Concepts on bone regeneration

Novel synthetics and traditional xenografts

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As recent medical and dental studies in bone reconstruction are gradually shifting their focus onto biodegradable and bioactive materials, resorbable synthetic bone substitutes might be a potential alternative to autogenous bone or bovine xenografts in implant bone reconstructive procedures. The purpose of this article is to present the latest concepts on bone regeneration, supplemented with histological and radiological data gathered from the authors' clinical and experimental research background.

In contemporary oral and implant surgery bone grafting procedures are performed for the augmentation of the bone around dental implants, the management of osseous defects of the jaws due to pathological processes or trauma, and the preservation of the alveolar ridge after extractions. Such measures involve the use of a wide variety of bone substitutes, barrier membranes and growth-factor preparations, and several different surgical methods have been proposed [1,2].

Among bone grafts, autogenous bone is still considered to be the gold standard. Autografts possess osteoconductive, osteoinductive and osteogenetic properties, they do not transmit diseases nor trigger immunologic reactions, while they are gradually absorbed and replaced by newly-formed high quality osseous tissue. The disadvantages of using autogenous bone include the restricted availability, the need for additional surgical site, the increased morbidity and the extended operating time [3,4].

As an alternative solution, bone graft substitutes are widely used in bone reconstructive surgeries and the science of biomaterials has become one of the fastest growing scientific fields in recent years [5]. Bone substitutes can be defined as "a synthetic, inorganic or biologically organic combination which can be inserted for the treatment of a bone defect instead of autogenous or allogenous bone" [6]. This definition applies to numerous materials which vary in terms of origin, composition and biological mechanism of function regarding graft resorption and new bone formation, thus the selection of biomaterials in clinical practice must be based on their inherent biocompatibility, bioactivity, biodegradability, and mechanical properties, as well as the resulting cell behavior [7–11]. Moreover, parameters like the physicochemical characteristics, molecular weight, and hydrophilicity/hydrophobicity may influence the handling and performance of bone substitutes [12,13]. In general, the ideal grafting material should also act as a substrate for bone ingrowth into the defect, and to be ultimately totally replaced by host bone having an appropriate resorption time in relation to new bone formation for complete regeneration up to the condition of restitutio ad integrum [1,14]. In parallel it should be able to maintain the volume stability of the augmented site [1].

Bioactivity is a characteristic of chemical bonding between bone biomaterials and biological tissues. Calcium phosphate ceramics and calcium sulfates are considered bioactive materials as they have the ability to elicit a controlled action and reaction to the host tissue environment with a controlled chemical breakdown and resorption, to ultimately be replaced by regenerating tissue [5,15].

Among bioactive ceramics, β-tricalcium phosphate (β-TCP) and hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] are frequently utilized in dental bone regenerative procedures [13]. They have compositions similar to that of natural bone, exhibit good biocompatibility and osteoconductivity, can osseointegrate with the defect site, and are free of any risk of transmitting infections or diseases by themselves [16–21]. Moreover, the degradation products and released ions can participate in the human metabolism and create an alkaline environment to

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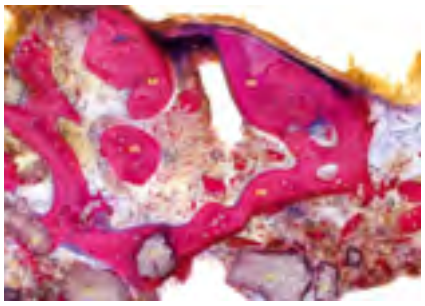
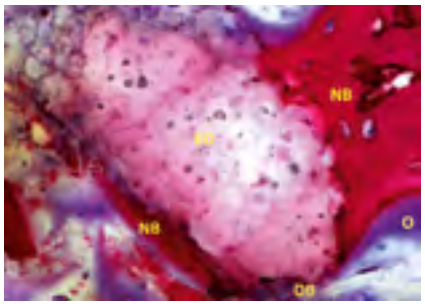
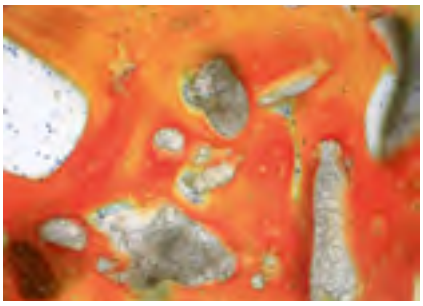


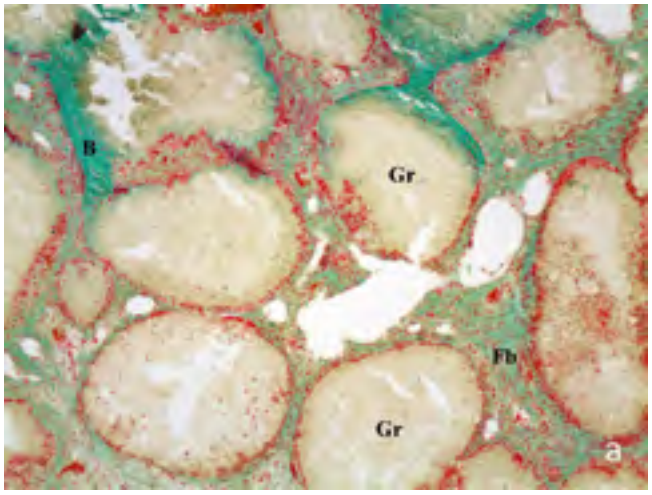
1 I Human bone biopsy after twelve weeks of healing. Socket grafting with β-TCP/CS (EthOss). Newly-formed bone trabeculae (NB) and disintegrating EthOss particles (EO). Histomorphometric analysis of the sample revealed 48.12 per cent newly-formed bone and 8.11 per cent residual EthOss particles. Undecalcified ground sections; azure II / pararosaniline staining. Original magnification x50.

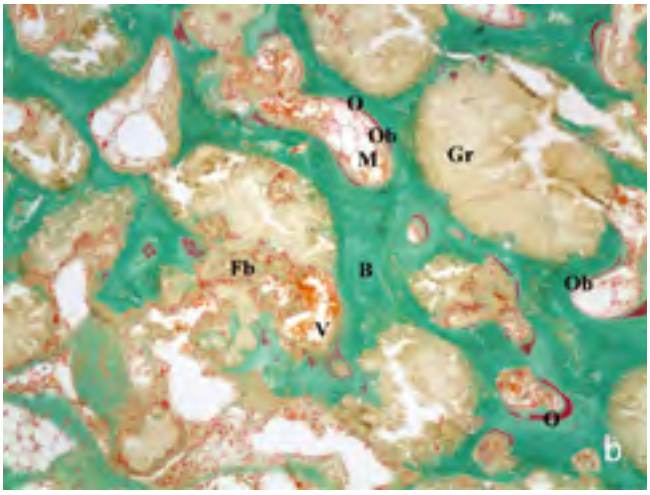


2 I Histological picture showing the breakdown and the reaction of a β-TCP/CS (EthOss) granule (EO) in newly-formed bone formation after ten weeks of healing. The β-TCP/CS particle is partially disintegrated, allowing osteoblasts (OB) to actively form osteoid (O) and add new bone trabeculae (NB) in contact and inside the pores of the bioactive grafting material. Undecalcified ground section; stain azure II / pararosaniline staining, original magnification x400.



3 I Human bone biopsy after ten weeks of healing. Socket grafting with β-TCP/CS (EthOss). Histological picture showing tight integration of β-TCP/CS particles (Gr) in newly-formed bone trabeculae (NB). Van Gieson's staining.



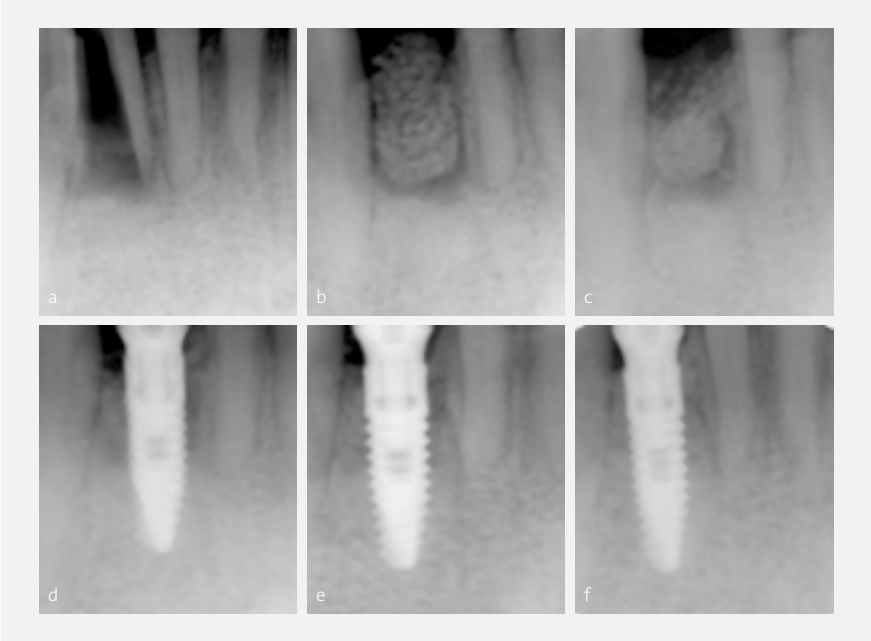


4a and b I Histologic microphotograph of rabbit calvaria defect filled with β-TCP/CS (Fortoss Vital): a) three weeks of healing revealing newly formed mineralized bone (B), remaining graft particles (Gr), and fibrous connective tissue (Fb); b) six weeks of healing revealing newly formed mineralized bone (B), osteoid (O), ream of osteoblasts (Ob), remaining graft particles (Gr), capillary blood vessels (V), marrow (M), and fibrous connective tissue (Fb). A statistically significant material resorption and new bone regeneration was found. Goldner's trichrome staining. Original magnification x10 [33].

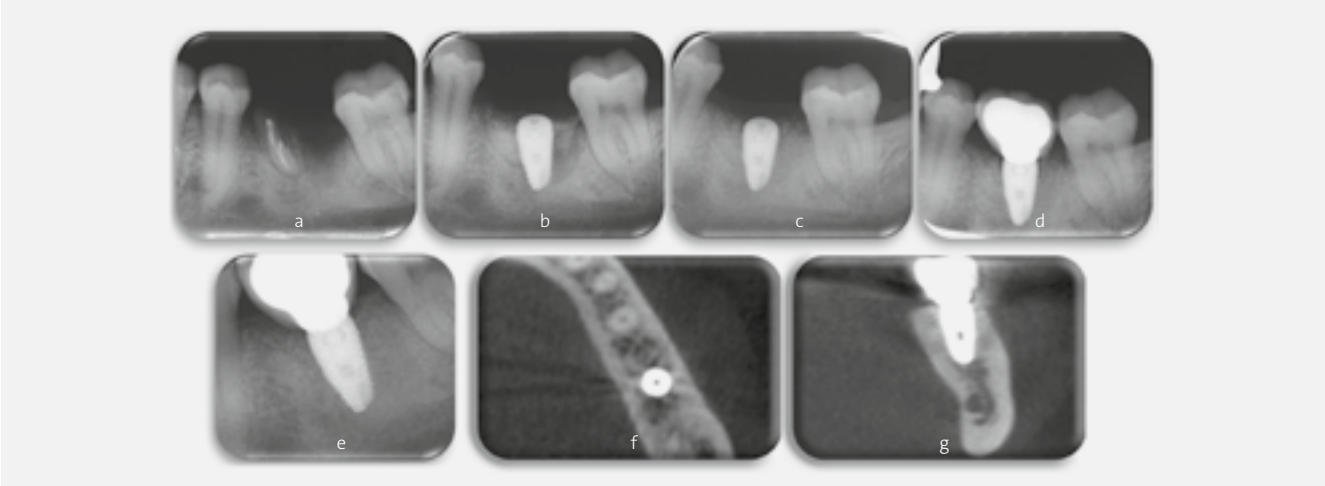
enhance cell activity and accelerate bone repair [13]. Recent in vitro and in vivo experimental studies have demonstrated that such alloplastic bone substitutes can also stimulate osteogenic differentiation of stem cells, as well as ectopic bone induction [23–27]. It is also important that β-TCP may promote the proliferation and differentiation of endothelial cells, and improve neovascularization in the grafted site, having clear benefits for osteogenic processes [13,28].

The ability of the bacteriostatic calcium sulfate (CS) to set is well documented. Adding CS to β-TCP produces a compound in situ hardening alloplastic biomaterial that binds directly to the host bone, maintains the space and shape of the grafted site, and acts as a stable scaffold [29–35]. As mechanical stability is a crucial factor for bone healing and differentiation of mesenchymal cells to osteoblasts [36], the improved stability throughout the bone graft seems to further improve the quality of the bone that will be regenerated [37,38]. Moreover, the CS element creates a nano-porous cell occlusive membrane that may prevent the early stage invasion of unwanted soft tissue cells into the graft [39–41].

Both CS and β-TCP are fully resorbable bone substitutes, leading to the regeneration of high quality vital host bone without the long-term presence of residual graft particles (Figs. 1 to 6g). The CS element will resorb over a three to six week period, depending on patient physiology, thus creating a vascular porosity in the β-TCP scaffold for improved vascular ingrowth and angiogenesis, while the β-TCP element will resorb by hydrolysis



5a to f I Socket grafting with β-TCP (Guidor easy-graft Classic) in a 65-year-old patient. Periapical x-rays of the case showing the modeling, ongoing remodeling and gradual resorption of the grafting material:
a) initial situation
b) immediately post-op
c) after four months
d) at implant placement
e) three months loaded
f) four years loaded.



6a to g I Implant placement with simultaneous bone grafting with β-TCP/CS (EthOss) in a 60-year-old patient. Periapical x-rays of the case showing the modeling, ongoing remodeling and gradual resorption of the grafting material: a) initial situation; b) immediately post-op; c) ten weeks post-op; d) twelve weeks post-op; e) one year loaded. Two years loaded (f and g), axial and coronal planes of the CBCT showing the preservation of the dimensions of the regenerated bone [55].

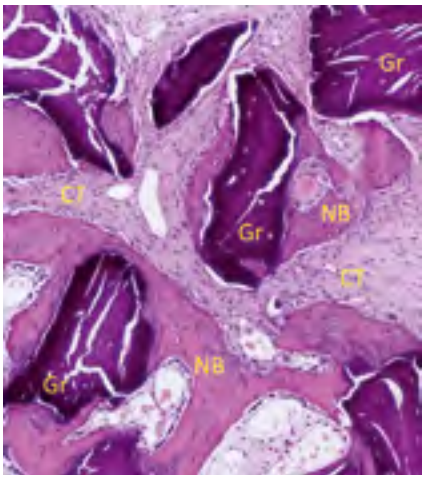
and enzymatic and phagocytic processes, usually over a period of 9 to 16 months. Although it is difficult to evaluate these resorptive mechanisms, it seems that cell-based degradation might be more important than dissolution, and macrophages and osteoclasts may be involved in phagocytosis, again largely dependent on host physiology [22,41–43].

Bovine xenografts have been extensively studied and documented in preclinical studies and clinical trials in dentistry, largely owing to their established osteoconductive properties and their ability to maintain the volume of the augmented site in the long-term (Fig. 7). However, controversy still remains as to whether this graft source is truly resorbable [44–52]. In a histological and histomorphometrical analyses of human biopsies harvested eleven years after sinus floor augmentation with deproteinized bovine and autogenous bone, *Mordenfeld* et al. (2010) found that the xenograft particles were well integrated in lamellar bone, showing no significant changes in particle size with no obvious signs of resorption [53].

Another important issue is that there are still significant concerns that bovine-derived bone grafts may carry a risk of prion transmission to patients. According to *Kim* et al. (2016) the limited ability to screen prions within the animal genome, along with a long latency period to manifestation of bovine spongiform encephalopathy (1 to over 50 years) in infected patients, provides a framework for discussing possible long-term risks of the xenografts that are used so extensively in dentistry. The authors suggest abolishing the use of bovine bone and highlight that

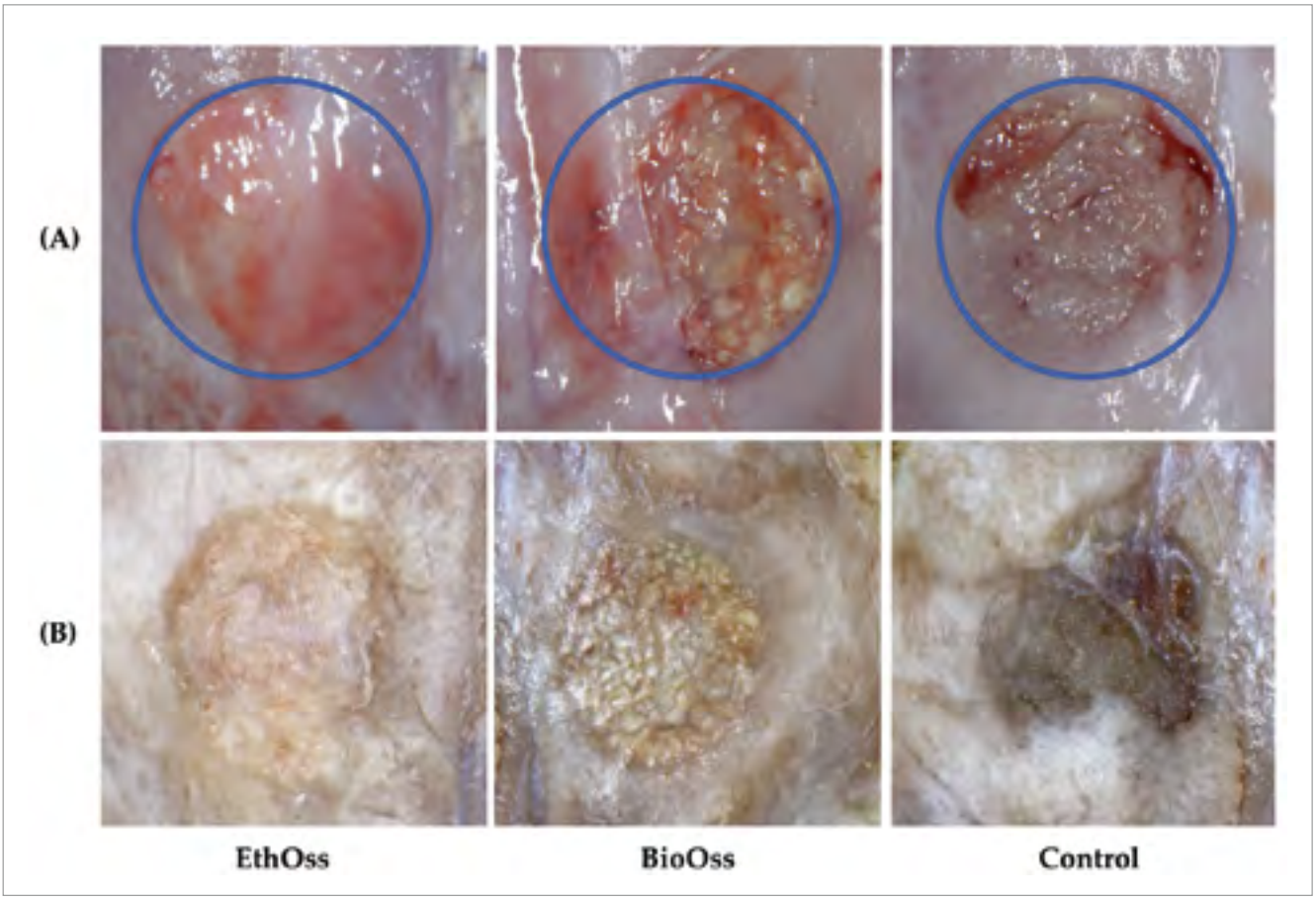
patient counseling should always include a clear description of the origin of the bone grafting materials used [54].

It is without doubt that bone quality is of paramount importance in successful implant therapy. It is doubtful whether an alveolar ridge preservation method should be claimed successful, if it only preserves the external contour of the alveolar ridge, but the newly-formed hard tissue is of inferior quality and quantity (percentage of matured trabecular bone) to what is spontaneously achieved following a tooth extraction. Contemporary literature reports conflicting results with the use of the widely-used xenografts, with changes in the percentage of vital bone ranging from -22 per cent (decrease) to 9.8 per cent (increase), while considerable residual hydroxyapatite and xenogenic particles (15 % to 36 %) remained at a mean of 5.6 months after socket grafting procedures [7]. Although



7 I Bone biopsy after eight weeks of healing. Rabbit calvaria bone defect filled with xenograft (BioOss). The xenograft particles (Gr) are embedded in well perfused connective tissue (CT) and newly-formed bone (NB), showing the osteoconductive properties of the material. H&E staining.

it remains unknown whether these changes in bone quality will affect implant success and peri-implant tissue long-term stability, *Chan* et al. (2013) state that there is a concern that firstly the long-term presence of residual non-resorbable or slowly resorbable graft particles might interfere with normal bone healing and remodeling, secondly it may reduce the bone-to-implant contacts and thirdly have a negative effect on the overall quality and architecture of the bone that surrounds implants [7]. In a recent systematic review of randomized controlled clinical trials analysing the outcomes of flapless socket grafting, *Jambhekar* et al. [10] reported that after a minimum healing period of twelve weeks, sockets filled with synthetic biomaterials had the maximum amount of vital bone (45.53 %) and the least amount of remnant graft material (13.67 %) compared to xenografts and allografts.



8 I Gross observations of rabbit calvaria bone defect sites after eight weeks of healing. (A) Freshly harvested rabbit calvaria; (B) after removing the dura mater and fixed in formalin (10 %) for 24 hours. Clinical observation reveals the different pattern of healing of the osseous defect between groups resulting in different geometry and architecture of the newly-formed hard tissue (ongoing experimental studies, [56]).

The use of grafting materials to treat bone defects might have an important effect on the amount of the regenerated bone, while the presence of the graft particles may alter the bone geometry and microarchitecture of the newly-formed hard tissue (Figs. 8 and 9). Although the grafting of the bone defects seems to affect the bone healing mechanism and the geometry of the newly-formed tissue, such differences might have an effect on the overall quality of the reconstructed bone. Laboratory studies have demonstrated moderate to strong correlations between trabecular bone volume/architecture and biomechanical properties and the strength of the bone tissue. However, in dental implantology it is unclear how differences in structural parameters of trabecular bone and bone microarchitecture can influence the ability of the bone to resist mechanical loads. The ability of the regenerated bone to remodel and to adapt to the transmitted occlusal forces depends on the amount of bone, the spatial distribution of the bone mass (shape and microarchitecture), and the intrinsic properties of the materials that comprise the bone. So, the long-term presence of residual non-resorbable or slowly resorbable graft particles, as in the case of using bovine xenografts, might have an unclear effect on the overall strength and quality of the reconstructed bone, the stability of the placed implants, or the bone-to-implant contacts [7].

For all the above reasons, when regenerating alveolar bone around implants with the use of bone substitutes, the grafting material should ideally be ultimately replaced by host bone, having an appropriate resorption time in relation to new bone formation. Following such concepts and in line with their published protocol and research [31,33–35,55,56], the authors have focused on using novel synthetic resorbable materials for the last 16 years. With an in situ hardening β-TCP/CS synthetic bioactive bone substitute like EthOss, the authors feel that they have a material ideally suited to working in a biological way with host healing in order to regenerate quickly and in a predictable way true vital bone around implants in a wide spectrum of everyday clinical cases.

The references are available at www.teamwork-media.de/literatur

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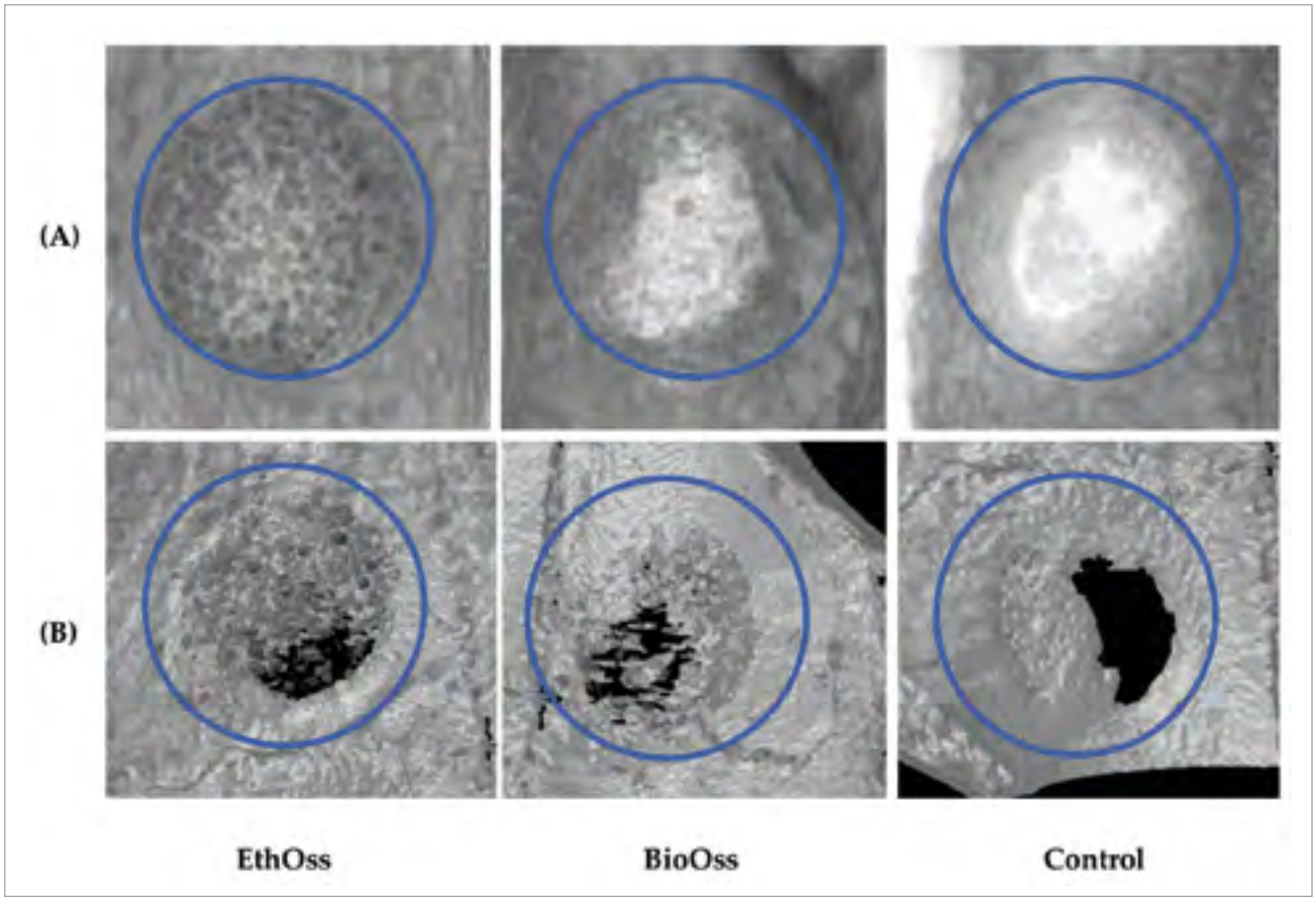

9 I Rabbit calvaria bone defect sites after eight weeks of healing. (A) Axial sections and (B) reconstructed 3D micro-CT images of the defect sites after eight weeks of healing, revealing the different micro-architecture of the newly-formed hard tissue according to each different group of treatment (ongoing experimental studies, [56]).