ORIGINAL ARTICLE

The histological study of osseous regeneration following implantation of various bone graft biomaterials

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Abstract

Background: While various biomaterials are used for bone regeneration, the relative comparative efficiency of them has not been thoroughly investigated.
Purpose: This study evaluated histopathological events during osseous healing after implantation of following bone grafts: Demineralized freeze-dried cortical bone powder (DFDB), natural coral implants, calcium sulfate-based putty containing demineralized bone matrix (CaS-DBM), and pure-phase beta tricalcium phosphate ceramic granules (β-TCP).
Materials and Methods: Fifty-six Wistar Albino rats were used for this study. The postimplantation osseous healing was evaluated at 3rd, 6th weeks after the operation.
Results: DFDB did not induce bone formation in 3 weeks period, but it showed a highly osteoinductive effect at the end of 6th week period. The effects of coral implants on bone formation both at 3 and 6 weeks period were much higher than the DFDB. CaS-DBM showed higher bone formation than β-TCP at 3rd, 6th weeks. It was found that coral and CaS-DBM had a more beneficial impact on early bone healing compared to β-TCP and DFDB. All these graft biomaterials are useable in human bone defects. The main difference in the ossous healing properties of these materials is observed early postimplantation with the delayed healing outcome being similar.

Key words: Graft materials, histological observation, osseous healing

Introduction

Bone defects resulting from trauma, infection, bone tumors or congenital malformation are considered a serious surgical challenge. Bone grafting is a surgical procedure that replaces the missing bone with material from the patient's own body, artificial, synthetic, or natural substitutes.[1] The final fate of a bone graft is determined by three parallel phenomena, namely osteoconduction, osteoinduction, and osteogenesis.[2] In osteoconduction, the graft material provides a scaffold for adjacent osteoblasts to grow on its surface. Osteoinduction is stimulation of differentiation of primitive, undifferentiated, and pluripotent cells to

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osteoblasts.[3] Osteogenesis occurs when graft material contains viable osteoblasts capable of participating in repair of the bony defect.[4]

Autologous grafts are considered the gold standard in terms of osteogenic potential. However, the morbidity and limited availability associated with autografts, and the potential for disease transmission, immunogenic response, and variable quality associated with allografts, emphasize the need for alternative approaches.[4] Consequently, significant strides have been made to search ideal bone graft substitutes. Recently, tissue engineering approach has been shown to be very effective in bone regeneration. This new therapeutic technology induces bone regeneration by employing various growth factors, osteogenic cells, and biocompatible scaffolds for a combination of these approaches.

The remodeling process for osteoconductive materials is well characterized. Differences amongst osteoconductive materials lead to differential rates of remodeling with varied levels of secondary inflammation and fibrosis at the remodeling sites.[5] Osteoconductive materials when placed in regions of good vascularity with exposure to medullary elements have abundant access to growth factors and proteins important to bone production and remodeling as well as osteoprogenitor cells.[6] There have been several studies investigated the osteogenetic capacity of biomaterials. However, the difference in reparative response of bone affected by osteoconductive properties of various synthetic and natural materials has not been thoroughly described.

In the present study, we analyzed the histological and cellular events in response to implantation of demineralized freeze-dried cortical bone powder (DFDB), calcium sulfate-based putty containing demineralized bone matrix (CaS-DBM), pure-phase beta tricalcium phosphate ceramic granules (β-TCP), and natural coral implants underlying osteoinductivity and bioresorption in rat femurs. Moreover, we aimed to compare these materials with each other in terms of osteogenesis.

Materials and Methods

In this study, for the comparison of bone healing property DFDB (Dembone, Demineralized Human Bone Powder, Pacific Coast Tissue Bank, CA, USA), CaS-DBM (AlloMatrix Injectable Putty, Wright Medical Technology, Inc., Arlington, Tenn), β-TCP (Cerasorb, Curasan AG, Kleinostheim, Germany) and natural coral implants (Biocoral, Inoteb, Saint-Gonnery, France) were used. In addition, for surgical procedures ketamine (Ketalar, Yuhan Co., Korea), xylazine-hydrochloride (Rompun, Bayer Korea Ltd., Korea), povidone-iodine solution (Betadine, Samil Pharm. Co., Korea), Vicryl 4-0 (Ethicon, Somerville,

NJ), gentamycin (Gentamycin, Kukje Pharm. Co., Korea) were also used.

Surgical procedure

This study was approved by the Animal Ethics Committee at University of Dicle, Diyarbakir, Turkey. Fifty-six Wistar Albino rats (4 months, weight range: 200–240 g,) were included in the study. Implantation surgery was performed in an operating theater using aseptic technique. The animals were anesthetized with intramuscular injections of 0.2 ml ketamine and 0.1 ml xylazine-hydrochloride.

The skin of the right leg was shaved and cleaned with povidone-iodine solution. 1 cm longitudinal incision was made with no. 15 scalpel over the femur and then flap elevation was done. Blunt dissection was then carried out until the femur was visualized. A 3 mm deep, 2 mm wide and 10 mm long cavity was prepared in the femur under NaCl irrigation with a stainless steel round bur [Figure 1].

Cavities were filled in 14 rats with 3.5 mg DFDB [Figure 2a], in 14 rats with coral [Figure 2b], in 14 rats with β-TCP [Figure 2c] and the other 14 cavities were filled with CaS-DBM [Figure 2d]. Periosteum and skin were closed with Vicryl 4-0, subsequently. To prevent infection, gentamycin was administrated intramuscularly into all experimental animals at the ratio of 0.05 mg/kg/day for 3 days. After surgery, they were left in separate cages to prevent injury to the operation sites.

At the time of 3 and 6 weeks after the surgical procedure, seven rats from each group were anesthetized with the same method as above. Femurs were dissected and removed. The animals were euthanized with an overdose of Urethane. The specimens were decalcified in formic acid and embedded in paraffin. The paraffin blocks were sectioned in 5.0 of thickness and stained with hematoxylin-eosin to examine under a light microscope.
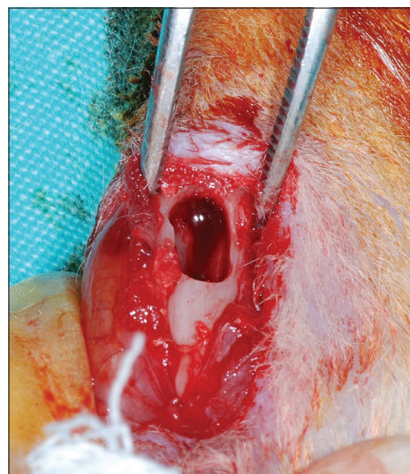

Figure 1: Rats femur after preparation of the defects

Clinically, all of the implant sites healed normally except one which had an infection in the implant sample and no important reactionary change was noted between the period of 3 and 6 weeks. Otherwise, the surgical procedure was well tolerated by the animals; healing was uneventful.

Results

Histological examination

End of the 3rd week

Demineralized freeze-dried cortical bone powder

DFDB was surrounded by connective tissue [Figure 3a]. This connective tissue was highly cellular with new capillaries. A combination of spindle-shaped cells with elongated nuclei and small rounded cells with large nuclei were observed within the connective tissue. There were also extravasated erythrocytes and few giant cells in some samples.

Naturel coral implants

Coral lamellas were seen between the compact bones at the 3rd week following coral implantation [Figure 3b]. Pores in the structure of coral were completely filled by bone marrow cells and capillary vessels. Very few osteoclasts and osteoblasts were also observed.

Calcium sulfate-based putty containing demineralized bone matrix

A weak osteogenic response was observed at the defect area [Figure 3c]. The graft material was vascularized and surrounded by fibrous tissue and fibroblast-like cells which indicates anatomical integrity. There was no new bone marrow formation and graft resorption. To the end of this period, inflammatory cell number decreased, and these cells began to accumulate at specific areas. Graft material was biocompatible, and there was no foreign body reaction.
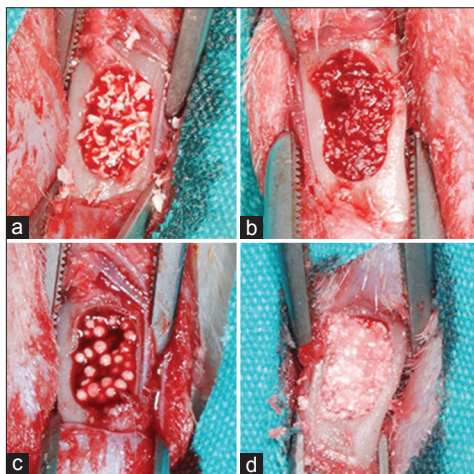
Pure-phase β-tricalcium phosphate ceramic granules

At the 3rd week, the osteogenic response and anatomical integrity is less than calcium sulphate-included CaS-DBM graft materials [Figure 3d]. During this period, fibrogenesis was partially developed, and fibrous tissue did not exhibit rich vasculogenic profile as observed in CaS-DBM grafts. Moreover, inflammatory cells were fewer, and foreign body reaction was not observed. There was a small amount of resorption in the grafts. In general, biocompatibility of β-TCP was good.

End of the 6th week

Demineralized freeze-dried cortical bone powder

Six weeks after DFDB implantation, highly cellular connective tissue was observed similar to that described at the 3rd week [Figure 4a]. Most of the DFDB were surrounded by well-developed bone and a peripheral rim of cartilage. This cartilage consisted of hypertrophied chondrocytes embedded in a highly basophilic matrix. The maturation of cartilage increased as it approached bone. The anatomical integrity of DFDB particles to each other and to the surrounding bone was maintained by this tissue. In some areas, bone marrow was observed and some osteoblast-like cells were seen adjacent to the newly-formed bone.

Natural coral implants

There was a new bone formation in juxtaposition to coral implants during 6 weeks [Figure 4b]. The lamellas that formed the porous structure of coral implant become thickened by new developing bone tissue. In this new bone, haversian structure was observed. On the coral surface, there were more osteoblastic activity and a reduction in osteoclastic activity. The space between the trabeculae of bone was completely filled by the bone marrow cells.
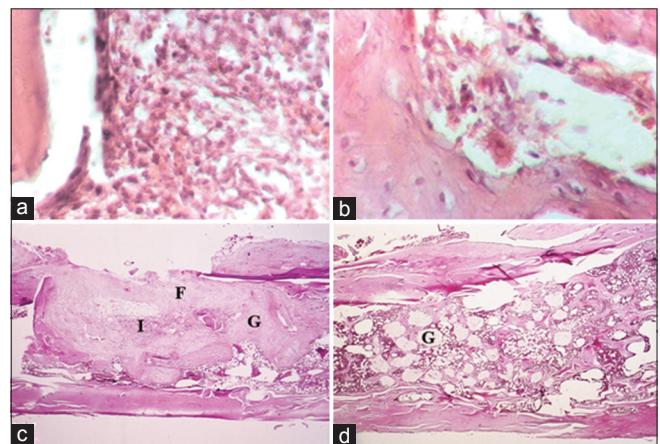

Figure 2: Views of the defects filled with (a) demineralized freeze-dried cortical bone powder, (b) natural coral implants, (c) beta tricalcium phosphate and (d) calcium sulfate-based putty containing demineralized bone matrix

Figure 3: Photomicrographs indicating the histopathologic view of H and E stained tissue sections taken at the end of 3rd week. (a) Demineralized freeze-dried cortical bone powder (×252), (b) natural coral implants (×252), (c) calcium sulfate-based putty containing demineralized bone matrix (×40) and (d) beta tricalcium phosphate (×40). F: Fibrotic tissue growth, G: Graft material, I: Inflammation
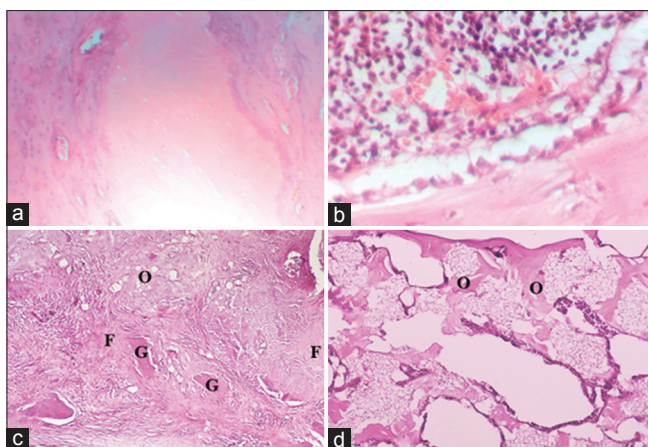
Figure 4: Photomicrographs showing the histopathologic view of H and E stained tissue sections taken at the end of 6th week. (a) Demineralized freeze-dried cortical bone powder (×252), (b) natural coral implants (×252), (c) calcium sulfate-based putty containing demineralized bone matrix (×100), and (d) beta tricalcium phosphate (×100). F: Fibrotic tissue growth, G: Graft material, and O: Osteogenesis

Calcium sulfate-based putty containing demineralized bone matrix

Resorption was observed in graft body and osteogenesis began adjacent to bone graft [Figure 4c]. Newly-formed bone trabeculae showed an immature lamellar pattern. New bone formation replaced fibrous tissue and the number of inflammatory cells decreased. It was seen that graft materials were biocompatible, and there was no foreign body reaction. During this period, high osteoblastic activity was observed in newly-formed bone and bone marrow.

Pure-phase β-tricalcium phosphate ceramic granules

The osteogenesis was observed between the granules and newly-formed bone directly attached to the porous structures [Figure 4d]. Reposition process is higher besides the resorbed bone the new bone marrow and lamellar structure and osteoblastic activity takes place.

Discussion

In augmentation of skeletal healing, bone grafting has become one of the most common techniques in surgical practice. Different types of biomaterials are applied for reconstructive indications and their popularity rises. Materials for bone engineering should satisfy various criteria. They should be: biocompatible, absorbable, osteoconductive, easy to manufacture and sterilize; and have beneficial mechanical properties.[7] In these days autograft, allograft, xenograft, and alloplastic materials are commonly used. A healing period of graft materials may vary by age, type of experimental animals, shape and form of the implant materials.[6]

The graft materials that we used in this study have different shapes such as DFDB, and β-TCP implants have an irregular

shape, CaS-DBM has putty form, and coral implants is round shaped. Some literature reported that in round shaped implant materials, healing is faster compared to other shapes.[4,5] In our study, the reason for the superiority of coral and CaS-DBM implants can be explained on the basis of their shape.

DFDB is a material that lacks antigenicity, can be easily obtained and stored, and stimulates the adjacent tissue to form new bone. It is also biologically compatible. Demineralization causes unmasking of the osteoinductive proteins and reduces antigen expression of the bone matrix to a minimum. DFDB in rats can induce cartilage and bone formation by stimulating undifferentiated mesenchymal cells to differentiate to chondroblasts and osteoblasts.[8]

Sharawy reported that, if there are more capillary vessels in the connective tissue around DFDB particles, more undifferentiated mesenchymal cells are directly differentiated to osteoblasts, while in the contrary condition the cells are first differentiated to chondroblasts, then to osteoblasts.[9] In our study, since there were not many capillaries around the DFDB, undifferentiated mesenchymal cells were first differentiated to chondroblasts in all animals.

Einhorn *et al.*[10] observed healing in 12 weeks after applying demineralized bone matrix in the region of the osteoperiostal segment from the rat femurs. Gepstein *et al.*[11] applied DFDB in the diaphyseal defects in the rats, and observed bone formation in 15–24 days and found that the defect was completely healed by the 35th day.[11] In our study, the DFDB group showed abundant osteoid tissue in the 6th week but none in the 3rd week.[12]

An ideal graft material reduces just as new bone matures into it in order to the stability of the construct is preserved during the complete period of bone healing.[13] The resorption rate of a graft material depends on some parameters such as the chemical configuration, location, chosen model, scaffold dimensions, defect size, porosity, and surface area per unit volume.[14] Careful consideration requires to be given to the porosity, material and resorption rate of the bone graft when using the different surgical procedure.[13] Glazer *et al.*[15] used CaS as a bone graft material for treatment of lumbar spinal fusion in a rabbit study model and reported that it was unsuccessful possibly because of its quick resorption, resulting in the nonappearance of an osteoconductive material left in the middle of the intertransverse process space until new bone formation extended this level.

The coral implant is a resorbable bone substitute material. Although it has been demonstrated to resorb more quickly than hydroxyapatite, the resorption speed is not optimal for oral surgery and in some cases it is too slow. Cellular and interstitial fluids have been implicated in coral resorption

though the exact mechanism remains unclear. Regardless of the resorption rate, bone healing process is enhanced parallel to accelerated resorption.[16] Kujala *et al.*[16] also reported that the mineral content of coral implant resembles that of bone, and they do not cause immunologic reactions. Coral implant resorbs in intraosseous and subperiosteal regions. On the other hand, in avascular conditions coral implant does not resorb sufficiently leading to sequestration of the implant between the bone graft and bone followed by inhibition of healing process.

Calcium sulfate (CaS), known as plaster of Paris, is used as bone graft and expander. It is rapidly resorbed and replaced by bone. It has been proposed that fast-resorbing CaS-based putties are effective for successful restoration of bony defects.[17,18] Handling characteristics of CaS can be improved by combining it with demineralized bone matrix.[19] Hu *et al.*[20] reported that the injectable calcium sulfate/phosphate cement have substantial clinical advantages, and might have been potentially applied in orthopedic, reconstructive and maxillofacial surgery, especially in minimally invasive surgical procedures.

Pure-phase β-TCP is a suitable material for the filling of bone defects in the alveolar region because of its versatility, low postoperative complication rate, and favorable long-term results.[21,22] The lack of foreign body response or toxicity supports the usefulness of implant as a suitable alternative bone graft to repair the defect.[7] β-TCP particles exhibited minimal new bone formation with loose connective tissues consisting of collagen fibers and fibroblast-like spindle-shaped cells with macropores.[21]

Studies done with DFDB and CaS-DBM showed that in the early period fibrous encapsulation found around graft materials, by the way, there were no encapsulation around coral implants.[19,23] In our study, the fibrous tissue around DFDB and CaS-DBM was found especially in the 3rd week. In the 6th week, the new bone formation had been done instead of fibrous tissue. The early period of β-TCP had middle degree fibrotic tissue without good vascularization in late period new bone formation was made. In the coral implants neither in the early nor late periods, no fibrous tissue was found. This fact explains the difference of the new bone formation periods between these materials.

After DFDB and CaS-DBM implantation, the undifferentiated mesenchymal cells of the host are differentiated to chondroblasts and osteoblasts to form an initial chondroid tissue. However, coral implants undergo resorption and are replaced directly by the bone cells without chondroid tissue formation.[7,8,20]

Moreover, in this study, coral implants showed a rapid resorption and new bone formation. Though chondroid tissue formation was observed in DFDB and CaS-DBM implants and loose collagen fibers in β-TCP, not any chondroid tissue observation recorded around natural coral implants.

Osteoinductive materials induce cartilage and new bone formation by stimulating undifferentiated mesenchymal cells. Hence, they induce new bone formation in all mesenchymal tissue.[4] On the other hand, it is reported that coral implants are resorbed in the soft tissue ever in small fragments.[24] Furthermore, in our study very little DFDB escaped to the soft tissue around the femur caused changes like in the bone, but in coral implants we have not observed such changes. This can be another factor to choose coral implants.

Bony substitution of the materials depends on individual patient factors, such as defect size, implant site, and individual osteogenetic bone potential, in addition to factors relating to the materials used.[4,8]

Our study shows that no foreign body response or toxicity was elicited and hence it was confirmed that the used graft materials were accepted as a suitable alternative bone grafts to fill the defects. This could be attributed to the fact that all the graft materials used in the present study were biocompatible and subsequently had low or no inflammatory response after transplantation.

Lack of osteoinduction also limits their use in large bone defects that would require interposition grafts. It is possible to confer osteoinductive properties to biomaterials by combining them with osteoinductive growth factors, such as bone morphogenic proteins.[25]

It may be concluded that CaS-DBM and coral implants accelerate the healing response of osseous defects more than DFDB, β-TCP. However, the quality of newly-formed bone is similar with respect to the angiogenic profile and anatomical structure.

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Conflicts of interest
There are no conflicts of interest.

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