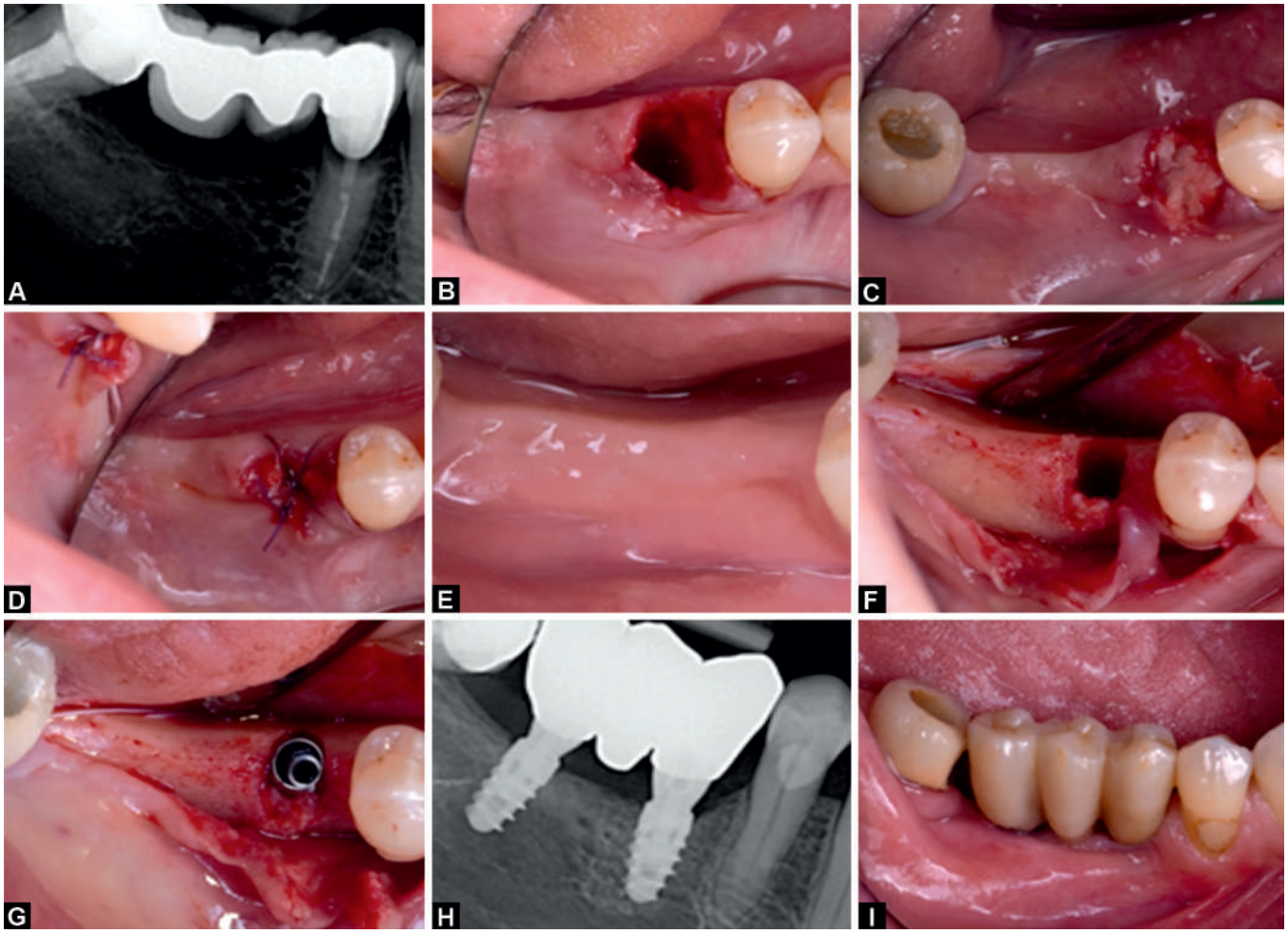
of bone collagen and extracellular matrix components, which are therefore totally preserved. Equine bone components in the Activabone paste used in this study are equine cancellous bone micro-granules (<0.2 mm of diameter) and equine cancellous bone granules having 0.5–1 mm diameter. The carrier, namely Exur®, is a polymeric hydrogel, consisting of a mixture of water and PEG/HPMC. The mixture is combined with a subsidiary amount of vitamin C, acting as a visco-modulator agent (patented). Vitamin C, indeed, is able to limit the intramolecular and intermolecular rearrangements of PEG and HPMC polymeric chains produced by the sterilization process, thus maintaining nearly unaltered the visco-elasticity of gels and injectability of bone fillers.[25] The paste undergoes freeze-drying, beta-sterilization at 25 kGy, and is provided to the oral surgeon in sterile-packaged vials.

Histological and Histomorphometric Analysis

The test tube containing the biopsy in buffered 10% formalin was marked with an alphanumeric code and sent to the histologists. After 3 days of fixation, bone cores were decalcified for 5 days using a chelating agent (Osteodec, Bio Optica, Milano, Italy). The sample was subsequently dehydrated in ascending concentrations of ethanol at room temperature, clarified with xylene, infiltrated, and finally embedded in paraffin orientated to further obtain longitudinal sections (Bio-Plast, Bio Optica, Milano, Italy). For histological preparation, serial longitudinal sections of 6 µm were obtained in the central portion of the block with a microtome (Leica Biosystems, Milano, Italy) equipped with blade R35 (Bio Optica, Milano, Italy) and optimized for the cutting of the mineralized tissue. For each experimental site, two sections were stained manually with Mayer's Hematoxylin and Eosin, and images were captured by Aperio CS2 (Leica Biosystems) to perform qualitative and quantitative analyzes (Image Scope software, Leica Biosystems, Milano, Italy).

The qualitative assessment aimed at recognizing the amount of inflammatory, fibrous, and fatty tissue infiltrate, as well as areas of necrosis, following ISO-10993-6:2007 annex E.

The histomorphometric evaluation of the tissue components was performed using a standard stereologic method. A digital counting grid was placed over each microscopic image section, and the tissue underlying each grid intersection was recorded as either mineralized matrix, osteoid tissue, medullary spaces, or residual biomaterial.[28] The volume fraction percentages were obtained by



Figs 2A to I: Patient B: (A) The patient presented with tooth 4.5 to be extracted because of a destructive caries; (B) After extraction; (C) the socket was filled with EDEBEX; (D) Gingival rims were stabilized with a cross stitch and soft tissues left to heal by second intention; (E) Healing occurred uneventfully; (F) After 6 months, a biopsy was collected from position 4.5; (G) At the same time, two implants were placed in positions 4.5 and 4.7; (H) Radiographic results at 18 months from grafting show mature bone; (I) The patient was rehabilitated with a three-element monolithic-zirconia implant supported prosthesis

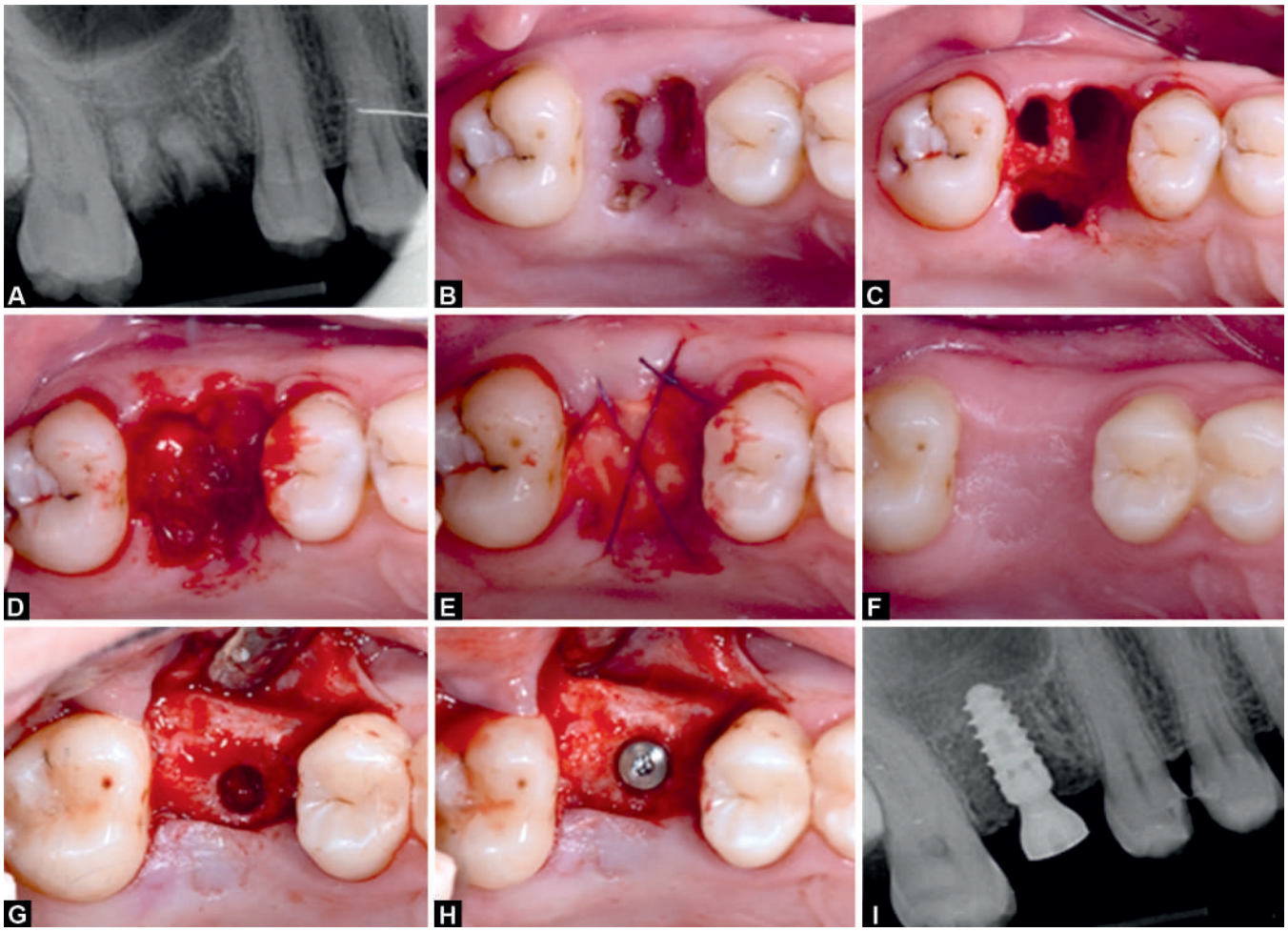
the ratio of the intersection points that fall down on each type of tissue to the total intersection points.[29]

Results

All patients healed uneventfully, and their final rehabilitation was both functionally and esthetically satisfactory. At the last follow-up, all prostheses were working effectively, and no gingival recessions could be observed. The marginal bone loss for all implants placed into the regenerated sockets was within the success threshold defined by Albrektsson and Zarb.[27]

Qualitative Histologic Assessment

Under qualitative histologic examination, no biopsy showed any sign of inflammation or other tissue reaction, such as, fibrous tissue formation or necrosis (Figs 4 and 5). Concerning patient A, whose biopsy was collected 4 months after grafting at position 3.5, his bone tissue appeared to be still undergoing intense remodeling, with a prevalence of bone formation (anabolism) over bone degradation (catabolism) (Fig. 6). Within the sample, wide immature bone areas could be observed displaying a high cell density, round-shaped osteocytes within ample lacunae, and woven, less mineralized bone tissue. Some bone graft traces could be observed, but (see the following paragraph) the biomaterial could be considered as being fully resorbed. The biopsy of patient B, which was collected 6 months after grafting at position 4.5, showed the presence of bone in a quite advanced stage of maturation. Most of the sample presented compact lamellar bone, with well-defined lamellae surrounding Haversian and Volkmann's canals. A biopsy fragment, possibly corresponding to the coronal area of the socket, displayed more immature bone. Finally, the biopsy of patient C, collected 7 months after grafting at position 1.6, displayed an intermediate degree of maturation in an active anabolic phase: bone areas were made of lamellar, mineralized bone, with newly formed osteons; osteocytes were still large and many active osteoblasts could be observed. In all the specimens, in the internal parts of some bone trabeculae, it was possible to observe a peculiar structure characterized by a dense concentration of osteocytes and possible hydrogel remnants fully incorporated in the newly formed bone (Figs 4 and 5).



Figs 3A to I: Patient C: (A to C) The patient presented with residual tooth roots to be removed at position 1.6; (D) After removal, the post-extractive socket was filled with EDEBEX and protected with a three-dimensional collagenic matrix; (E) Gingival rims were stabilized with a cross stitch, and soft tissue was left to heal by second intention; (F) Healing occurred uneventfully; (G) After 7 months, a biopsy was collected, (H) an implant was placed and the patient is waiting to be rehabilitated with a single, implant-supported monolithic crown; (I) Radiographic results at 10 months from grafting show a newly formed bone

Quantitative Histomorphometric Assessment

The results of the histomorphometric assessment are provided in Table 1. The amount of mineralized matrix was 63.3–70.7%, whereas medullar spaces were 26.0–30.7%. Osteoid (non-mineralized) tissue (9.0%) was present only in the biopsy taken from patient C. No residual biomaterial was observed in the biopsy taken from patient A; instead, in the biopsies taken from patients B and C, the residual biomaterial was about 1.8%.

Discussion

No doubt exists, when reading the literature published on ridge preservation, that ridge preservation procedures indeed result in clinically significant less horizontal and vertical ridge bone loss, even if a certain degree of bone remodeling cannot be, however, avoided.[30] It is also known that appropriate flap management and the use of barrier membranes favorably contribute to bone preservation.[31] Yet, questions still exist concerning the surgical procedure, the biomaterial, or the combination of the two that should be preferred.[32–34] In 2015, a Cochrane meta-analysis concerning several randomized clinical trials and more than 200 extraction sites concluded that different grafting procedures and biomaterials did not show any clinically significant difference and called for more clinical research on the subject.[35] The results of the present retrospective pilot case series study show that the dry paste EDEBEX used provided good clinical and histomorphometric results. Our data may be regarded as fairly in line with those of the recently published case report by Di Stefano et al.[26] where the amount of the newly formed bone was about 60%. In our study, we observed a slightly higher amount of mineralized matrix (i.e., the newly formed bone, being the residual biomaterial absent, or minimal). Interestingly, Di Stefano et al.[26] still found, 3.5 months after grafting, a significant amount of biomaterial (about 20.5%); instead, in the present study none, or almost none, was observed. Overall, the results of our study and those of Di Stefano et al.[26] seem to indicate that the bone substitute under examination is capable of favoring bone formation within a relatively short period; this is consistent with previous clinical and histomorphometric evidence concerning the particulate component this equine bone paste is made of, that is, particulate EDEB.[16,36,37] *In vitro* studies have shown that EDEB's proregenerating property might be linked to the way osteoclasts interact with it — that is, by adhering and remodeling

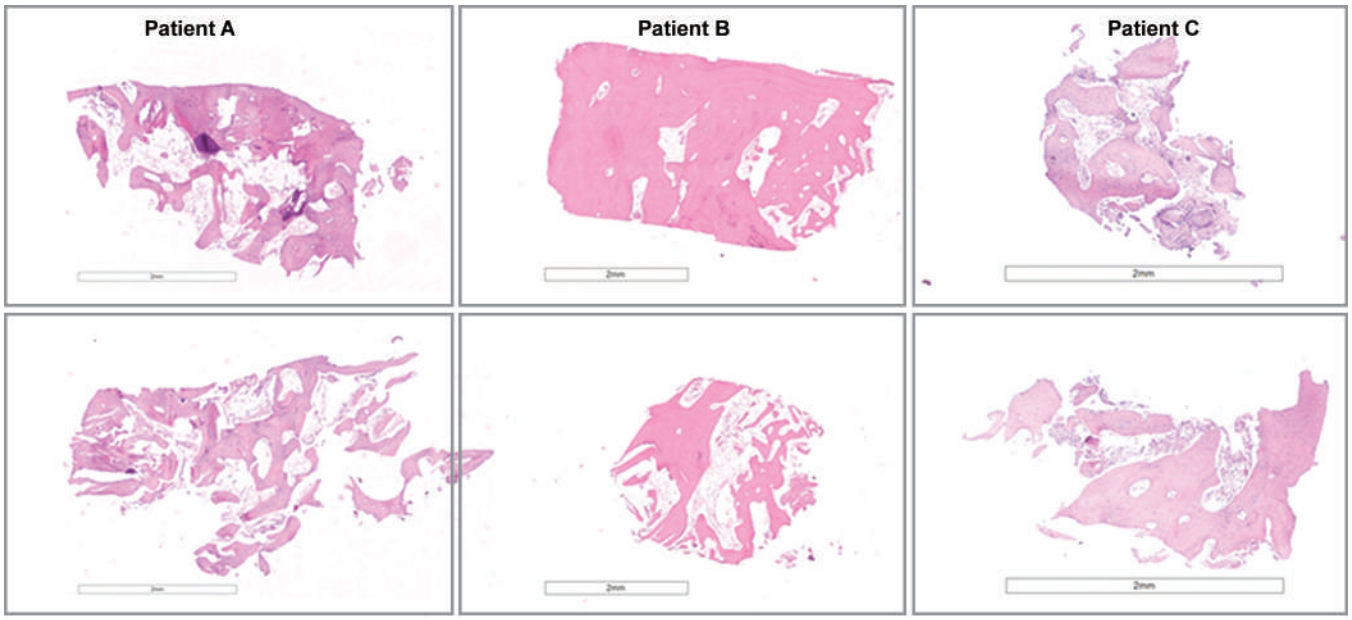


Fig. 4: Histologic assessment of the two fragments (first and second rows) of the bone cores collected from the three patients. Hematoxylin-eosin staining. First and second rows: 10×, 30×, and 18× magnifications

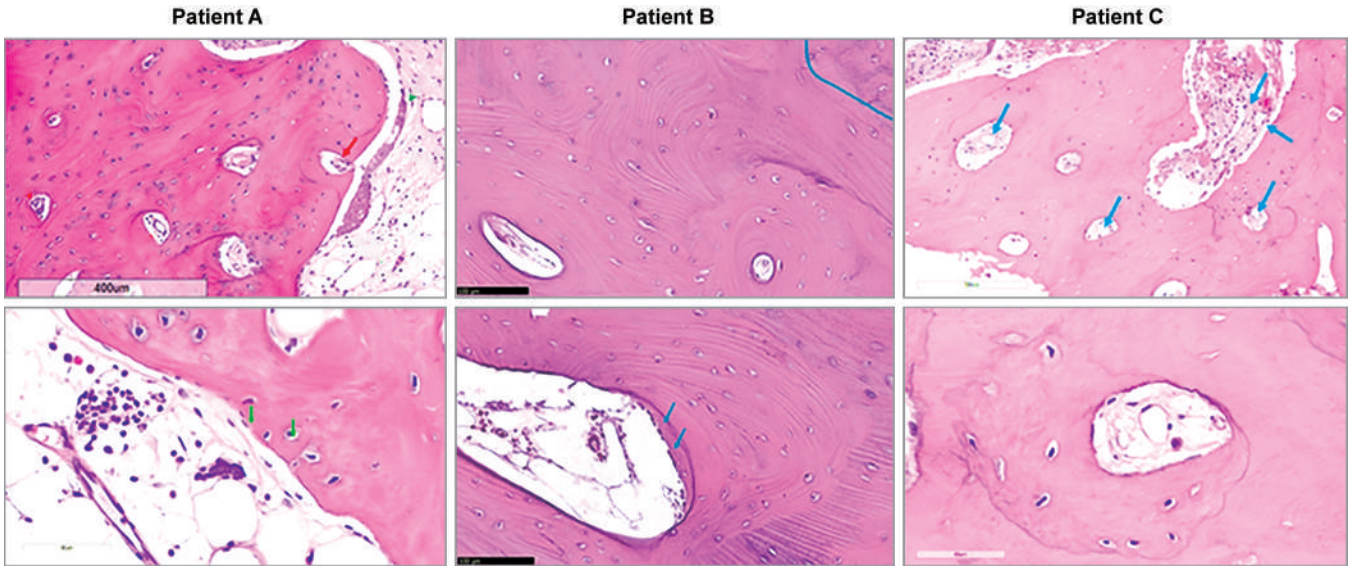
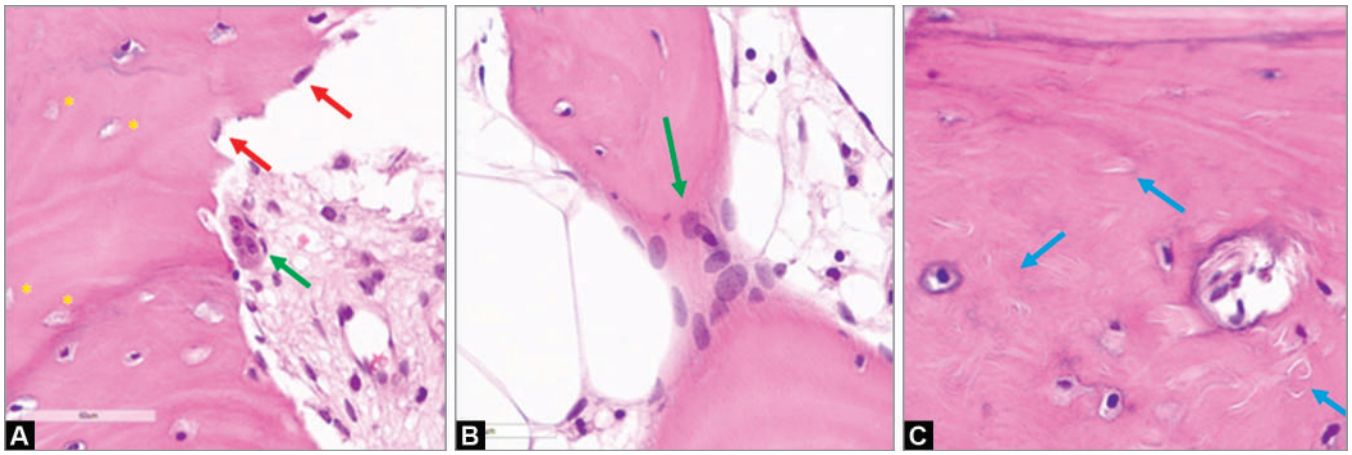


Fig. 5: Histologic assessment of the two fragments (first and second rows) of the bone cores collected from the three patients. Hematoxylin-eosin staining: First row, 120×, 250×, 100× magnifications. Patient A, the red arrows mark the Haversian canals; the green arrows mark the osteoid tissue; Patient B, the blue arrows mark the Haversian canals, and the bone above the blue line is more immature; Patient C, the blue arrows mark some blood vessels within the Haversian canals. Second row, 400×, 200×,400× magnifications. Patient A, the green arrows mark active osteoblasts becoming embedded in their own matrix; Patient B, the blue arrows mark a region undergoing new bone deposition; Patient C, a detail, showing again a blood vessel within an Haversian canal

the equine bone[38] in a physiological way (and differently from what they do on ABB[39]). Most probably, EDEB preserves type I bone collagen unaltered in its native conformation,[15,16,35,38] which, in turn, might allow its modulation of several processes related to bone regeneration.[40–43] These observations are consistent with the histomorphometric ones comparing EDEB and ABB (which is collagen-free):[12] when the two materials were grafted in prospectively recruited and randomly allocated patients who underwent sinus augmentation, biopsies collected at 6 months from sinuses grafted with EDEB systematically contained more newly formed bone and less residual biomaterial than those collected from sinuses grafted with ABB.[36] A further confirmation comes from the study by Di Stefano et al. in 46 patients seeking implant-supported rehabilitation and treated with EDEB or ABB.[44] In that study, no collagen or other proteins were detected in ABB samples by the attenuated total reflection Fourier transform



Figs 6A to C: Focus on histologic assessment of Patient A: (A) The active remodeling is showed by a new matrix synthetized by osteoblasts (red arrows). The osteoclasts' activity creates resorption areas (Howship lacunae, green arrows) nearby a biomaterial residual, marked by empty osteocyte lacunae (asterisks); (B) The panel reveals active osteoblasts (green arrows) filling a new matrix connecting two areas of the newly formed bone; (C) The panel shows the presence of a newly formed bone alongside comma-shaped low-density areas (blue arrows) that might resemble carrier residuals

Table 1: Results of histomorphometric analyzes on the three bone samples collected from patients A, B, and C

Patient	*Mineralized matrix (%)*	*Osteoid tissue (%)*	*Medullar spaces (%)*	*Residual biomaterial (%)*
A	69.29 ± 3.15	–	30.71 ± 1.15	–
B	70.70 ± 2.21	–	27.46 ± 1.70	1.85 ± 0.52
C	63.27 ± 0.09	9.00 ± 1.58	25.98 ± 0.98	1.76 ± 0.51

infrared (ATR-FTIR) analysis. On the contrary, both the ATR-FTIR and the SDS-PAGE analyzes showed the presence of collagen in its native conformation in EDEB samples.[44] Furthermore, histomorphometric examination demonstrated a significantly higher amount of newly formed bone in sites grafted with EDEB compared to those grafted with ABB. Similarly, a fewer amount of residual biomaterial was detected in sites grafted with EDEB.[44] Another study retrospectively analyzed histomorphometric data of biopsies collected at different times from sinuses grafted with EDEB and concluded that bone formation at 4 months after grafting was not significantly different from those observed at later time points.[37] These results are in accordance with the results of the present study, supporting the evidence that native type I bone collagen in EDEB may have favored bone regeneration. The equine bone paste (EDEBEX) used in this pilot investigation also contains vitamin C, which is a requested cofactor of prolyl-hydroxylase and lysyl-hydroxylase, two enzymes that catalyze collagen fibril assembly and are essential during bone formation.[45] This might further explain the early bone deposition and bone substitute remodeling observed in our study. Interestingly, the histological investigations performed in the present study highlighted a peculiar structure in the inner part of some bone trabeculae, which can be ascribable to hydrogel remnants and, therefore, the original grafting site of the biomaterial. These areas are characterized by a dense concentration of mature osteocytes, possibly suggesting that the grafting material has been fully populated by the cells first, and then acted as the starting point for new bone formation. However, all these observations and speculations should be considered as preliminary. Our histomorphometric data differ from those by Di Stefano et al.[26] concerning the amount of residual biomaterial observed; our data concerning patient C, whose biopsy was collected at the latest time point (7 months), showed that bone tissue was still undergoing deposition, while bone remodeling have already reached a balance between anabolism and catabolism in patient B, whose biopsy was collected 1 month earlier. These results indicate that more factors, other than the bone paste, may modulate the kinetic of its remodeling. This should definitively be the subject of further studies. Our observations also confirm that freeze-dried EDEBEX may be used in post-extractive sockets not undergoing any flap preparation, that is — left to heal by second intention. This property, together with its easy-handling, should also be further investigated with appropriate studies.

Conclusion

The results of this preliminary, pilot histomorphometric investigation on a novel freeze-dried equine-derived bone paste show that it allows successful socket preservation, promoting the formation of a large amount of bone, therefore reinforcing prior indications that it might be a promising bone graft for ridge preservation surgeries. These preliminary findings should be the subject of further targeted studies.

Clinical Significance

Ridge preservation using bone substitutes as an alternative to autogenous bone is known to be effective, yet available clinical evidence still does not indicate the biomaterial, if any, that should be preferred to carry it out. The equine bone paste used in the present study appears to be a good candidate for further investigation given the bone formation rate it seems to display and its easy-handling property in the clinical setting.

References

1. Van der Weijden F, Dell'Acqua F, Slot DE. Alveolar bone dimensional changes of post-extraction sockets in humans: a systematic review. J Clin Periodontol 2009;36(12):1048–1058. DOI: 10.1111/j.1600-051X.2009.01482.x.
2. Araújo MG, Lindhe J. Ridge alterations following tooth extraction with and without flap elevation: an experimental study in the dog. Clin Oral Implants Res 2009;20(6):545–559. DOI: 10.1111/j.1600-0501.2008.01703.x.

3. Rasperini G, Canullo L, Dellavia C, et al. Socket grafting in the posterior maxilla reduces the need for sinus augmentation. Int J Periodontics Restorative Dent 2010;30(3):265–273.
4. De Risi V, Clementini M, Vittorini G, et al. Alveolar ridge preservation techniques: a systematic review and meta-analysis of histological and histomorphometrical data. Clin Oral Implants Res 2015;26(1):50–68. DOI: 10.1111/clr.12288.
5. Misch CM. Maxillary autogenous bone grafting. Oral Maxillofac Surg Clin North Am 2011;23(2):229–238. DOI: 10.1016/j.coms.2011.01.003.
6. Nkenke E, Weisbach V, Winckler E, et al. Morbidity of harvesting of bone grafts from the iliac crest for preprosthetic augmentation procedures: a prospective study. Int J Oral Maxillofac Surg 2004;33(2):157–163. DOI: 10.1054/ijom.2003.0465.
7. Esposito M, Grusovin MG, Felice P, et al. Interventions for replacing missing teeth: horizontal and vertical bone augmentation techniques for dental implant treatment. Cochrane Database Syst Rev 2009;4:CD003607. DOI: 10.1002/14651858.CD003607.pub4.
8. Aerssens J, Boonen S, Lowet G, et al. Interspecies differences in bone composition, density, and quality: potential implications for in vivo bone research. Endocrinology 1998;139(2):663–670. DOI: 10.1210/endo.139.2.5751.
9. Zizzari VL, Zara S, Tetè G, et al. Biologic and clinical aspects of integration of different bone substitutes in oral surgery: a literature review. Oral Surg Oral Med Oral Pathol Oral Radiol 2016;122(4):392–402. DOI: 10.1016/j.oooo.2016.04.010.
10. Baldini N, De Sanctis M, Ferrari M. Deproteinized bovine bone in periodontal and implant surgery. Dent Mater 2011;27(1):61–70. DOI: 10.1016/j.dental.2010.10.017.
11. Jensen SS, Terheyden H. Bone augmentation procedures in localized defects in the alveolar ridge: clinical results with different bone grafts and bone-substitute materials. Int J Oral Maxillofac Implants 2009;24(Suppl):218–236.
12. Benke D, Olah A, Möhler H. Protein-chemical analysis of bio-Oss bone substitute and evidence on its carbonate content. Biomaterials 2001;22(9):1005–1012. DOI: 10.1016/S0142-9612(00)00323-9.
13. Felice P, Piana L, Checchi L, et al. Vertical ridge augmentation of an atrophic posterior mandible with an inlay technique and cancellous equine bone block: a case report. Int J Periodontics Restorative Dent 2013;33(2):159–166. DOI: 10.11607/prd.1098.
14. Pistilli R, Signorini L, Pisacane A, et al. Case of severe bone atrophy of the posterior maxilla rehabilitated with blocks of equine origin bone: histological results. Implant Dent 2013;22(1):8–15. DOI: 10.1097/ID.0b013e3182777239.
15. Di Stefano DA, Andreasi Bassi M, Cinci L, et al. Treatment of a bone defect consequent to the removal of a periapical cyst with equine bone and equine membranes: clinical and histological outcome. Minerva Stomatol 2012;61(11–12):477–490.
16. Artese L, Piattelli A, Di Stefano DA, et al. Sinus lift with autologous bone alone or in addition to equine bone: an immunohistochemical study in man. Implant Dent 2011;20(5):383–388. DOI: 10.1097/ID.0b013e3182310b3d.
17. De Angelis N, Scivetti M. Lateral ridge augmentation using an equine flex bone block infused with recombinant human platelet-derived growth factor BB: a clinical and histologic study. Int J Periodontics Restorative Dent 2011;31(4):383–388.
18. Ludovichetti M, Di Stefano DA, Pagnutti S, et al. Vertical ridge augmentation using a flexible heterologous cortical bone sheet: Three-year follow-up. Int J Periodontics Restorative Dent 2011;31(4):401–407.
19. Di Stefano DA, Artese L, Iezzi G, et al. Alveolar ridge regeneration with equine spongy bone: a clinical, histological, and immunohistochemical case series. Clin Implant Dent Relat Res 2009;11(2):90–100. DOI: 10.1111/j.1708-8208.2008.00104.x.
20. Stievano D, Di Stefano A, Ludovichetti M, et al. Maxillary sinus lift through heterologous bone grafts and simultaneous acid-etched implants placement. five year follow-up. Minerva Chir 2008;63(2):79–91.
21. Santini S, Barbera P, Modena M, et al. Equine-derived bone substitutes in orthopedics and traumatology: authors' experience. Minerva Chir 2011;66(1):63–72.
22. Fontana F, Rocchietta I, Dellavia C, et al. Biocompatibility and manageability of a new fixable bone graft for the treatment of localized bone defect: preliminary study in a dog model. Int J Periodontics Restorative Dent 2008;28(6):601–607.
23. Ten Heggeler JM, Slot DE, Van, et al. Effect of socket preservation therapies following tooth extraction in non-molar regions in humans: a systematic review. Clin Oral Implants Res 2011;22(8):779–788. DOI: 10.1111/j.1600-0501.2010.02064.x.
24. Roddy E, DeBaun MR, Daoud-Gray A, et al. Treatment of critical-sized bone defects: Clinical and tissue engineering perspectives. Eur J Orthop Surg Traumatol 2018;28(3):351–362. DOI: 10.1007/s00590-017-2063-0.
25. Giannoni P, Villa F, Cordazzo C, et al. Rheological properties, biocompatibility and in vivo performance of new hydrogel-based bone fillers. Biomater Sci 2016;4(11):1691–1703. DOI: 10.1039/C6BM00478D.
26. Di Stefano DA, Arosio P, Cinci L, et al. Ridge preservation using an innovative enzyme-deantigenic equine bone paste: a case report with 36-month follow-up. J Contemp Dent Pract 2019;20(10):1229–1234. DOI: 10.5005/jp-journals-10024-2664.
27. Albrektsson T, Zarb G, Worthington P, et al. The long-term efficacy of currently used dental implants: a review and proposed criteria of success. Int J Oral Maxillofac Implants 1986;1(1):11–25.
28. Canullo L, Pellegrini G, Canciani E, et al. Alveolar socket preservation technique: effect of biomaterial on bone regenerative pattern. Ann Anat 2016;206:73–79. DOI: 10.1016/j.aanat.2015.05.007.
29. Canullo L, Wiel Marin G, Tallarico M, et al. Histological and histomorphometrical evaluation of postextractive sites grafted with mg-enriched nano-hydroxyapatite: a randomized controlled trial comparing 4 versus 12 months of healing. Clin Implant Dent Relat Res 2016;18(5):973–983. DOI: 10.1111/cid.12381.
30. Vignoletti F, Matesanz P, Rodrigo D, et al. Surgical protocols for ridge preservation after tooth extraction. A systematic review. Clin Oral Implants Res 2012;23(Suppl 5):22–38. DOI: 10.1111/j.1600-0501.2011.02331.x.
31. Lee J, Lee JB, Koo KT, et al. Flap management in alveolar ridge preservation: a systematic review and meta-analysis. Int J Oral Maxillofac Implants 2018;33(3):613–621. DOI: 10.11607/jomi.6368.
32. Al Yafi F, Alchawaf B, Nelson K. What is the optimum for alveolar ridge preservation? Dent Clin North Am 2019;63(3):399–418. DOI: 10.1016/j.cden.2019.02.007.
33. Bassir SH, Alhareky M, Wangsrimongkol B, et al. Systematic review and meta-analysis of hard tissue outcomes of alveolar ridge preservation. Int J Oral Maxillofac Implants 2018;33(5):979–994. DOI: 10.11607/jomi.6399.
34. Iocca O, Farcomeni A, Pardiñas Lopez S, et al. Alveolar ridge preservation after tooth extraction: a bayesian network meta-analysis of grafting materials efficacy on prevention of bone height and width reduction. J Clin Periodontol 2017;44(1):104–114. DOI: 10.1111/jcpe.12633.
35. Atieh MA, Alsabeeha NH, Payne AG, et al. Interventions for replacing missing teeth: alveolar ridge preservation techniques for dental implant site development. Cochrane Database Syst Rev 2015;2015(5):CD010176. DOI: 10.1002/14651858.CD010176.pub2.
36. Di Stefano DA, Gastaldi G, Vinci R, et al. Histomorphometric comparison of enzyme-deantigenic equine bone and anorganic bovine bone in sinus augmentation: a randomized clinical trial with 3-year follow-up. Int J Oral Maxillofac Implants 2015;30(5):1161–1167. DOI: 10.11607/jomi.4057.
37. Di Stefano DA, Gastaldi G, Vinci R, et al. Bone formation following sinus augmentation with an equine-derived bone graft: a retrospective histologic and histomorphometric study with 36-month follow-up. Int J Oral Maxillofac Implants 2016;31(2):406–412. DOI: 10.11607/jomi.4373.

38. Perrotti V, Nicholls BM, Piattelli A. Human osteoclast formation and activity on an equine spongy bone substitute. Clin Oral Implants Res 2009;20(1):17–23. DOI: 10.1111/j.1600-0501.2008.01608.x.
39. Perrotti V, Nicholls BM, Horton MA, et al. Human osteoclast formation and activity on a xenogenous bone mineral. J Biomed Mater Res A 2009;90(1):238–246. DOI: 10.1002/jbm.a.32079.
40. Liu G, Hu YY, Zhao JN, et al. Effect of type I collagen on the adhesion, proliferation, and osteoblastic gene expression of bone marrow-derived mesenchymal stem cells. Chin J Traumatol 2004;7(6):358–362.
41. Mizuno M, Fujisawa R, Kuboki Y. Type I collagen-induced osteoblastic differentiation of bone-marrow cells mediated by collagen-alpha2beta1 integrin interaction. J Cell Physiol 2000;184(2):207–213. DOI: 10.1002/1097-4652(200008)184:2<207::AID-JCP8>3.0.CO;2-U.
42. Regazzoni C, Winterhalter KH, Rohrer L. Type I collagen induces expression of bone morphogenetic protein receptor type II. Biochem Biophys Res Commun 2001;283(2):316–322. DOI: 10.1006/bbrc.2001.4813.
43. Green J, Schotland S, Stauber DJ, et al. Cell-matrix interaction in bone: type I collagen modulates signal transduction in osteoblast-like cells. Am J Physiol 1995;268(5 Pt 1):C1090–C1103. DOI: 10.1152/ajpcell.1995.268.5.C1090.
44. Di Stefano DA, Zaniol T, Cinci L, et al. Chemical, clinical and histomorphometric comparison between equine bone manufactured through enzymatic antigen-elimination and bovine bone made non-antigenic using a high-temperature process in post-extractive socket grafting. A comparative retrospective clinical study. Dent J (Basel) 2019;7(3):70. DOI: 10.3390/dj7030070.
45. Grinnell F, Fukamizu H, Pawelek P, et al. Collagen processing, crosslinking, and fibril bundle assembly in matrix produced by fibroblasts in long-term cultures supplemented with ascorbic acid. Exp Cell Res 1989;181(2):483–491. DOI: 10.1016/0014-4827(89)90105-5.