Methodological Approach for the Evaluation of Homologous Bone Graft Use in Post-Extractive Atrophic Alveolar Ridges

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KEY WORDS: allograft bone, post-extractive site, bone regeneration, bone histology.

Abstract

The aim of this study is to investigate the in vivo efficacy of Puros® cancellous particulate allograft bone in the regeneration of post-extractive sites.
Twelve molar or premolar sites were selected. Seven days after minimally invasive tooth extraction, Puros® cancellous particulate allografts were inserted into the elected sites. TC Cone-Beam investigation was performed the day after extraction and at 4 months from surgery; bone height and width were evaluated. Five months after surgery, biopsy samples of the regenerated sites were taken and histologically analyzed to qualitatively evaluate bone regeneration. TC analysis showed, a mean *bone gain of 4.1 mm* ***in height*** *and 2.02 mm in width.*
The histological analysis of the samples showed intense *bone metabolic activity with active osteoblasts on the implant surface, at the level of the native bone-graft interface and in the grafted area.*
The radiographic and histological analyses demonstrate an optimal bone regeneration, both in terms of quality and quantity using ***Puros®.***

Introduction

In recent years the demand for bone graft materials to address deficits of bone tissue is gradually increasing,[1-3] in particular in the areas of orthopedic, dental and maxillofacial surgery
requiring the use of grafting biomaterials may be possible [4-8].

The ideal characteristics of biomaterials are: biocompatibility, mechanical strength, flexibility, osteoconductivity, osteoinductivity, osteogenicity, biodegradability, sterilizability, easy availability and easy storage [9,10].
Possibilities to compensate the lack of bone tissue are currently diverse. New techniques are being tested almost daily [11,12], thanks to the rapid broadening of scientific knowledge; at present traditional surgery involves the use of four methods of bone replacement: the use of autologous bone grafts (with intraoral or extraoral donor site sample), homologous or allogeneic grafts (human bone bank), allografts or xenografts (animal bones: cattle, sheep, horses) and grafts with alloplastic materials (synthetic biomaterials) [13-17].
OBJECTIVE: The aim of this paper is to investigate the in vivo efficacy in the regeneration and maintenance of post-tooth extraction peak bone before implant surgery using cancellous bone particulate graft Puros® (Zimmer Dental) [18,19].
In particular, our aim is to identify a reproducibility methodology for the analysis and the collection of data about the pre-implant bone regeneration obtained by the above material.

Materials and method

Patients

Ten patients were enrolled in the study between May 2010 and May 2012 from the Department of Prosthetic Dentistry, University of Genoa. Contraindications were various both relative (e.g. renal artery or kidney disease, autoimmune diseases, uncontrolled diabetes, smoking, uncontrolled endocrinopathies, osteoporosis, polyarthritis, valvular heart disease, psychiatric illnesses, therapeutic immunosuppression, drug addiction) and absolute (e.g. pregnancy, immune deficiencies, cancer, hemophilia, patients awaiting organ transplantation) to advanced implant surgery. All patients underwent undergoes an accurate first visit after the collection of the anamnestic history, and

were included in the research program after having given *informed*.
During objective clinical examination, the integrity, the absence of infection and the periodontal health of the teeth to be extracted using the patient's Panoramic view were assessed.
Twelve teeth for extraction were identified – 6 from the upper jaw and 6 from the lower jaw.

First-stage surgery

The first surgical phase after anesthesia (mepivacaine + epinephrine 1:100,000) consisted in the minimally invasive extraction of the dental element; in this regard the piezoelectric tool from Mectron® which permits selective micrometric cutting so as to obtain an extremely conservative avulsion with the preservation of the alveolus walls was used.
If the extracted tooth showed a periapical infection the alveolus surface was cleaned and an antibiotic (Zimox® for 6 days) administered. The wound was left to heal by secondary intention.
The following day the patient underwent a Cone-Beam X-ray CT investigation (low dose of radiation) only at the level of the post-extraction site to assess the true integrity of the alveolus walls as well as the height and thickness of the post-extraction site itself.

Second-stage surgery

Seven days after surgery the patient underwent a second operation in which, after elevation of a full-thickness flap, all the residual tissues were removed, thus eliminating the granulation tissue, and the alveolus was curetted to permit bleeding, useful for the engraftment of the graft. Finally the filling material was positioned.
The material used for this purpose was cancellous bone particulate for Puros® allograft from Zimmer Dental Inc. (Warsaw, Indiana, USA); this product is homologous bone treated with the Tutoplast® process which ensures collagen maintenance and tissue integrity preservation.
The material was inserted with the use of pluggers. The post-extraction site, where pre-implant bone regeneration is needed, was filled to at least 2/3s of the alveolus. For proper healing and protection of the graft a reabsorbable bovine CopiOs® Pericardium Membrane from Zimmer Dental was inserted. The handling of soft tissue was crucial also for a correct coverage and immobilization of the membrane during the suture phase (silk 3/0).
Comparative investigation by Cone-Beam X-ray CT at 4 months after the grafting.
Radiographic Cone-Beam CT control of the site was performed four months after the intervention with the evaluation of the regeneration in height and thickness (amount of bone) (Fig.1-2).

Histological analysis at 5 months after the grafting.

After 5 months, samples of the regenerated sites were taken thanks to bone drills (Trephine Bur 2mm ID 3 mm ED, Biomet 3i®) (Fig.3) and an implant was contextually inserted in each regenerated site. The samples (approximately 8 mm of bone) were histologically analyzed to assess the morphology and the characteristic of bone regeneration.

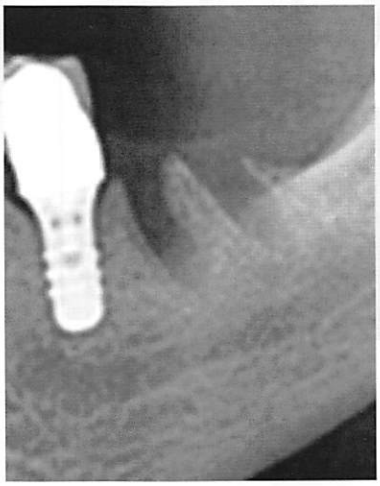

Figure 1. X-ray after tooth extraction

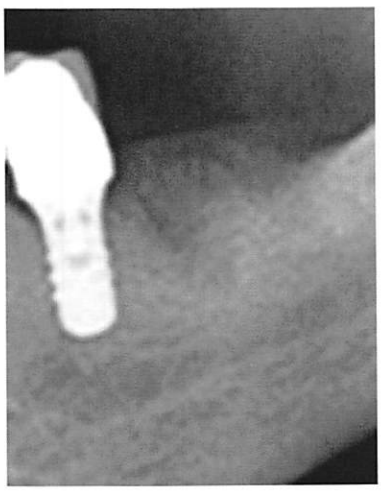

Figure 2. X-ray 4 months after the grafting.

Figure 3. Trephine bur drilling the bone in the regenerated site.

For this study the histological specimens were prepared adopting an acrylic resin embedding method ideal for bone biopsies not previously decalcified [30-31-32]. This method preserves tissue morphology overcoming the limits of the routine processes of decalcification and paraffin embedding, avoiding manipulations involving the contraction and distortion of the material; the mineralized component maintains the correct steric relationship especially in those areas where implanted bone takes the form of spicules and fragments. The resin used for the embedding of the biopsy material is a cold polymerizing resin (4° C). It is a combination of a water-soluble resin (HEMA) and a plasticizer (Hydroxyethane). Each sample was collected, fixed in ether for at least 24 hours, then dehydrated in absolute ethanol at room temperature. The material was placed in the infiltration solution (resin + hardener I -accelerator benzoyl peroxide hydrate) under vacuum for at least 72 hours. Samples were then embedded in resin. The embedding solution was prepared by adding in the infiltration solution the hardener II (accelerator - tetramethylaniline). A special Teflon mould was used for embedding in which the polymerization reaction occurs in the absence of air at 4 ° C. After polymerization, the specimens were prepared fixing the block of resin on a support with a special fast-acting resin glue. Semi-thin sections were obtained from each block using a semi-automatic rotary microtome with a tungsten blade. Then the sections were stained with Gill's Hematoxylin, Toulidine blu and Goldner's Trichromic. The Goldner method is a modified Masson's Trichrome stain specifically modified for bone tissue as is stains mineralized bone in green, osteoid in red and nuclei in blue.

Results

TC Cone-Beam analysis results

The TC analysis of the 12 sites showed, a mean bone gain of 4.1 mm in height and a mean bone gain of 2.02 mm in width.

Histological analysis results

Histological analysis of the 12 samples showed intense bone metabolic activity with active osteoblasts on the implant surface, at the level of the native bone-graft interface and in the grafted area (Fig.4). The grafted

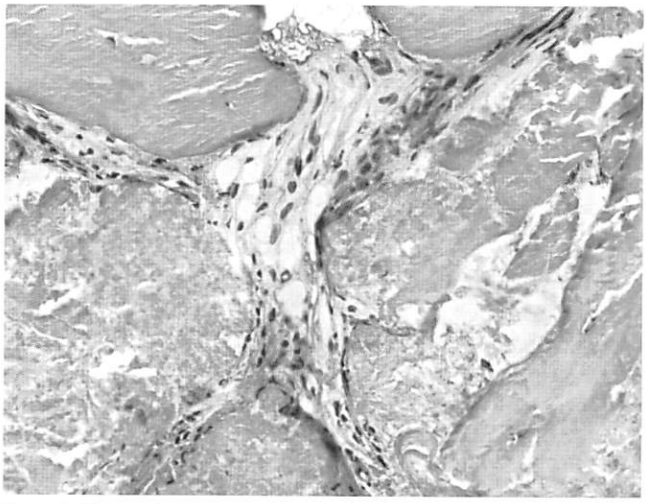

Figure 4. The grafted material was partially replaced by new regenerated bone and a partially mineralized osteoid matrix (40X).

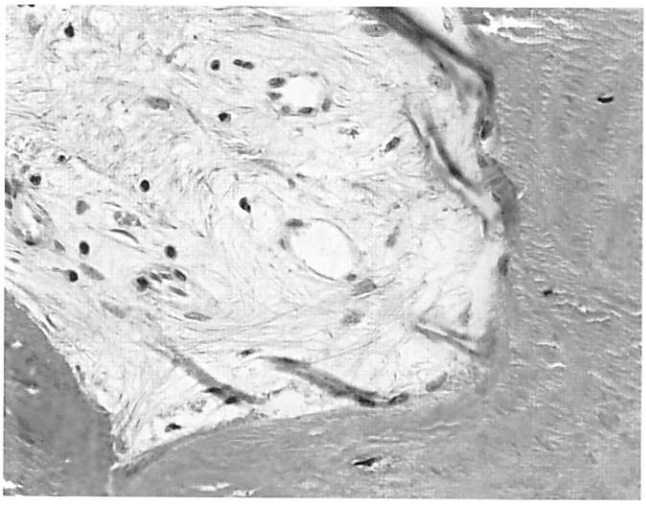

Figure 5. Mature Connective tissue with active osteoblasts and numerous vascular structures were observed interspersed in this connective tissue matrix (40x).

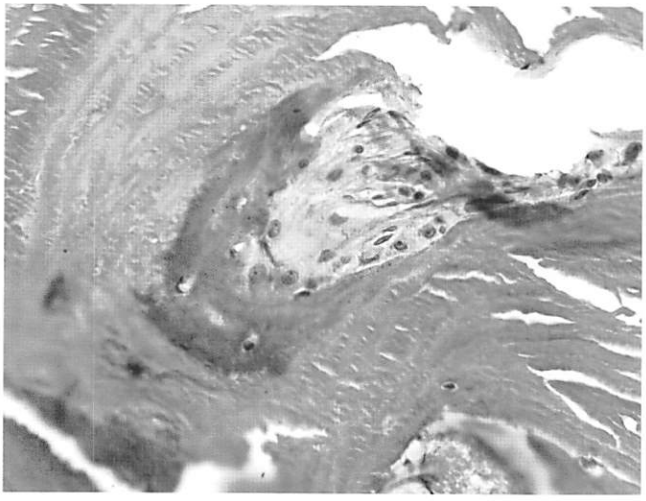

Figure 6. Osteoblasts in an intense metabolic activity surrounded by osteoid matrix and lamellar bone (40x).

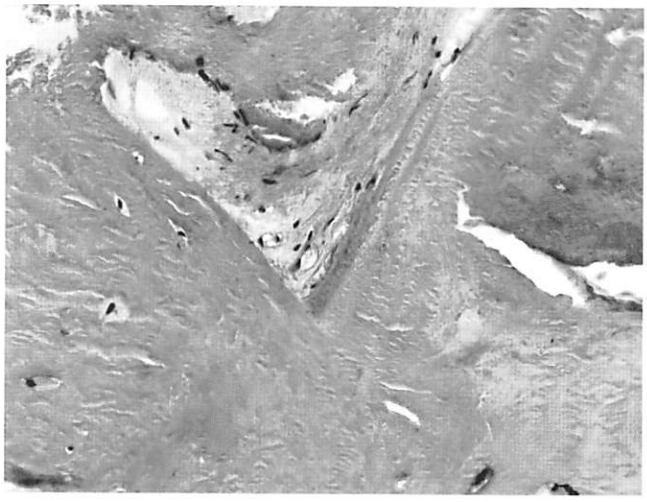

Figure 7. Intense bone metabolic activity with osteoblasts and lamellar structure with Osteocytic nuclei lacunae (40X).

material was partially replaced by new regenerated bone and a partially mineralized osteoid matrix was visible with new vessels. In only 3 cases connective tissue with new vessels (Fig.5) was prevailed over mineralized bone tissue. The active osteoid matrix edge (Fig.6-7) was observed in 10 samples. In 2 samples the grafted material was totally replaced by new regenerated bone. No samples presented histological signs of inflammation; only 2 samples presented a less active rearrangement.

Discussion

This study focuses on the identification of reliable methods for the evaluation of bone regeneration in post-extractive sites after using in vivo Puros® homologous bone [19-21]. In particular radiographic evaluation of allograft integration (by TC Cone-Beam to understand real vertical and horizontal bone gain) and the histological analysis of the bone were investigated. Only partial methods of radiographic evaluation for this kind of procedure are present in the Literature [22-28] and usually only with the radiographic evaluation of vertical bone augmentation. Histological analysis is even more rarely considered for the evaluation of the quality of the new regenerated bone even though this is an extremely important parameter for the survival of the implant-prothesis. A specific staining protocol is also described and identifies metabolically active grafted bone. This is more difficult to analyze with a classical stain like Hemotoxylin-eosin as used in the other studies on this topic [22-28].
In conclusion this study establishes a reliable method [29] to study bone regeneration in post-extractive sites. The radiographic and histological analyses demonstrate optimal bone regeneration, both in terms of quality and quantity using Puros®. Additional studies are needed, involving a greater number of patients as well as comparing different graft materials.

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