CLINICAL AND HISTOLOGICAL EVALUATION OF SOCKET PRESERVATION USING SMART-BONE®, A NOVEL HETEROLOGOUS BONE SUBSTITUTE: A CASE SERIES STUDY

F. MANDELLI[1], G. PERALE[2], S. DANTI[3,4], D. D'ALESSANDRO[4], P. GHENSI[5]

[1] *Private Practice, Milano, Italy*
[2] *Industrie Biomediche Insubri SA (IBI SA), Mezzovico-Vira, Switzerland*
[3] *Creative Engineering Design Area, The Biorobotics Institute, Scuola Superiore Sant'Anna, Pontedera (PI), Italy*
[4] *Department of Surgical, Medical, Molecular Pathology and Emergency Medicine, University of Pisa, Pisa, Italy*
[5] *Centre for Integrative Biology (CIBIO), University of Trento, Trento, Italy*

SUMMARY
Objectives. The aim of this case series study was to evaluate, clinically and histologically, the performances of a novel composite xenohybrid bone substitute.
Methods. Ten non-restorable teeth were extracted and socket preservation was performed with a bovine heterologous graft enriched with collagen and resorbable biopolymers (SmartBone®). The socket was covered with a collagen membrane firmly sutured. After five months of healing, implant site was prepared by means of a trephine bur and a dental implant was inserted. Specimens were sent for histological analysis. After three months of healing, patients received a provisional restoration followed by a definitive crown.
Results. All socket preservations healed uneventfully and, after five months, it was possible to insert implants with no additional bone augmentation procedures. All placed implants osseointegrated successfully and were in function after a minimum follow-up period of 30 months.
Conclusions. The tested biomaterial confirmed good clinical performance and, even if left exposed to the oral cavity covered with a collagen membrane, did not show signs of infection. Further research is desirable with a larger sample and variations of socket preservation technique to better understand the potential of this novel bone substitute.

Key words: socket preservation, biomaterial, heterologous, histological, dental implants, bone substitute.

Introduction

Many studies have evaluated how bone remodels after tooth extraction, both vertically and horizontally (1-3). Post-extraction alveolar bone resorption varies among subjects and sites and involves anterior and posterior teeth (4-6). A recent systematic review on human studies reported a vertical dimensional change of 11-22% at 6 months and a horizontal dimensional change of 32% at 3 months and 29-63% at 6-7 months after tooth extraction (7). It is a well-known concept that resorption of the buccal compartment of the ridge – after tooth removal – is more pronounced than the amount of tissue that is lost in the lingual/palatal portion. The alteration of the ridge occurs during the process of healing of the soft and hard tissues but the process of remodeling may continue for several months (8). Alveolar ridge resorption in height and width may cause problems with implant placement, especially in the anterior maxillary region, where

bone volume is important for both biologic and aesthetic reasons. For this reason, over the past twenty years, different ridge preservation techniques have been proposed aiming both to maintain an ideal ridge profile in aesthetic sites, and to prevent alveolar ridge collapse, preserving adequate dimensions of bone in order to facilitate implant placement in prosthetically driven positions (9). Autogenous bone, demineralized freeze-dried bone allograft (DFDBA), xenografts, alloplastic materials and bone morphogenetic proteins (BMP) used alone or in combination with a membrane have been used to fill the tooth socket immediately following tooth extraction (10-14). While recent studies confirmed that by augmenting the extraction socket with bone substitute material, resorption processes are reduced and further treatment steps are simplified (15, 16), it was suggested that graft materials often interfere with physiological healing processes and the quality of the new hard tissue in the socket can vary broadly (11, 12, 16). Hence, there is a need to develop and test graft materials that will predictably enhance the healing of extraction sockets and, at the same time, immediately induce a consistent remodeling, that allows a consistent formation of new bone. The present study was therefore conducted to investigate the effectiveness of socket preservation but mainly to determine the composition of the tissue formed after 5 months of healing in extraction sites that had been grafted with SmartBone®, a newly developed heterologous cancellous bovine-derived bone substitute, enriched with collagen and biopolymers (17-20).

Materials and methods

Case series

This case series study included 10 partially edentulous patients in need for posterior tooth extraction and subsequent implant prosthetic rehabilitation and that were treated with the same alveolar socket preservation technique prior to implant placement. All subjects were informed of the study and their informed consents were duly recorded. Good clinical practice was always followed.

For antibiotic prophylaxis, 2 g of amoxicillin (Zimox, Pfizer Inc., USA) were administered to all patients 1 hour before surgery. Patients also rinsed for 2 minutes with chlorhexidine 0.20% mouth rinse (Dentosan, Pfizer Consumer Healthcare, Rome, Italy) and received 600 mg of a non-steroidal-anti-inflammatory drug (Brufen 600, Abbott Laboratories). Local anesthetic was administered by means of infiltration into the oral mucosa with 4% Articain hydrochloride with epinephrine 1:100000 (Oralbloc, Pierrel S.p.A, Italy).

In order to minimize soft and hard tissue trauma, extraction was performed by separating the tooth in two or more fragments with a surgical bur and a high-speed handpiece. Particular care was given to avoid damaging surrounding bone walls and root pieces were then extracted separately. No flap was raised in order to keep intact soft tissue architecture and not to interrupt the vascularization of the buccal bone. If present, endodontic lesions were thoroughly removed and the socket was rinsed with sterile saline water.

After the extraction, the alveolar socket was grafted with SmartBone® 0.25-1 mm granules (IBI SA, Mezzovico-Vira, Switzerland), up to the top of the bone walls. An equine resorbable collagen membrane (Parasorb Forte membrane, Resorba, Nuremberg, Germany) was trimmed to the size of the socket, placed covering the graft and firmly sutured with a 6/0 Nylon suture (Resotex Oral, Resorba, Nuremberg, Germany). No antibiotic was given after surgery and ibuprofen was prescribed three times a day for three days. The patients were instructed not to brush the wound and to apply a 1% chlorhexidine gel (Corsodyl, SmithKline Beecham, Milan, Italy) twice a day for fifteen days. Sutures were removed seven days after surgery.

A periapical radiograph was performed just after the extraction, before implant placement at the time of implant loading and during follow-up visits. The day of implant surgery, previously described pre-operative protocol and anesthesia were performed. A full-thickness flap was raised,

the implant osteotomy was prepared with a 2.5-mm internal diameter trephine bur (Thomas, Bourges, France) and the harvested bone core was sent for histomorphometric analysis. A 3.5 or 4.5 mm-wide implant were inserted (Ankylos, Dentsply Sirona Implants, Mölndal, Sweden) with a healing abutment. After three months of healing, all the implants received a standard abutment (Ankylos, Dentsply Sirona Implants, Mölndal, Sweden) of either 1,5 mm or 3 mm transmucosal height that was tightened at 25 N/cm and never removed afterwards. A poly-methyl-methacrylate (PMMA) provisional crown was kept in place for eight weeks, before the definitive porcelain crown was delivered (Figure 1 A-Q).

Bone substitute

SmartBone® is a composite xeno-hybrid bone graft, composed of bovine-derived cancellous

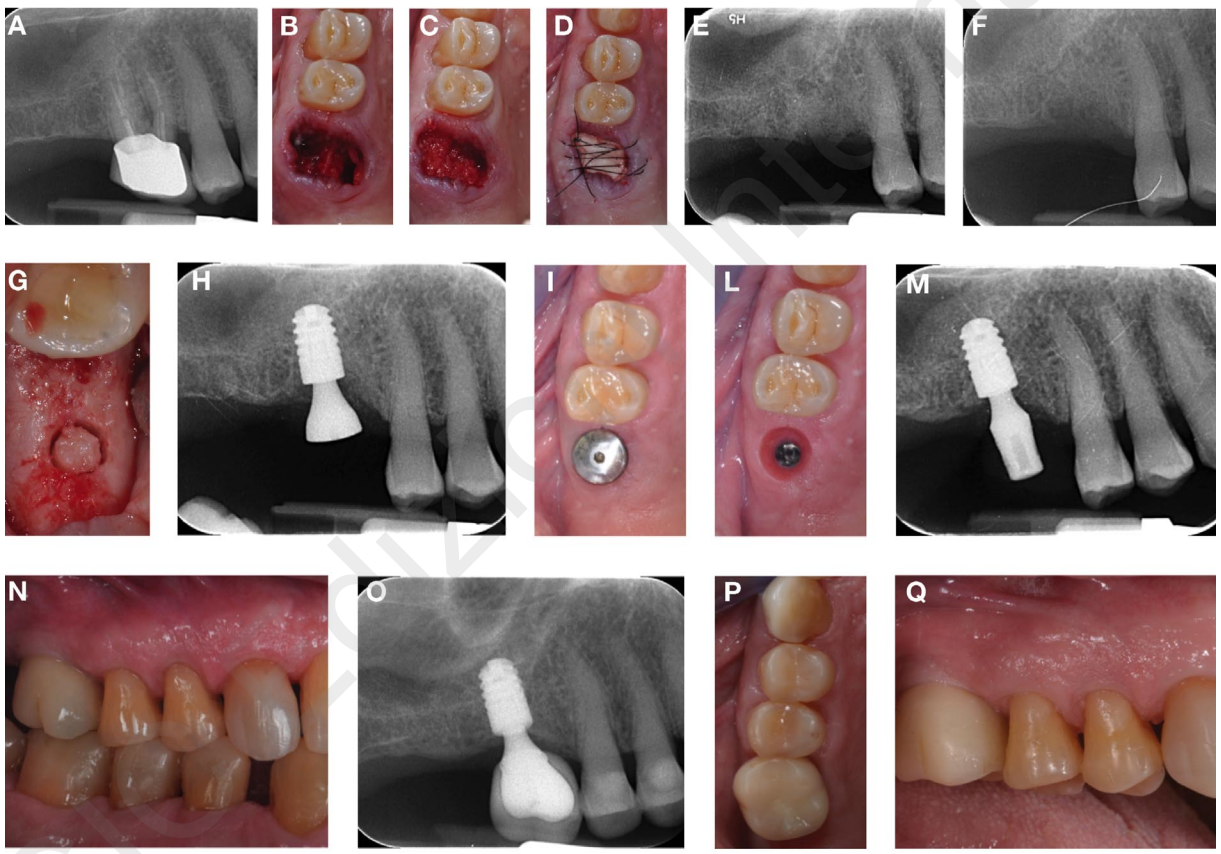

Figure 1
Clinical phases of socket preservation, implant placement and prosthetic rehabilitation.
A) Non-restorable first right upper molar with class III furcation. B) Atraumatic tooth extraction without flap elevation. C) Socket grafting with SmartBone® 0.25-1 mm granules. D) An equine resorbable collagen membrane trimmed to the size of the socket, placed covering the graft and firmly sutured with a 6/0 Nylon suture. E) Periapical radiograph performed just after tooth extraction and socket preservation. F) Periapical radiograph performed after 5 months of healing and before implant placement. G) Implant osteotomy prepared with a 2.5-mm internal diameter trephine bur. H) Periapical radiograph showing the insertion of an Ankylos dental implant with a healing abutment. I) One-month healing after implant insertion. L) Soft tissue conditions after three months of healing. M) Periapical radiograph showing implant with a standard abutment tightened at 25 N/cm. N) A poly-methyl-methacrylate (PMMA) provisional crown in place. O) Periapical radiograph showing the definitive implant-prosthetic rehabilitation. P) Occlusal view of the definitive implant-prosthetic rehabilitation after 36 months. Q) Lateral view of the definitive implant-prosthetic rehabilitation after 36 months.

bone mineral matrix, reinforced with a proprietary blend of biopolymers (*i.e.* resorbable aliphatic block-co-polyesters) and collagen fragments (in the form of hydrolyzed gelatin), intended for use in bone regeneration applications in reconstructive surgeries. Its intrinsic composition, together with its microstructure, make of SmartBone® a good autologous bone mimicking substitute: it was demonstrated to sparkle and withstand bone remodeling process, being finally able to be completely substituted by living healthy bone from the host (18-20).

Histologic processing

Sample preparation

After collection, the biopsies were fixed in 4% w/v formaldehyde diluted in 1× phosphate buffered solution (PBS) (Bio-Optica, Milan, Italy) overnight at 4°C, then washed in 1× PBS and decalcified in a ddH2O solution containing 10% ethylenediaminetetraacetic acid (EDTA, Sigma-Aldrich, Saint Louis, MO, USA) for 15 days at 4°C, replacing the solution each 3-4 days. After washing, samples were stored in 70% ethanol at 4°C, dehydrated with an ascending graded series of ethanol up to absolute ethanol (Sigma-Aldrich) and clarified in xylene twice for 45 min. All the steps were performed in a thermostatic bath at 40°C; therefore, the biopsies were rinsed in liquid paraffin at 60°C for 2 h and finally paraffin-embedded. Sections (5 micron-thick), performed by standard microtome, were mounted on glass slides and stored overnight at 37°C. Before each staining/reaction, the sections were deparaffinized in xylene and rehydrated in absolute ethanol.

Histochemistry

The following tests were performed for each sample: Hematoxylin-Eosin (H&E) staining to evaluate general morphology; Periodic Acid Schiff (PAS) reaction to detect glycoproteins; Alcian Blue staining at pH 2.5 to show the presence of glycosaminoglycans (GAGs); Van Gieson staining to highlight the presence of the total collagen. After each staining, the sections were dehydrated in absolute ethanol and clarified in xylene, then they were mounted by DPX mounting medium (Sigma-Aldrich)

Immunohistochemistry

Sections were incubated with 0.2% Triton X-100 (Sigma-Aldrich) in 1× PBS for 10 min and quenching of endogenous peroxidases was performed by incubating them with 0.6% H2O2 in methanol (Sigma-Aldrich) for 20 min in the dark. The samples were thus incubated with 5% Goat Serum (Vektor Lab, Burlingame, CA, USA) diluted in 1× PBS for 20 min at 37°C, to block aspecific binding sites of the secondary antibody. The sections were finally incubated with primary antibodies diluted in a 1× PBS solution containing 0.1% Bovine Serum Albumin (BSA, Sigma-Aldrich), in moist chamber overnight at 4°C, namely: rabbit anti-collagen I 1:2000 (ab34710, abCam, Cambridge, MA, USA), rabbit anti-osteocalcin 1:800 (sc30044, Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit anti-TGFbeta1 1:500 (sc-146, Santa Cruz), and mouse anti-fibronectin 1:1000 (sc-59826, Santa Cruz). Negative controls were obtained by incubating two sections for each biopsy with BSA/1× PBS solution only. After each step, washings with 1× PBS were performed. The following day, the specimens were incubated with goat anti-rabbit or goat anti-mouse biotinylated secondary antibodies (Vektor Lab, USA) diluted 1:200 in 1.5% goat serum-1× PBS solution for 60 min and then with streptavidin according to manufacturer's instructions (Vectastain Elite ABC Kit Standard, Vektor Lab, USA). The sections were incubated in the substrate-chromogen solution (0.5 mg/mL 3,3'-diaminobenzidine tetrahydrochloride containing 0.02% H2O2) (Amresco, Solon, OH, USA) for 5 min in the dark to reveal the reaction, counterstained in hematoxylin, dehydrated and finally mounted as previously described. In the second part of the reaction, after each step, washings in 0.01% Triton/1× PBS and 1× PBS were performed. Histological analyses were evaluated under a Nikon Eclipse Ci microscope (Nikon Instruments, Amsterdam, The Netherlands) equipped with a digital camera.

Results

A total of 10 patients (6 males, 4 females; average age 58 years, range 44 to 82 years) was consecutively enrolled in this case series study and the mean follow-up was of 35.8 months (range 30-43). From an overall point of view, 9 completely successful cases were recorded (one subject moved abroad and did not come for the follow-up visit); no failures, nor accidents, nor adverse event nor poor results were recorded. All extractions healed uneventfully and the patients were visited every week for four weeks, after two months and just before the implant placement. Periapical radiographs performed five months after tooth extraction suggested a good healing of the sockets. One patient did not receive the implant because he moved to another city and was lost to follow-up. Nine implants were placed and all of them osseointegrated. A total of nine Ankylos implants was inserted. Implants were rehabilitated with cemented crowns and no detachments of the prostheses were reported during the evaluation period. At every follow-up visit, the rehabilitations were checked for signs of peri-implant inflammation, radiographic signs of bone loss, fractures, and mobility of the prosthesis or of abutments, none of which was ever recorder for all 9 patients.

At the time of writing this paper, after a minimum follow-up period of 30 months, soft tissue contours showed no significant changes and radiographic examination highlighted maintained bone levels around implant platforms.

From a histological point of view, biopsies collected five months after tooth extraction and socket preservation showed the simultaneous presence of SmartBone®, new bone and fibrous tissue with formation of new vessels, which are essential for the regeneration process, that is indeed well depicted and fully on-going at that time of biopsies. The histological analysis showed that the tested bone substitute acted as an efficient scaffold for new bone formation. Bone extracellular matrix molecules were highly expressed in the newly formed bone and osteoblasts were visible on granules surfaces. Overall, histological analysis proved a robust ongoing remodeling process on harvested sites, in good agreement with radiological evidences (Figure 2 A-H).

Discussion

It is well known that tooth extraction is invariably followed by loss of height and width of the alveolar process; socket preservation procedures have been proposed to reduce shrinking of residual bone (21, 22). At the same time, socket preservation is an excellent risk-free surgical study model and, for this reason, it was employed in this case series study to clinically and histologically evaluate the performance of SmartBone®, a novel heterologous bone substitute enriched with collagen and biopolymers (23). Minimally invasive protocol was followed. Extraction and socket grafting were performed without raising a flap, the augmented site was covered with a resorbable membrane and not with a primary closure or a soft tissue punch. This approach was selected in order to minimize patient morbidity and surgical time, but mostly attempting not to displace the mucogingival junction (24). Despite the clear limitations of a case series study, it was possible to obtain interesting considerations on this new biomaterial. From a clinical and radiographic point of view, in all 9 cases the healing took place without complications and soft and hard tissues at five months appeared more than satisfactory. Soft tissues appeared healthy and free of scarring, while bone tissue at the time of implant insertion resulted positively preserved, compact and almost completely healed with only small fractions of biomaterial adherent to the primary flap: in fact, no further augmentation procedures where needed. These findings are interesting because the waiting time was only five months, shorter than many other studies in the literature performed with other biomaterials (21, 25, 26). Moreover, it should be emphasized that a second intention healing with a resorbable collagen membrane intentionally left exposed did not in any case lead to infection, mucosal inflammation or to unsatisfactory healing.

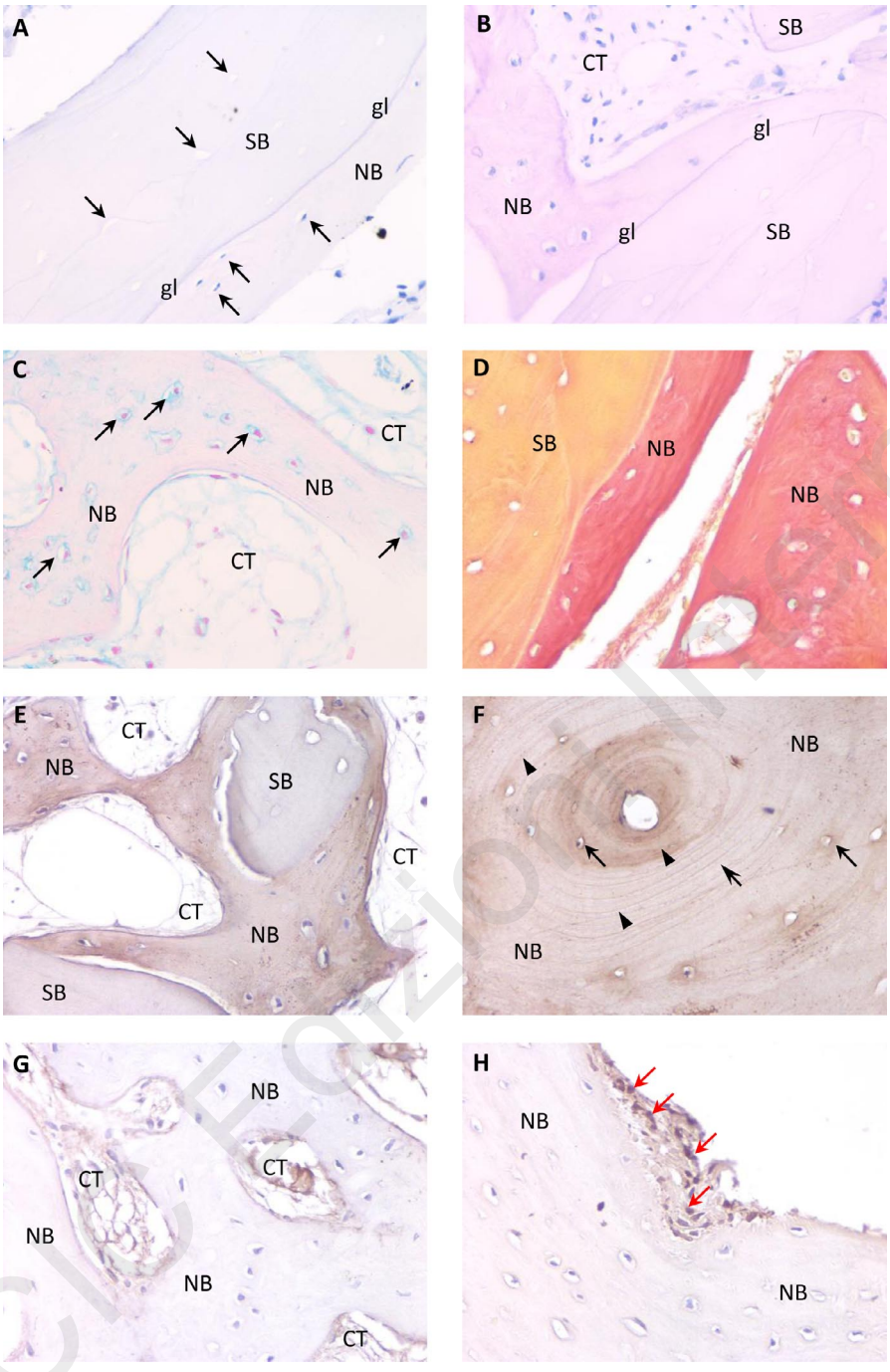

Figure 2
Histologic analysis of osteogenic marker and extracellular matrix molecules expressed in bone biopsies. A-D) Histochemistry. A) Hematoxylin-Eosin shows general morphology. Cell nuclei are in blue-violet and bone areas in pink. B) Periodic Acid Schiff reaction reveals the presence of glycoproteins in magenta. Cell nuclei are in blue-violet. C) Alcian Blue pH 2.5 staining highlights the presence of glycosaminoglycans in cyan. Cell nuclei are in red. D) Van Gieson staining shows the presence of total collagen fibers in red; non-collagenous structures are in yellow and cell nuclei in brown-black. E-F) Immunohistochemistry. Osteogenic markers are in brown, cell nuclei are in blue-violet. E) Collagen Type I. F) Osteocalcin. G) Fibronectin. H) Transforming Growth Factor beta 1. A-H) Black arrows = bone lacunae; gl = growth line; SB = SmartBone; NB = New Bone; CT = Connective Tissue; arrowhead = bone lamellae; red arrows = osteoblasts. Original Magnification x400.

From a histological point of view, in most of the samples new bone areas were predominantly detected, while graft areas were scarce. Osteoblasts were observed along the borders of the new bone and a large amount of well-structured connective tissue was present, which surrounded both new bone and graft areas; moreover, in most of the samples no fibrotic areas and inflammatory cells were detected. Bone remodeling therefore appeared remarkable with reduced

amounts of residual biomaterial, being almost all already substituted by new bone already after five months from grafting.
These results are interesting, although limited to nine clinical cases and without the presence of positive and negative controls, but in good agreement with biomaterial clinical claims and mechanism of action (27). It would be useful to consider the behavior of this relatively new biomaterial even after waiting times both shorter and longer but, above all, in cases of alveolar socket preservation with primary wound closure through the flap or a soft tissue punch.

Conclusions

The tested biomaterial confirmed good clinical performance and, even if left exposed to the oral cavity, covered with a collagen membrane did not show signs of infection. Its regenerative action mechanism and its being an adequate support to new bone formation was confirmed through clinical and histological evidences. Further research is desirable with a larger sample and variations of socket preservation technique to better understand the potential of this novel bone substitute.

Conflict of interest

Dr. Giuseppe Perale declares a conflict of interest with the biomaterial brand used in this case series study as co-owner of the company IBI S.A. All the other Authors declare no conflict of interest.

References

1. Moya-Villaescusa MJ, Sanchez-Perez A. Measurement of ridge alterations following tooth removal: a radiographic study in humans. Clin Oral Implants Res. 2010;21:237-242.
2. Araujo MG, Lindhe J. Dimensional ridge alterations following tooth extraction. An experimental study in the dog. J Clin Periodontol. 2005;32:212-218.
3. Malchiodi L, Cucchi A, Ghensi P, Nocini PF. Evaluation of the esthetic results of 64 nonfunctional immediately loaded postextraction implants in the maxilla: correlation between interproximal alveolar crest and soft tissues at 3 years of follow-Up. Clin Implant Dent Relat Res. 2011;15:130-42.
4. Leblebicioglu B, Salas M, Ort Y, Johnson A, Yildiz VO, Kim DG, et al. Determinants of alveolar ridge preservation differ by anatomic location. J Clin Periodontol. 2013;40:387-395.
5. Scala R, Ghensi P, Cucchi A, Pistoia E. Postextraction implant placement with immediate provisionalisation and finalisation, using a simplified technique: technical notes and a case report. Open Dent J. 2012;6:164-9.
6. Malchiodi L, Corrocher G, Cucchi A, Ghensi P, Bissolotti G, Nocini PF. Long-term results of immediately loaded fast bone regeneration–coated implants placed in fresh extraction sites in the upper jaw. J Oral Implantol. 2010;36:251-61.
7. 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;36:1048-1058.
8. Schropp L, Wenzel A, Kostopoulos L, Karring T. Bone healing and soft tissue contour changes following single- tooth extraction: a clinical and radiographic 12-month prospective study. Int J Periodontics Restorative Dent. 2003;23:313-323.
9. Darby I, Chen S, De Poi R. Ridge preservation: what is it and when should it be considered. Aust Dent J. 2008;53:11-21.
10. Becker W, Becker BE, Caffesse R. A comparison of demineralized freeze-dried bone and autologous bone to induce bone formation in human extraction sockets. J Periodontol. 1994;65:1128-1133.
11. Becker W, Urist M, Becker BE, Jackson W, Parry DA, Bartold M, et al. Clinical and histologic observations of sites implanted with intraoral autologous bone grafts or allografts. 15 human case reports. J Periodontol. 1996;67:1025-1033.
12. Froum S, Cho SC, Rosenberg E, Rohrer M, Tarnow D. Histological comparison of healing extraction sockets implanted with bioactive glass or demineralized freezedried bone allograft: a pilot study. J Periodontol. 2002;73:94-102.
13. Artzi Z, Tal H, Dayan D. Porous bovine bone mineral in healing of human extraction sockets. Part 1: histomorphometric evaluations at 9 months. J Periodontol. 2000;71:1015-1023.
14. Serino G, Biancu S, Iezzi G, Piattelli A. Ridge preservation following tooth extraction using a polylactide and polyglycolide sponge as space filler: a clinical and histological study in humans. Clin Oral Implants Res. 2003;14:651-658.

15. Shakibaie MB. Comparison of the effectiveness of two different bone substitute materials for socket preservation after tooth extraction: a controlled clinical study. Int J Periodontics Restorative Dent. 2013;33:223-228.
16. Heberer S, Al-Chawaf B, Hildebrand D, Nelson JJ, Nelson K. Histomorphometric analysis of extraction sockets augmented with Bio-Oss Collagen after a 6-week healing period: a prospective study. Clin Oral Implants Res. 2008;19:1219-1225.
17. Pertici G, Carinci F, Carusi G, Epistatus D, Villa T, Crivelli F, et al. Composite Polymer-Coated Mineral Scaffolds for Bone Regeneration: From Material Characterization to Human Studies. J Biol Regul Homeost Agents. 2015;29:136-148.
18. D'Alessandro D, Perale G, Milazzo M, Moscato S, Stefanini C, Pertici G, et al. Bovine bone matrix/poly(llactic- co-epsilon-caprolactone)/gelatin hybrid scaffold (SmartBone(R)) for maxillary sinus augmentation: a histologic study on bone regeneration. Int J Pharm. 2017;523:534-544.
19. Grecchi F, Perale G, Candotto V, Busato A, Pascali M, Carinci F. Reconstruction of the Zygomatic Bone with Smartbone(R): Case Report. J Biol Regul Homeost Agents. 2015;29:42-47.
20. Stacchi C, Lombardi T, Ottonelli R, Berton F, Perinetti G, Traini T. New bone formation after transcrestal sinus floor elevation was influenced by sinus cavity dimensions: A prospective histologic and histomorphometric study. Clin Oral Implants Res. 2018 Mar 23. [Epub ahead of print].
21. Maiorana C, Poli PP, Deflorian M, Testori T, Mandelli F, Nagursky H, et al. Alveolar socket preservation with demineralised bovine bone mineral and a collagen matrix. J Periodontal Implant Sci. 2017;47:194-210.
22. Avila-Ortiz G, Elangovan S, Kramer KW, Blanchette D, Dawson DV. Effect of alveolar ridge preservation after tooth extraction: a systematic review and meta-analysis. J Dent Re. 2014;93:950-958.
23. Willenbacher M, Al-Nawas B, Berres M, Kammerer PW, Schiegnitz E. The Effects of Alveolar Ridge Preservation: A Meta-Analysis. Clin Implant Dent Relat Res. 2016;18:1248-1268.
24. Fairbairn P, Leventis M, Mangham C, Horowitz R. Alveolar Ridge Preservation Using a Novel Synthetic Grafting Material: A Case with Two-Year Follow-Up. Case Rep Dent. 2018;2018:6412806.
25. Patel K, Mardas N, Donos N. Radiographic and clinical outcomes of implants placed in ridge preserved sites: a 12-month post-loading follow-up. Clin Oral Implants Res. 2013;24:599-605.
26. Iorio-Siciliano V, Blasi A, Nicolo M, Iorio-Siciliano A, Riccitiello F, Ramaglia L. Clinical Outcomes of Socket Preservation Using Bovine-Derived Xenograft Collagen and Collagen Membrane Post-Tooth Extraction: A 6-Month Randomized Controlled Clinical Trial. Int J Periodontics Restorative Dent. 2017;37:e290-e296.
27. Roato I, Belisario DC, Compagno M, Verderio L, Sighinolfi A, Mussano F, et al. Adipose-Derived Stromal Vascular Fraction/Xenohybrid Bone Scaffold: An Alternative Source for Bone Regeneration. Stem Cells International. 2018;2018:11.

Correspondence to:
Paolo Ghensi DDS
Oral Surg MClin Dent, Clin Msc
E-mail: dr.ghensi@gmail.com