

## Case Report

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# A New Surgical Protocol For Horizontal Ridge Augmentation: Simplified Apposition Technique. Human Histologic and Radiographic Analysis After 2.5 Years of Follow-Up, A Case Report

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## Abstract

Following tooth extraction, alveolar ridge undergoes a physiological and inevitable remodeling process that could influence the prosthodontic rehabilitation. Therefore, augmentation of an insufficient bone volume is often indicated, although it remains a significant challenge in clinical practice. The aim of this case report was to scientifically validate a new minimally invasive surgery technique called “Simplified Apposition Technique” (S.A.T), which consists in the use of deproteinized bovine bone, human fibrin glue and collagen membrane in association with a post-extraction socket, in order to obtain an increase of the horizontal ridge dimension in the nearby atrophic bone crest.

A 69-years-old man patient was enrolled in this study. The hopeless tooth 4.7 was extracted and the ridge reconstruction and the socket preservation were carried out following S.A.T. After 11 months, the formation of newly bone was evaluated through radiographic analysis (Cone Beam Computed Tomography, CBCT) comparing the status before and after surgery and at 2.5 years of follow-up. Simultaneously, an implant was placed in the regenerated site and the bone chips remaining in the drills were used for the histological evaluation. The CBCT revealed an increase of the bone crest width from 3.7 to 6.5 mm and, after 2.5 years of follow-up, the bone width has remained stable. The histological evaluation showed various stages of bone formation and maturation with a good integration of the regenerated area. According to these results, S.A.T can be considered a good surgical technique for horizontal ridge augmentation in the atrophic bone, near the extraction site.

**Keywords:** Bone regeneration; Collagen membrane; Bone remodeling; Ridge reconstruction; Human fibrin glue

## **1. Introduction**

After tooth extraction, morphological and dimensional changes of the alveolar ridge occur because of qualitative and quantitative alterations at the edentulous site [1-3], resulting in significant three-dimensional bone resorption and shrinkage [4-8]. However, post-extraction dimensional alterations appear to be related to several additional factors, including surgical trauma due to flap elevation, lack of functional stimulus on the remaining bone walls and a lack of periodontal ligament and genetic information [9]. Studies in literature reported a reduction of the horizontal ridge dimension in post-extraction sockets ranging from 2.6 to 4.6 mm [10-12]; while the mean vertical reduction in ridge height was about 1.24 mm [13].

Implant placement in patients with atrophic mandible or maxilla is becoming more prevalent and it is therefore important to maintain or recreate a natural contour of the post-extraction socket and of the residual ridge, especially in the esthetic zone [14]. Socket grafting has shown to modify these modeling events and to partially counteract the marginal ridge shrinkage that occurs following tooth extraction [15-17]. A large variety of biomaterials have been employed for ridge preservation and tested in several studies [15, 18-21], but the autologous bone graft is considered the gold standard, because it combines osteogenic, osteoinductive and osteoconductive properties [22, 23]. However, autograft is often associated with complications at the harvesting site and limited in quantity.

Recent studies have shown that grafting of extraction sockets with xenogenous biomaterials and the use of barrier membranes are able to reduce the degree of dimensional alterations and to promote the ridge augmentation procedures, by the osteoconductive and osteoinductive properties [6, 15, 24-29]. In addition, a good long-term stability has been demonstrated due to the slow resorption characteristic [24, 30]. In a recent systematic review, it was reported that the horizontal augmentation procedures using xenografts are feasible, presenting significant bone gain and low rates of complications [31]. Measurements performed in histological sections demonstrated that socket grafting with the use of deproteinized bovine bone (BO, Bio-Oss, Geistlich Biomaterials, Korea) allows preserving most of the dimension of the ridge. In a systematic review on ridge conservation after tooth extraction, Vignoletti et

al. [32] concluded that socket grafting with biomaterials may result in less vertical and horizontal contraction of the bone crest, but also that there is no clear guideline supported by scientific evidence to indicate the type of biomaterial to be used. Moreover, similar conclusions were reported in the randomized clinical trial of Cardaropoli et al. [33].

The biological interactions occurring at the bone-biomaterial interface are critical for long-term clinical success [34]. Bio-Oss, also used in this study, has a natural, nonantigenic porous matrix and is chemically and physically identical to the mineral phase of human bone; it has been reported to be highly osteoconductive and to show a very low resorption rate [35-38].

In the last few years, fibrin sealants derived from human blood plasma have been used in various surgeries (abdominal, thoracic, vascular, oral, endoscopic) due to their wound healing, tissue adhesion and blood clotting properties [39]. Human fibrin glue (HFG), used in this study, mimics the last step of the coagulation cascade through activation of fibrinogen by thrombin, resulting in a clot of fibrin with adhesive properties. HFG could also be used as scaffolds for cell culture and transplantation due to their biocompatibility, biological degradation and cell attraction properties [40]. It contains factors, such as thrombin, fibrin, fibronectin and PDGF, which are known to retain biological activities, in particular on cell proliferation and differentiation, and which may also stimulate wound healing by acting as a scaffold for the proliferation of mesenchymal and endothelial cells [41]. Only a few publications questioned the osteoinductive properties of HFG [22, 42].

Although the topic of “ridge regeneration and preservation” has been widely debated in the scientific literature, establishing clear guidelines on the biomaterials and the technique to use for achieving predictable treatment outcomes remain a significant challenge in the clinical practice [25, 43].

Therefore, the aim of this case report was to scientifically validate a new minimally invasive surgery technique called “Simplified Apposition Technique” (S.A.T). This technique consists in the use of BO (Bio-Oss, Geistlich Biomaterials, Vicenza, Italy), HFG (Tisseel, Baxter, Deerfield, Illinois, US.) and collagen membrane (CM, Bio-Gide compressed, Geistlich Biomaterials, Vicenza, Italy) in association with a post-extraction socket, in order to obtain an increase of the horizontal ridge dimension with new hard tissue formation. A comparison was made between the bone width, by the use of CBCT, before surgery, after 11 months and at 2.5 years of follow-up. Furthermore, the bone quality after 11 months of follow-up was evaluated through histological analysis.

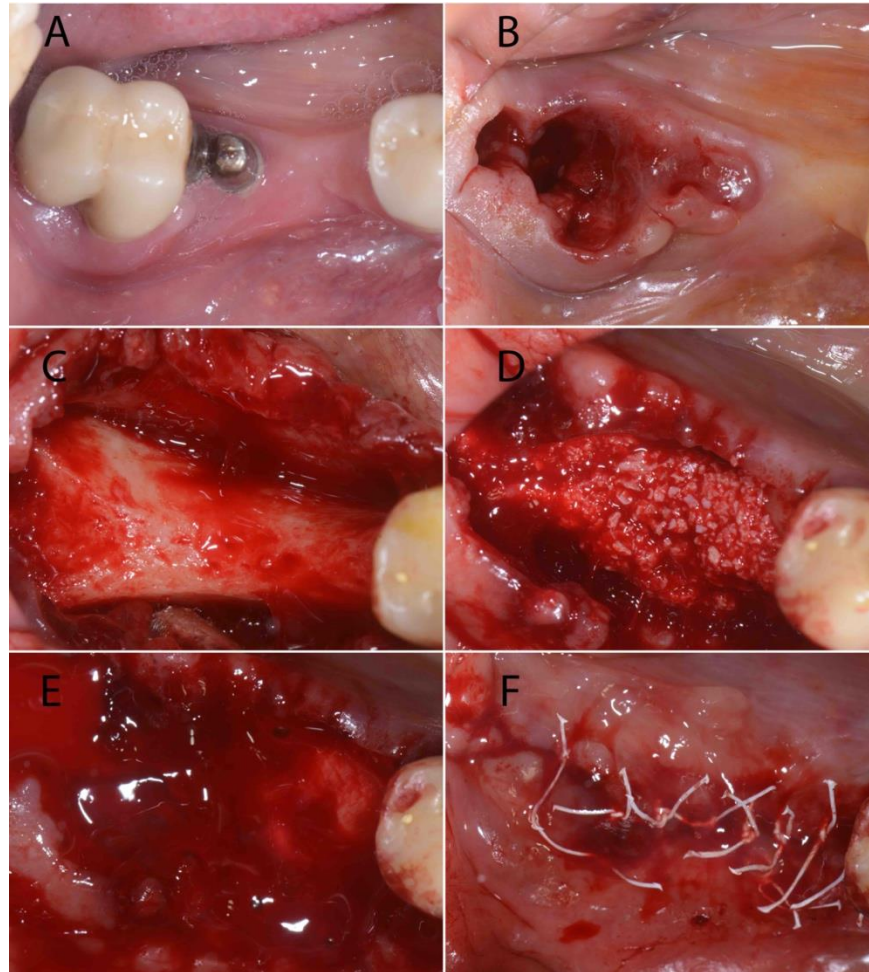
## **2. Materials and Methods**

A 69-years-old man patient was enrolled in this study. He did not suffer from systemic disease and was not taking drugs influencing bone metabolism. The systemic exclusion criteria were: (i) existence of metabolic bone disease, (ii) history of malignancy, (iii) history of radiotherapy or chemotherapy for malignancy in the past 5 years, (iiii) history of autoimmune disease, and long-term steroidal or antibiotic therapy. The patient was provided oral and

written information regarding the surgery and the study, and written informed consent was obtained. He was subjected to oral hygiene to lower the bacterial charge and favour bone healing. He was treated with a new non-invasive technique, S.A.T, that consists in the use of BO, HFG, CM in association with a post-extraction socket, in order to obtain an increase of the horizontal ridge dimension with new hard tissue formation in the nearby atrophic crest.

## **2.1 Surgical protocol**

The patient clinically presented a prosthetic bridge in zone 4.5-4.7, without the presence of the tooth 4.6, and an atrophic bone crest (Figure 1A). The tooth 4.7, highly compromised and hopeless, was extracted. One hour before surgery, the patient received 2 gr of amoxicillin, and then, just before surgery, 0.2% chlorhexidine mouthwash was performed for 2 minutes. Moreover, 1 g of amoxicillin twice a day for 5 days was prescribed. The patient was subjected to local anaesthesia with Articaine, 40 mg/ml 1:100.000, administrated near the nerve, and Articaine, 40 mg/ml 1:1.200.00, administered by infiltration in the areas adjacent to the surgery. Tooth 4.7 was gently luxated with periotomes, and then carefully extracted with extraction forceps while attempting to minimize trauma to the surrounding bone. The tooth socket was carefully debrided to remove granulation tissue. Special attention was paid to avoid damaging the soft tissues. The extraction was performed using a flapless procedure (Figure 1B). After the extraction, a mucoperiosteal flap was raised, using a 15C blade, making a crestal incision in the edentulous mandibular. The crestal incision was continued as sulcular incision on the adjacent teeth. Full-thickness flaps were reflected in the buccal and palatal sides to maximize visualization of the underlying alveolar ridge defect and in order to allow a good positioning of the graft material (Figure 1C). According to the S.A.T, the ridge reconstruction and the filling of the socket was performed using BO, HFG, and CM (Figure 1D). HFG was used after diluting the thrombin with physiological solution from 500 international units to about 5 international units. Then, using the device provided by the manufacturer, the diluted thrombin was mixed with human fibrinogen, in order to achieve more working time and reduce the early polymerization of the glue with the possible subsequent encapsulation of the biomaterial. BO has been wet with some drop of physiological solution and with diluted HFG to obtain a handy sticky paste. The sticky paste was placed into the socket and upon the ridge for the horizontal augmentation. Subsequently, a collagen membrane was trimmed and dropped with HFG, and it was gently placed over the graft (Figure 1E). Finally, a modified horizontal mattress and single sutures were used to secure the membrane and the graft material in place, using 5-0 PTFE non resorbable sutures (Omnia S.r.l., Parma, Italy). Some drops of HFG were placed over the sutures to increase the protection and the rapidity of the soft tissue healing (Figure 1F). Sutures were removed after 10 days. In the post-extraction site, there was a secondary healing; on the other hand, in the horizontal ridge augmentation site, there was a first healing. The patient was asked to continue the chlorhexidine rinses until complete gingival closure, and he was monitored weekly during the first month of healing.



**Figure 1:** (A) Mandibular site at baseline on the day of the extraction. The hopeless tooth 4.7 was extracted using a flapless procedure (B). After the extraction, (C) a mucoperiosteal flap was raised and the bone crest was exposed. According to S.A.T, the ridge reconstruction and the filling of the socket has been performed with Bio-Oss (D), human fibrin glue and collagen membrane (E). A modified horizontal mattress and single sutures were used to secure the membrane and the graft material in place, covered by some drops of HFG (F).

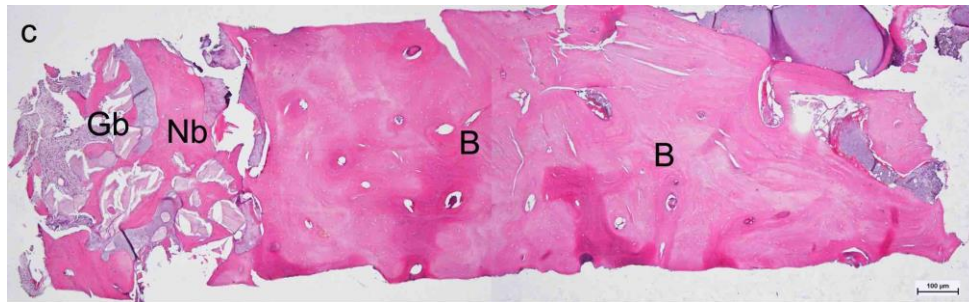
## 2.2 Radiographic evaluations: CBCT

Before starting the surgical phase, the patient was subjected to a CBCT, produced by Carestream 8100 3D, so as to evaluate the bone width of the analysed site. The radiographic examination was repeated after 11 months of healing and after 2.5 years of follow-up. The images were acquired by means of the Carestream software and processed by a computer. Acquisition was performed according to the acquisition protocol with a small voxel size. The cortical buccal and lingual bone were taken as landmarks. The width of the bone ridge was determined by measuring in perpendicular direction the distance between the buccal and lingual walls. A dentist who was unaware of the treatment protocol performed the measurements.

Moreover, after 11 months, after local anesthesia, a flap was raised, and the graft appeared well integrated and hard. An implant was placed in zone 4.6 (4.1 mm. x 11.5 mm., T3 Biomet 3i), completely into the bone crest. At the same time, the bone chips remaining in the drills were preserved and used for the histological evaluation.

### 2.3 Histological evaluation

The specimen was immediately fixed in 4% neutral buffered formaldehyde solution for 48 h, decalcified by Biodec R (Bio-Optica, Milan, Italy) for 6 hours and then routinely processed for paraffin embedding at temperatures not exceeding 56°C. Six- $\mu$ m-thick paraffin-embedded tissue sections were deparaffinized and rehydrated with xylene and a graded series of ethyl alcohols (from 100% to 50%), and stained by Haematoxylin & Eosin (Figure 2). Section were observed under Nikon Eclipse E600 light microscope.

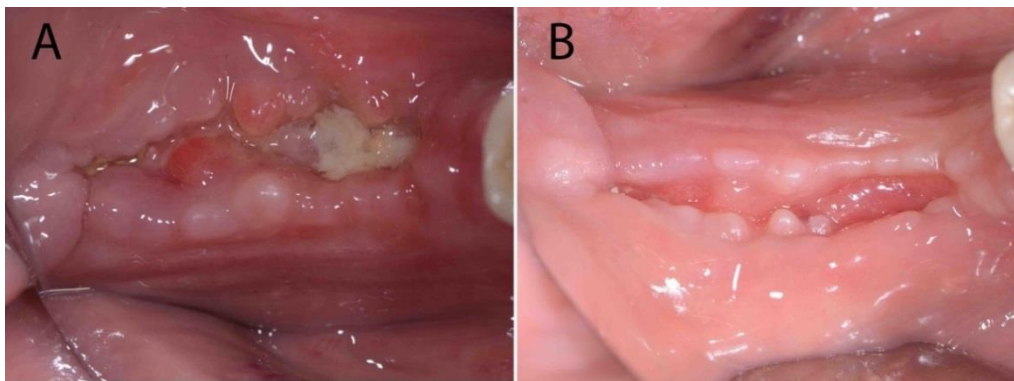


**Figure 2:** Histological evaluation of the grafted area, Haematoxylin & Eosin staining, original magnification 4x. These composed low magnification light micrographs show an overview of the good quality of the lamellar compact bone of the retrieved specimen. Newly-bone (Nb) apposition can be observed immediately below the regenerated area (Gb), with a very good integration with the pre-existent bone (B). No inflammatory cells or signs of adverse reactions are evident.

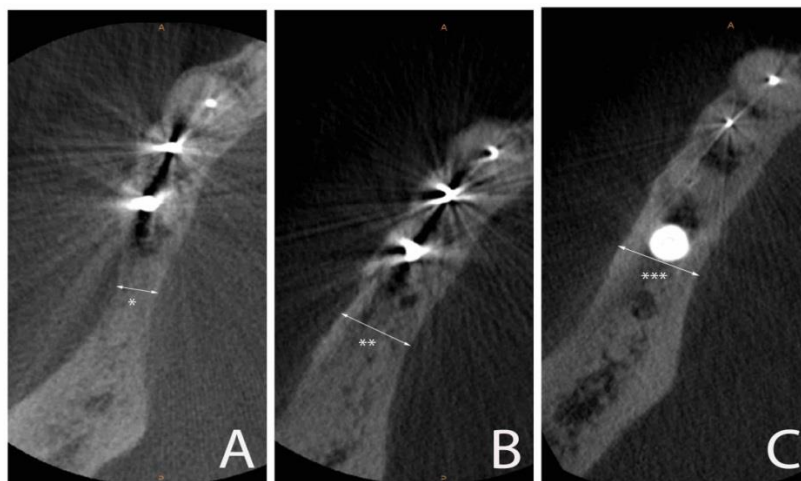
### 3. Results

In this case report a 69-years-old man patient was enrolled. The surgical procedures were performed successfully as planned, without complications. The follow-up evaluation performed at the time of suture removal, on day 10, and after 1 month, showed uneventful healing, no signs of inflammation and soft tissue closure, which was partly obtained by means of a second intention healing, using the association of collagen membrane and human fibrin glue [44] (Figure 3A,3B). HFG allows creating a seal that stabilizes the clot and facilitates tissue healing [22]. CBCT analysis, performed at 11 months of follow-up, revealed no residual radiolucency and a good integration of the graft material. At baseline, the width of the crest, between the buccal and the lingual walls, was 3.7 mm. After 11 months, the analyzed width became markedly increased, reaching 6.5 mm (Figure 4A,4B). The CBCT carried out after 2.5 years of follow-up showed maintenance of the reconstructed volumes (Figure 4C).

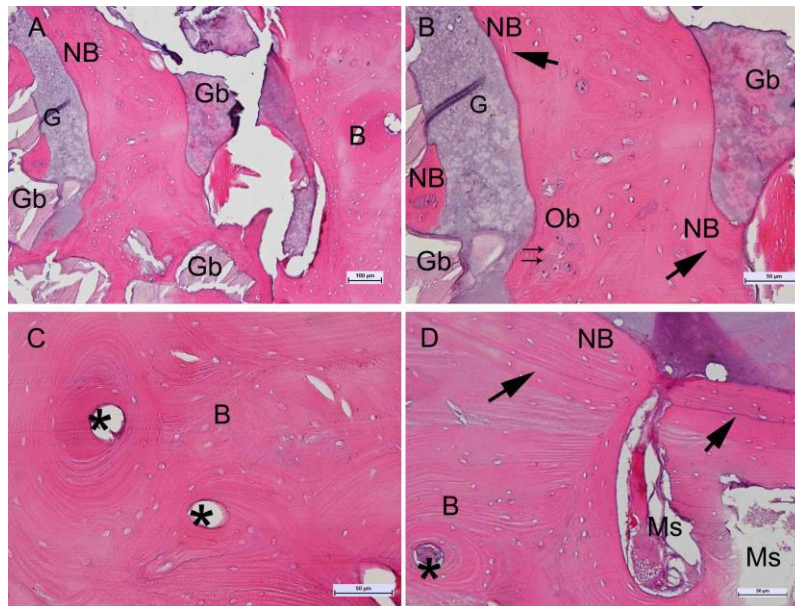
After flap elevation, after 11 months, in the extraction zone, the grafted material appeared to have a hard consistency and no fibrointegrated graft particles could be observed in the site. At the light microscopy evaluation, the bone chips exhibited various stages of bone maturation and formation, showing a good quality of lamellar compact bone without any inflammatory response (Figure 2C). The area regenerated using BO and HFG demonstrated newly formed bone and very good integration with the pre-existent bone. There were remnants of the residual particles and amorphous grafted biomaterials in contact with the newly-formed bone. The lamellar compact osseous tissue presented several osteoblasts and numerous osteocytic lacunae; the native bone areas were characterized by well-distinguished osteonic structures, demonstrating a high degree of mature bone features (Figure 5).



**Figure 3:** (A) Clinical appearance of grafted site after 10 days of healing, at the time of suture removal. (B) Clinical appearance of grafted site after 1 month of healing. The soft tissue closure occurred partly with a second intention healing, without sign of inflammation.



**Figure 4:** CBCT carried out immediately after the extraction of 4.7 (A) and after 11 months of healing, after applying S.A.T (B). No residual radiolucency was noticed, and the graft material was well integrated. The increase of the bone width was evident, comparing the width in A (\*) with the one in B (\*\*). The CBCT carried out after 2.5 years of follow-up showed a maintenance of the reconstructed horizontal volumes (\*\*\*), compared with B (C). The radiopaque pois in C is the implant placed completely into the bone crest, which was not possible before the S.A.T reconstruction.



**Figure 5:** High magnification light micrographs of the regenerated area. (A) Numerous residual particles and amorphous structures of the grafted biomaterials (Gb and G) are evident in close contact with the newly-formed bone, which is intensively stained by Haematoxylin & Eosin (NB), being woven in some regions, especially near the grafts, and already acquiring features of mature lamellar bone in the majority of the regions (NB in B, C, D). Some cement lines demarking the passage between newly formed bone (NB) and pre-existent bone (B) are present (arrows). The lamellar compact osseous tissue presents several osteoblasts (Ob pointed by tiny arrows) and numerous osteocytic lacunae; the native bone areas (B) are characterized by well-distinguished osteonic structures (\*), demonstrating a high degree of mature bone features. A few marrow spaces are present (Ms). Original Magnification of A: 10x; of B, C, and D: 20x.

#### 4. Discussion

Residual bone defects are often found under prosthetic bridge. When the hopeless abutment needs to be extracted, clinicians have to find a prosthetic solution in the atrophic bone crest, near the extracted socket. Bone grafting materials for alveolar bone deficits have markedly improved in recent years, increasing the applicability and success of oral implantology and bone ridge augmentation procedures [45]. Bone substitutes should be flexible to properly fit the defect shape, but also suitable to contain soft tissue migration and prevent the wound from collapsing. In guided bone regeneration, various strategies can be used to increase the rate of bone formation and to augment bone volume [46, 47].

Bio-Oss is a bovine-derived xenograft enriched with bovine collagen that combines the flexibility of purified collagen with the mechanical strength of inorganic bone derivatives, and it becomes plastic but cohesive when hydrated [48]. Lindhe et al. (2014) performed a study in man and reported that the tissue of sockets that had been



grafted with Bio-Oss Collagen after 6 months of healing contained graft particles surrounded by a richly vascularized provisional matrix and newly formed woven bone [49]. The above observations are in agreement with findings reported from studies in humans and dogs, showing that the BO particles during wound healing integrated with the newly formed host bone, remained virtually intact, and hence retained the volume of the hard tissue defect [15, 50, 51].

A previous study shows results in agreement with the scientific literature: after 6 months, BO does not show signs of resorption and it is well integrated into the host tissues; in addition, the newly-formed bone presents features similar to pre-existing osseous tissue, thus indicating the good osteoconductive properties of BO [35]. In order to increase the osteoconductive properties of BO, it was associated with HFG and with a post-extraction socket. HFG is used in surgery due to its hemostatic, chemotactic and mitogenic properties and also as scaffolds for cell culture and transplantation. In the context of bone reconstruction, the association of the BO to a binding agent such as HFG may produce an easy-shaped material without empty spaces, which could increase the osteoconductive and osteoinductivity properties, associated with the healing factors [22]. Moreover, these biomaterials were covered by a collagen membrane, in order to avoid the colonization of the epithelial cells and create space for the blood clot. The use of an occlusive barrier membrane to stabilize and protect the augmented volume is recommended [52].

Based on the data available from the scientific literature, this new surgical technique has been proposed. S.A.T allows us to obtain an increase of the horizontal ridge dimension of the atrophic bone crest with new hard tissue formation, exploiting the osteoinductive and osteoconductive properties of biomaterials (BO, HFG and CM), increased by the presence of healing factors deriving from the post-extraction socket. This protocol simplifies the surgery technique, obtaining good results, since:

1. No vertical discharged is made;
2. Post-extraction socket reconstruction and horizontal augmentation of the atrophic crest are performed simultaneously;
3. The association of BO, HFG, CM with a post-extraction socket, which releases growth factors, increases the regenerative properties of these biomaterials;
4. HFG and CM allow to keep BO in place, without using screws or pins;
5. HFG, associated with BO, allows to have osteoinductive and osteoconductive properties in the grafted site;
6. In the post-extraction site, this technique allows to have a secondary healing that not compromise the clinical results of the grafted material, thanks to the use of the association of CM and HFG, that stabilize and protect the regenerated site.

According to the obtained results, it was possible to see the formation of lamellar compact bone outside of the pre-existent cortical bone. It seems that cortical perforations are not required to create new bone with good integration with pre-existent bone. Besides, when a thin bone is present, that cortical hole could create a bone suffering, having

a worse result of bone regeneration [53]. The use of HFG is important in this technique, because of (i) the growth factors in the HFG, whose action is increased by the healing factors derived from the post-extraction socket, and (ii) the prolonged fixation of HFG into the surgical wound, that protects and stabilizes the regenerated site, in association with CM [41].

## **5. Conclusions**

The purpose of S.A.T is to recreate and maintain a favorable crest bone width for future dental implant placement, using BO, HFG and CM, in association with the healing factors which can be found in the post-extraction socket. Therefore, S.A.T allows simplifying the surgical technique of the horizontal ridge augmentation, obtaining good results in one surgery time and with less pain for the patient. The clinical relevance consists in its application in patients presenting atrophic bone crests. The good clinical, radiographical and histological features of the bone at 11 months suggest that this technique could be used successfully [44]. Further clinical cases have been collected in order to prepare a case series demonstrating the reliability of the S.A.T, and comparing this procedure with other reliable surgical techniques. Despite the limitation of the present study, reporting only one clinical case, the promising histological and surgical findings provide valuable proof that S.A.T could be considered a successful bone regeneration method, thus minimizing risks of postoperative morbidity, stably increasing horizontal bone over time, as demonstrated by the CBCT at 2.5 years of follow-up.

Although the autogenous bone is still considered the gold standard for graft procedures, due to its inducted osteogenesis [23,54,55], it has some obvious disadvantages [28] that can be overcome searching alternative bone materials from different origins, as well as simple and mini-invasive surgical techniques. S.A.T is an innovative procedure which may exploit a proper osteoinduction, thanks to the factors derived from the post-extraction socket [47], together with the advantageous properties exerted by BO, HFG and CM. Further studies are ongoing, in order to support this technique using different biomaterials, always in association with factors released from post-extraction sockets.

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## **Author Contributions**

Andrea Grassi performed the surgery and contributed to the acquisition of data and clinical follow-ups. Giovanna Orsini and Caterina Licini performed the histological evaluation and contributed to the interpretation of data and manuscript results, discussion and editing. Fabrizio Bambini, Lucia Memè and Giulia Orilisi contributed to the idea and performed radiographic evaluation and data interpretation. All authors participated to the writing of the present manuscript.

### Financial Disclosure

No financial relationships relevant to this article.

### Informed Consent

Written informed consent was obtained from the patient.

### Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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