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Original Article

Accelerated Reconstruction of Rabbit Calvarias Bone Defect Using OsvehOss Synthetic Bone Substitute

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Abstract

Bone grafts after blood transfusion are second place in organ transplants in the world. In recent years, the importance of bone grafts in the healing of bone fractures, delay union, and mal union is progressively gaining attention in clinical fields. The biphasic calcium phosphate (BCP: HA (hydroxyapatite) /TCP (tri-calcium phosphate)) scaffold is one of the commonly used synthetic bone substitutes, the application of which has been investigated both in vitro and in vivo. OsvehOss BCP (Osveh Asia Medical Instrument Co., Iran) is a synthetic, porous, osteoconductive, and bioactive bone substitute for the filling/or reconstruction of bone defects. In the current animal study, we used OsvehOss biphasic scaffolds to treat calvaria defects in rabbits and evaluated the histological results to investigate the bone regenerative potential of the OsvehOss scaffolds. According to the obtained results, the OsvehOss scaffold seems to be a suitable bio-ceramic and strongly an osteoconductive bone substitute which could serve as a useful novel bone substitute material for future bone defect treatments.

Keywords: OsvehOss, Bone substitute, Rabbit calvarias, Histological, regenerative

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Introduction

The emergence of synthetic bone graft substitutes as a viable option for the treatment of a range of bony defects has revolutionized the field of tissue engineering. Bone grafts have been used in a range of clinical applications to mend bone lesions, fill gaps in multi-fragmentary fractures, non-unions, cysts and voids resulting from tumor removal due to osteosarcoma, and also for traumatic bone defects. The necessity of bone graft substitutes is gradually increasing in the field of maxillofacial surgery and orthopedic surgery to improve bone defect healing and bone fusion (1, 2). Autogenous bone offers an optimal balance of osteogenic, osteoinductive, and osteoconductive capacities, structural stability, and biocompatibility. However, donor-site morbidity, such as the risk of infection, hematoma, fracture, wound healing problems, and donor-site pain limited use of autografts (1, 3, 4). Hydroxyapatite (HA), Tricalcium phosphate (TCP) and Biphasic (BCP) bone grafts, with a composition similar to mineral bone, are known to be safe and non-allergenic, with good bone bonding capacity and used as substitutes for bone grafts in orthopedic, maxillofacial and dental operations over the last 30 years (5). Advantages of ceramic bone grafts include low immunogenicity and toxicity, stability at physiologic pH levels, and the ability to withstand sterilization procedures without losing structural integrity (4). Although recent studies reported the utility of synthetic bone grafts in bone reconstruction surgeries, its efficacy in this situation is still a subject of controversy. Hydroxyapatite is brittle and hard to achieve complete remodeling due to a low level of resorption after implantation. Thus, bone graft extenders with a high resorption rate have been developed. While the material with high resorption has a low risk as a foreign material since it does not stay inside of the body, but bone fusion or bone healing rate can be lowered if the resorption occurs before new bone formation (2, 5-8). In general, β-TCP has a median resorption period of around 6−12 months, but its cell affinity is lower than that of HA.

The clinical study produced high satisfaction with clinical results both objectively and subjectively.

To complement the above-mentioned limitations, the composite materials of β-TCP with high resorption rate and HA with appropriate osteoconductive properties (OsvehOss BCP) have been examined in vitro and in vivo and shown the results about their usefulness. OsvehOss, a macro and micro porous synthetic bone graft (> 70% Porosity, 200-500 microns), used in spine, maxillofacial, periodontology and open fracture surgeries is available as granules, powder, block, wedge and paste as bone regeneration material. This study was undertaken to evaluate the performance and clinical outcome of OsvehOss synthetic bone grafts in granular form and biphasic composition as a graft extender in the rabbit calvarias bone defect model.

Materials and Methods

OsvehOss bone graft substitutes (Osveh Asia Medical Instrument Co.) were produced with highly pure raw materials (Merck Millipore, Germany). Biphasic (BCP, HA: β-TCP= 60%:40%) granule and β-TCP granule were provided as specified in ASTM F1185-03 and ASTM F1088-04 respectively and followed the standard specification for surgical implants. In vitro and in vivo tests according to parts 3, 5,6,10, and 11 of ISO 10993, the standard for biological evaluation of medical devices, indicated that OsvehOss synthetic bone grafts were completely biocompatible. Characterization of the crystal structures of OsvehOss grafts was determined by x-ray diffraction (XRD), microstructure was evaluated with scanning electron microscope (SEM, Leo 1450VP, Germany), Content of HA and TCP in the BCP grafts composite was quantitated by the calculation method suggested in ISO 13779-03 and also X´Pert High Score software.

Implantation procedure and in vivo study performed in the Department of Clinical Sciences, Faculty of Veterinary Medicine (Ferdowsi University of Mashhad, Iran).

Twenty-four male rabbits were randomized into 4-week and 8-week groups. The animals were anesthetized with zoletil (0.4 mL/kg, Virbac Laboratories, Carros, France) and rum pun (10 mg/kg, Bayer Korea Ltd., Korea) and the region around scalp was shaved and antisepticised with betadine. For the calvaria defect model, the dorsal part of the cranium was shaved and a linear incision of approximately 20 mm was opened over the scalp of each animal. The periosteum was removed, and four full-thickness calvaria defects (5 mm in diameter) were prepared in the parietal bone using a dental drill (Figure 1). To prevent spontaneous bone healing, a 5 mm trephine burr was used to generate calvaria defect followed by saline irrigation. The same amount of OsvehOss bone grafts (BCP and TCP granule) was implanted into the calvaria defect, periosteum and scalp were sutured. Cefazoline (100 mg) was given to the animals by intramuscular injection immediately after the surgery for 2 days. The animals were raised at 22 ± 5∘C temperature and 50 ± 5% humidity without interruption and sacrificed at 4 weeks or 8 weeks after implant for analysis.

Figure 1: Surgical procedures of rabbit calvaria defect model and filling with bone substitute granules.

Histological evaluation was done by preparing block samples, including the surgical sites (Figure 2), which were decalcified in 10% EDTA (Ethylenediaminetetraacetic Acid) at room temperature, dehydrated using an ascending alcohol gradient and embedded in paraffin. Longitudinal sections were stained with Hematoxylin and Eosin (H&E) and visualized using an optical microscope.

Figure 2: Preparing block samples for histological evaluation and scarification of rabbits after 4 and 8 weeks for the histological and histomorphometry analyses.

Results

For bone defect healing, the bone graft substitutes should simulate the physicochemical properties of the bone ECM (Extracellular matrix). BCP bio-ceramics best represent the inorganic phase of the bone ECM with mineral composition similar to that of natural bone. Hydroxyapatite phase could function as a structural framework that supports the scaffold and newly formed bone, while the less stable TCP phase creates space for new bone ingrowth during the biodegradation process. Furthermore, some studies reported that formulations containing HA/β-TCP ratios of 50/50 (9) and 60/40 (10) enhanced cell proliferation to a higher extent.

OsvehOss is a synthetic bone graft substitute with a unique micro and macroporous structure that most closely resembles the architecture of natural human bone. It gradually dissolves in the body (various degradation rates for BCP and TCP granules), promoting new bone formation through the release of calcium and phosphate ions.

In the current study, XRD is used for phase identification and quantification of bioceramics materials using X-ray diffractometer machine (with Cu-Kα radiation) with scan range from 20° to 60°. Figure 3 shows XRD pattern of BCP (60 HA/40β-TCP) granules indexes main peaks corresponding to HA (JCPDS no. 09-0432) and β- β-TCP (JCPDS no. 09-169) in accordance with ICDD standard (The International Centre for Diffraction Data). The mean of approximating the HA/β-TCP ratio in the BCP grafts was determined using the ratio of intensities of the most intense diffraction peaks of the HA phase to those of the most intense diffraction peaks of β-TCP phase compared with the ratios obtained from calibrated standard mixtures of pure HA and β-TCP (11) and also X´Pert High Score software.

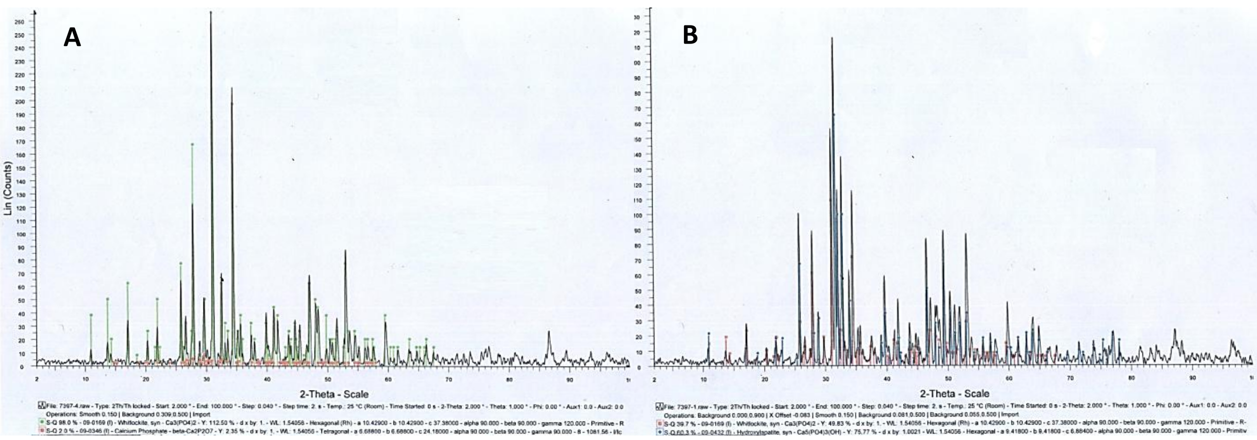

Figure 3: XRD analysis of (A) TCP and (B) BCP OsvehOss bone graft substitutes in granular shape used in the present research

SEM analysis was performed in order to examine the microstructure of OsvehOss bone grafts. In the microscopic image, the interconnected pores in the OsvehOss BCP sample were visible (Figure 4). In time, the porous structure becomes completely infiltrated with, and replaced by, healthy viable bone. Network of interconnected spaces that promote the biological infiltration and cellular colonization by osteoblasts and osteoclasts. The interconnection between macropores promoted in this way should be beneficial to cell migration and to the flow of nutrients and metabolism products.

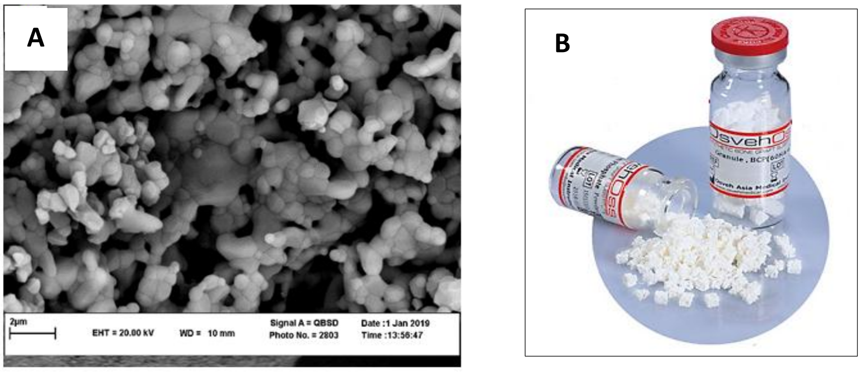

Figure 4: SEM images of (A) porous micro-structure of OsvehOss BCP granule and (B) granular form of product

For histological evaluation, samples were decalcified in 10% EDTA at room temperature, dehydrated using an ascending alcohol gradient and embedded in paraffin. Longitudinal sections were stained with H&E and visualized using an optical microscope (DXM200F Digital Camera; Nikon, Tokyo, Japan). The specimens were fixed, decalcified, embedded and sectioned into 5 mm thick sections. The sections were then stained with hematoxylin and eosin (H&E). Digital images of each slide were acquired using a digital camera mounted to a microscope. The histomorphometry analysis of slides, 8 weeks after surgery, illustrated a significantly larger amount of bone formed in the TCP (Figure 5C) and also the BCP groups (Figure 5B) than the control groups (Figure 5A). Compared to the empty control (Figure 5A), there were regions of osteoid matrix within the interiors of the implantation site in the OsvehOss BCP and TCP group, indicating the OsvehOss scaffolds can stimulate new bone formation.

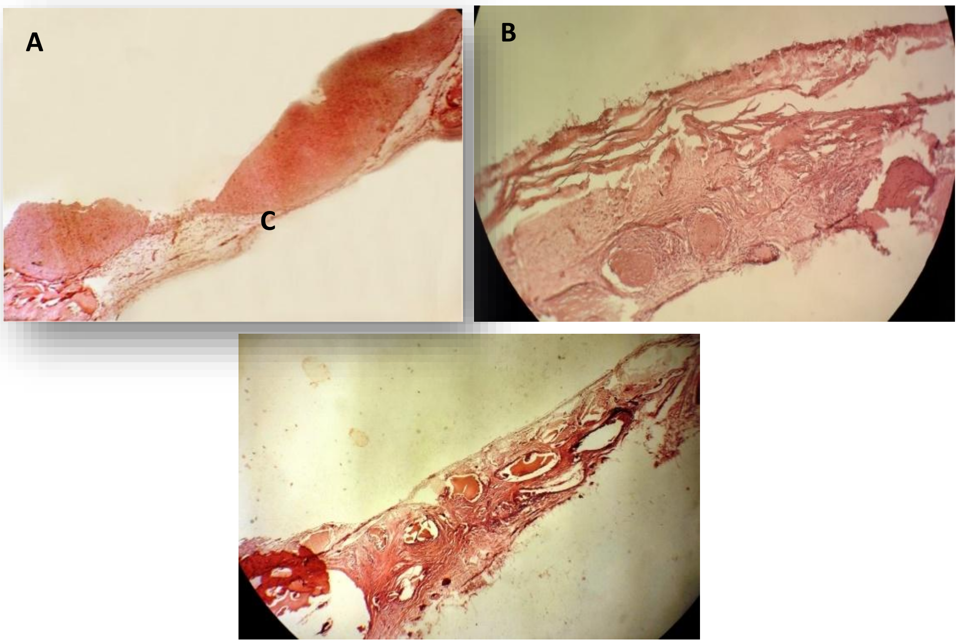

Figure 5: (A) Blood clot in control space, (B) Immature bone formation around resorbed OsvehOss TCP bone grafts, (C) Formation of mature bone in place of resorbed OsvehOss BCP bone grafts, 8 weeks postoperative. (H&E-40x).

Additionally, 4 weeks after implantation surgery, large amounts of BCP granules remained nearly unchanged, while the OsvehOss TCP granules were almost significantly absorbed and replaced by new immature bone, which induced a higher absorption rate of TCP. As shown in Figure 6, one month after implantation, immature (intertwined) bone fibrotic bridge was forming around the implanted granules. Also, multinucleated giant cells were visible around reabsorbing TCP granules.

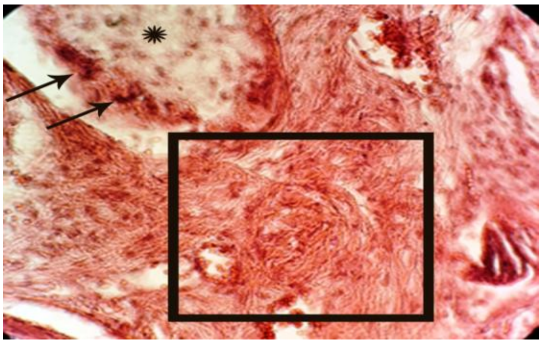

Figure 6: Bone defect in OsvehOss TCP group, 4 weeks after surgery. Multinucleated giant cells (arrows) and reabsorbing TCP granules (star), immature bone (square) (hematoxylin-eosin stain, magnification 400x).

In OsvehOss BCP group, 4 weeks after surgery, a bridge of connective tissue around the grafts particles and blood clot was detectable (Figure 7). Multinucleated giant cells reabsorbing bone graft particles were also recognizable. In some cases, an immature bone fibrosis bridge was forming and signs of resorption were observed, such as the formation of phagocyte cells and How·ship's lacuna.

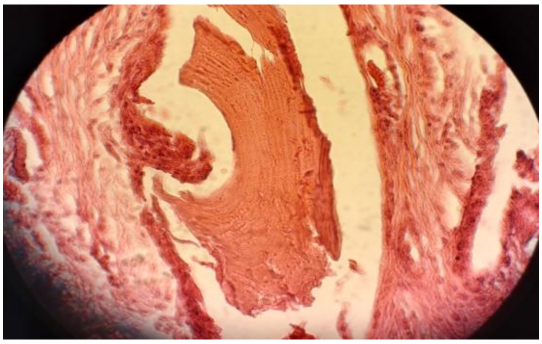

Fig 7. Bone defects in the BCP group, 4 weeks after surgery. Reabsorption of grafts particles by multinucleated giant cells (osteoclast) and formation of How·ship's lacuna (hematoxylin-eosin staining, magnification 400×)

Discussion

It has different ratios of HA/TCP, giving rise to balanced phases of activity, a more stable phase of HA, and a more soluble phase of TCP. Nery et al. concluded that biphasic calcium phosphate (BCP) with a high HA content may be desirable for repairing defects periodontal. For this reason, most ceramic bone grafts tend to be formulated with BCP (12). The X´Pert High Score software quantitative analysis determined the approximately relative values of 60%wt HA and 40%wt TCP for phase identification of OsvehOss bone grafts.

The results of the descriptive analysis of histopathology images show that the in vivo absorption rate of synthetic OsvehOss TCP bone grafts is higher than that of OsvehOss BCP granules. The absorption process of TCP granules had started within 4 weeks after the surgery. 8 weeks after the surgery, the absorption of TCP granules was done in almost all cases, and the immature fibrotic bone bridge replaced the grafts and filled the defect site. Regarding the bone defect areas filled with biphasic granules, 4 and 8 weeks after surgery, large amounts of residual grafts were still observed at the defect site, although there were signs of the beginning of the process of graft absorption in the period of 4 weeks with the presence of osteoclasts.

The OsvehOss TCP and BCP bone grafts exhibited robust osteogenic activity, with reasonable coverage of defects with newly formed bone, and the interface between new bone and host bone showed a close union without any gaps.

Conclusion

One of the most used models to study bone healing is the bone defects induced by pressing trephines against the calvaria bone of rabbits. The current research established for evaluation of the clinical outcome of OsvehOss synthetic bone grafts in rabbit calvaria bone defects. The results of the descriptive analysis of histopathology images in the present animal study show that in the case of synthetic OsvehOss grafts, the resorption rate of TCP granules is higher than the OsvehOss BCP granules. Within 4 weeks after the surgery, the absorption process of TCP granules had started, and 8 weeks after the surgery, the absorption of TCP granules was done in almost all cases, and the immature fibrotic bone bridge replaced the grafts and filled the defect site. Regarding bone defect areas filled with biphasic granules, 4 and 8 weeks after surgery, large amounts of residual grafts were still observed at the defect site, although there were signs of the beginning of the process of graft absorption in the period of 4 weeks with the presence of osteoclasts. We concluded that both OsvehOss BCP and TCP granules were able to improve bone healing and accelerate reconstruction of rabbit calvarias bone defect. The results indicate that these porous bone substitutes could provide a bioactive and osteoconductive carrier for growth factors in bone regeneration.

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