Two-Stage Rehabilitation with a Single Implant Placed in a Socket Grafted with New Generation Freeze-Dried Bone Paste

A clinical and histological case report

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Introduction

Following the extraction of a tooth, the alveolar socket undergoes a process of resorption, which occurs according to a precise spatial and temporal sequence [1,2]. Resorption can jeopardise both the aesthetic and functional success of the prosthesis, or even make implant placement impossible[2]. The development of atrophy can be countered by grafting the post-extraction socket with a bone substitute, according to the *ridge preservation* technique [3]. There are several bone substitutes available[4]. Their use makes it possible to avoid using autologous bone and the risks involved with it[5]. Autologous bone is still considered the gold standard today, as it has all the key factors of the tissue triad: adequate three-dimensional structure, cells and growth factors[6].

The current avenues of research to create new substitutes that allow effective bone regeneration are oriented in two directions. On the one hand, the in vitro reconstruction of complex grafts that have all three elements of the triad. For example, when autologous cells are taken from the patient, and then grown in vitro on appropriate substrates, possibly loaded with growth factors. Although promising, this approach requires further research and poses considerable challenges from a scientific, production, regulatory and certification point of view[7].

On the other hand, manufacturers of bone substitutes are bringing new biomaterials onto the market as a result of their research and development activities. Some of these appear to be simple clones of existing materials[4]. In other cases, they may actually be advanced bone substitutes, designed according to precise rationales that should be reflected in an equally precise improvement in clinical performance[8]. In this field, bone substitutes derived from non-human mammalian tissue are of particular interest, given their similarity in chemical composition, mineral content and three-dimensional structure to human bone tissue[9].

The first process used to inactivate animal antigens was the thermal process, which involves subjecting the source tissue to very high temperatures (>300°C)[10]. Today, however, it is known that there are alternative, more advanced biotechnological processes for antigen elimination, for example to preserve bone collagen - a molecule with multiple pro-regenerative functions - in the final bone substitute[11].

Heterologous bone substitutes obtained through these processes, which are based on selective antigen degradation by enzymatic means, show clear advantages in terms of histomorphometric outcome of regeneration compared to those obtained by thermal methods[11,13].

Recently, a bone paste containing cortical and cancellous bone granules of equine origin, made non-antigenic through an enzymatic process, and dispersed in a low-molecular-weight polymer hydrogel enriched with vitamin C, has been launched on the market[14]. This type of bone paste can facilitate graft placement as it avoids dispersion of the granular component[14]. It also offers the possibility of 3D modelling of the graft and increases the ease with which the graft adheres to the bone walls of the recipient site, favouring colonisation by cells and, ultimately, the entire bone regeneration process. This bone paste was the subject of a recent in vitro and animal model study[14], which showed its interesting properties and potential of facilitating bone regeneration. At present, its clinical use is described only in a few case reports[15,15]. The author has already used this bone paste in a number of cases, on which retrospective data collection is in progress. This bone paste is also available in a freeze-dried version that can further facilitate its use: the case report presented in this paper describes its use, also documenting the histological outcome.

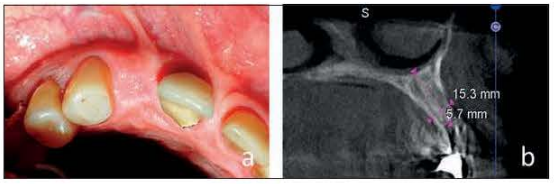
Case report

The 75-year-old male patient with no specific previous pathologies came to the author's attention complaining of pain at tooth 1.2, which had already been previously devitalised and rehabilitated with a crown after reconstruction with a fibre post, adjacent to a single-implant crown in position 1.1. The clinical and radiographic analysis showed a fracture of the tooth (Figs. 1a-1c). Given the aesthetic position of the tooth and the consequent need to ensure an adequate amount of hard and soft tissue for successful rehabilitation, the patient was proposed rehabilitation with a single implant, to be placed in two stages following a *ridge preservation* procedure. The patient gave his informed consent to the treatment.

Two days before surgery, the patient underwent complete oral hygiene. The surgery was performed under antibiotic coverage with Amoxicillin/Clavulanic acid 2 g (Augmentin, Glaxo-SmithKline, Verona, Italy) administered one hour before surgery, and then every 12 hours for eight days. The patient was also made to rinse for 2 minutes with Chlorhexidine 0.2% (Corsodyl, Glaxo-SmithKline, Verona, Italy), and was administered 100 mg of a non-steroidal anti-inflammatory drug (Aulin, Roche, Milan, Italy). Local anaesthesia was performed by infiltration of 1% Articaine and Epinephrine 1:100,000 (Molteni Dental, Milan, Italy). The tooth was extracted atraumatically (Fig. 1d) and the socket was curetted to remove any residual fibrous and inflammatory tissue. The freeze-dried bone paste (Activabone Dry Paste, Bioteck, Arcugnano, Italy) was then grafted (Fig. 1e) without prior hydration, exploiting its hygroscopic properties that allowed in situ imbibition by blood. The bone paste was then shaped and stabilised within the socket by applying gentle pressure with a blunt instrument. No flap was prepared.

After grafting, a cross stitch was placed (Fig. 1f), using a 5-0 non-absorbable suture (Monomyd, Butterfly, Cavenago, Italy). Gingival edges were left open in order to achieve healing by second intention. Implant placement surgery was performed 3 months later. The clinical appearance of the tissues, as well as the radiographic appearance of the grafted area, appeared satisfactory (Figs. 2a, 2b). Antibiotic prophylaxis and analgesic therapy were the same as described above for the first surgical phase. After lifting a flap to access the bony ridge and inspect the vestibular wall, a bone sample was taken from the implant tunnel using a special trephine burr. The bone sample was fixed in 10% buffered formalin. The implant site was prepared using the set of burrs in the sequence indicated by the manufacturer and the implant was then placed (3 x 13 mm, XiVE, Dentsply, USA), but not submerged (Fig. 3). The patient was definitively rehabilitated 5 months after the implantation surgery and is currently being followed at the author's practice (Fig. 4).

The biopsy sample was decalcified for 21 days in a solution of 0.76 M sodium formate and 1.6 M formic acid (Panreac Quimica, Spain), dehydrated in increasing concentrations of ethanol and embedded in paraffin. The sample was cut into thin slices (5 pm), which were spread on a glass slide, stained with haematoxylin-eosin dyes, and observed by optical microscopy also under polarised light (Fig. 5).



Figs. 2a, 2b - Check two months after ridge preservation surgery. Excellent healing of the soft tissue (a) and preservation of the size of the alveolar socket (a, b) can be observed.

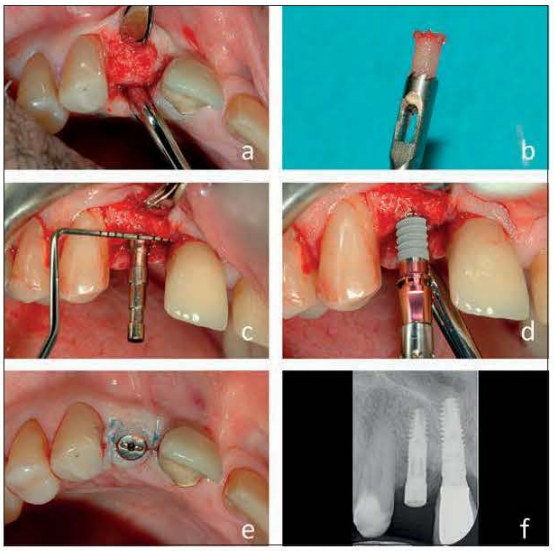
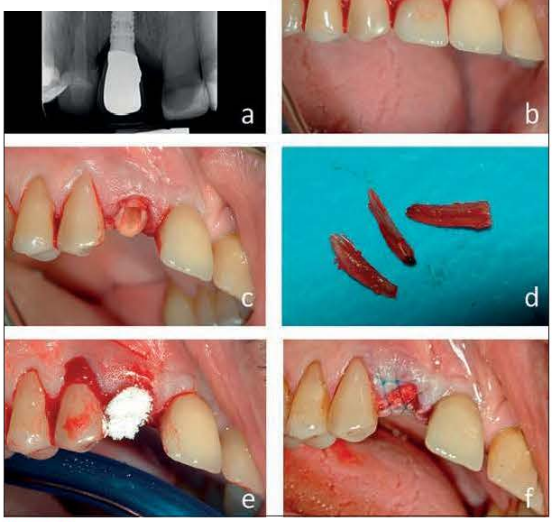


Fig. 3 - Implant placement, three months after bone regeneration surgery. During implant placement, a biopsy sample is taken for histological analysis.



Figs. 1a-1f - Tooth 1.2, which has a fractured root (a,d), is extracted autramatically. The socket is grafted with freeze-dried bone paste (e) and the soft tissue is allowed to heal by second intention (f).

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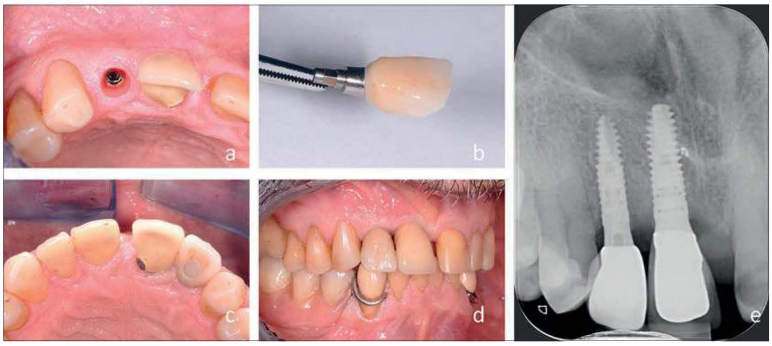

Fig. 4 - On delivery of the definitive prosthesis, 5 months after implant placement, the excellent conditioning of soft tissues and the good aesthetic result can be observed.

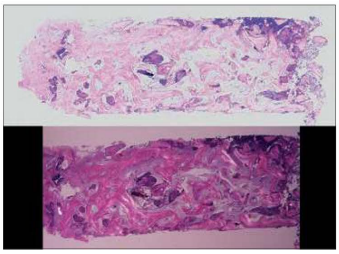

Fig. 5 - Haematoxylin-eosin histology under normal (top) and polarised (bottm) light. An area of newly formed tissue is observed over the entire surface of the sample; residual biomaterial particles are rare. No infiltrates of inflammatory origin are observed. Some areas of birefringence corresponding to mature lamellar bone tissue appear under polarised light.

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Results and discussion

The patient recovered without complications after both operations. The final appearance of the rehabilitation was satisfactory and completely met the patient's expectations. No ridge height contraction was observed between bone grafting and implant placement, thus confirming the effectiveness of the *ridge preservation* performed. Histological analysis showed a large area of newly formed bone tissue throughout the entire sample, with a small number of graft particles inside, all in continuous contact with the newly formed bone tissue. No inflammatory infiltrates were observed. Under polarised light, mature lamellar tissue could be observed in different areas of the sample.

The results presented are consistent with previous histological and immunohistochemical studies on the use of enzyme-deantigenic equine bone substitutes[12,13, 15-17]. The significant amount of newly formed bone tissue observed in the sample can be explained by both the effective osteoclastic remodelling action already observed in vitro with this biomaterial[18] and the presence of native bone collagen, a known positive modulator of bone regeneration[19-22]. A further explanation could lie in the presence, in the carrier contained in the bone paste used in this study, of a certain amount of vitamin C, which is a known cofactor necessary for the assembly of collagen fibrils[23]. A further advantage of using bone paste, compared to granular bone, is that the carrier gives the granules immediate spatial stabilisation, simultaneously facilitating contact of the graft with the recipient bone tissue. As with the hydrated extrudable version, the author particularly appreciated the manageability of this freeze-dried version.

Finally, this type of graft allows excellent second intention soft tissue healing to be achieved without placing a covering membrane, or simply using a thin layer of fibrin sponge. This characteristic can again be attributed to the presence of a carrier which, when hydrated, acquires an optimal density and is at the same time an excellent substrate for the regeneration of gingival tissue.