

Research Paper



Histological and Histomorphometric Evaluation of β -Tricalcium Phosphate-treated Mandibular and Femoral Defects in a Canine Model

Saeed Hesarakhi¹ , Davoud Sharifi² , Javad Ashrafi-Helan³ , Seyed Alireza Parhiz⁴ , Ehsan Fallah⁵ , Seyed Shahab Ghazi Mirsaeid⁶ 
Pooria Rooyan⁵ , Mohammad Hossein Shahrezaee^{7,8*} , Mostafa Shahrezaee^{6*} 

1. Department of Nanotechnology and Advanced Materials, Materials and Energy Research Center, Karaj, Iran.
2. Department of Clinical Sciences, Surgery and Radiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
3. Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.
4. Department of Oral and Maxillofacial Surgery, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran.
5. Department of Orthopedic Surgery, School of Medicine, AJA University of Medical Sciences, Tehran, Iran.
6. Department of Neurosurgery, School of Medicine, AJA University of Medical Sciences, Tehran, Iran.
7. School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran.
8. Department of Oral and Maxillofacial Surgery, TeMS.C. Islamic Azad University, Tehran, Iran.



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ABSTRACT

Background: Beta-tricalcium phosphate (β -TCP) is one of the most extensively studied synthetic bone substitutes because of its favorable biocompatibility, biodegradability, and osteoconductive properties. Despite widespread clinical application, the influence of anatomical site on the regenerative performance of β -TCP remains incompletely understood. This study aimed to evaluate histological bone regeneration and tissue response following implantation of β -TCP granules in experimentally created canine skeletal defects.

Methods: This experimental study included 10 adult dogs, divided into two groups of 5, one with standardized bone defects surgically created in the mandible (group 1), and one with defects surgically created in the proximal femur (group 2). Defects in each group were divided into subgroups of treatment (β -TCP treated) and control (untreated defects). Following a four-month healing period, specimens were harvested and subjected to histological and histomorphometric evaluation. New bone formation, graft integration, osteoblastic activity, inflammatory response, and tissue maturation were assessed.

Results: Substantial new bone formation was observed in β -TCP-treated defects compared with untreated controls. Histological study demonstrated extensive trabecular bone formation, active osteogenesis, and progressive replacement of implanted granules by newly formed bone. No evidence of infection, foreign-body reaction, granulomatous inflammation, or tissue necrosis was identified. Differences between the two groups were observed. Femoral bone defects exhibited more advanced maturation characterized by increased lamellar bone formation, whereas mandibular defects predominantly contained woven bone at the end of the 4-month period.

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* Corresponding Authors:

Mohammad Hossein Shahrezaee, DDS.

Address: Department of Oral and Maxillofacial Surgery, TeMS.C. Islamic Azad University, Tehran, Iran.

Phone: +98 (902) 5353974 E-mail: mohoshahrezaee@gmail.com

* Corresponding Authors:

Mostafa Shahrezaee, Professor.

Address: Department of Neurosurgery, School of Medicine, AJA University of Medical Sciences, Tehran, Iran.

Phone: +98 (912) 5353974 E-mail: Moshahrezaee@gmail.com



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Conclusion: β -TCP has excellent biocompatibility and effectively supports bone regeneration in canine skeletal defects. The regenerative response differs between the mandibular and femoral defect models, suggesting that local biological, biomechanical, and defect-model characteristics may contribute to the quality and maturation of regenerated bone.

Keywords: Beta-tricalcium phosphate (β -TCP), Bone regeneration, Osteoconduction, Histology, Canine model, Mandible, Long bone, Bone substitute

Highlights

- β -TCP increased mean new bone formation in both mandibular and femoral defect models compared to untreated controls
- Femoral defects showed more advanced structural maturation, including a greater lamellar-bone component, whereas mandibular defects retained more woven bone and osteoid.
- β -TCP showed favorable local biocompatibility, with no histological evidence of infection, granulomatous inflammation, foreign-body giant-cell reaction, or tissue necrosis.

Plain Language Summary

Beta-tricalcium phosphate (β -TCP) is a synthetic bone-graft material used to provide a temporary scaffold for new bone formation. This study examined how the β -TCP granules performed in two different skeletal defect models (mandibular and proximal femoral) in 10 adult dogs. In each animal, the right-sided defect was filled with β -TCP while the corresponding left-sided defect was left empty as a control. After 16 weeks, the tissues were assessed histologically and by histomorphometry. The treated sites showed greater mean new bone formation than their untreated sites in both defect models. Femoral bone regeneration also showed more advanced lamellar maturation, whereas mandibular sites contained more immature woven bone and osteoid. No major adverse local tissue reaction was identified. These findings support β -TCP as a biocompatible osteoconductive scaffold and indicate that regenerative performance can vary substantially by anatomical site and defect model.

Introduction

Reconstructing bone defects remains one of the most challenging problems in orthopedic surgery, maxillofacial surgery, and regenerative medicine. Bone loss resulting from trauma, tumor resection, infection, congenital anomalies, and revision surgery frequently requires grafting procedures to restore structural integrity and biological function [1]. Autogenous bone grafting remains the gold standard for skeletal reconstruction because of its osteogenic, osteoconductive, and osteoinductive properties. However, donor-site morbidity, limited graft availability, increased operative time, and postoperative complications have stimulated extensive research into alternative bone substitute materials [2]. Among currently available synthetic graft materials, calcium phosphate cements (CPCs) have attracted considerable attention because of

their chemical similarity to the mineral phase of native bone. Beta-tricalcium phosphate (β -TCP) is a biodegradable CPC characterized by favorable biocompatibility, interconnected porosity, and progressive resorption during bone remodeling. These characteristics allow β -TCP to function as an osteoconductive scaffold that supports cellular migration, vascular ingrowth, and new bone formation [3].

Previous experimental and clinical studies have demonstrated the successful use of β -TCP to treat bone defects in orthopedic, spinal, craniofacial, and dental applications [4, 5]. Nevertheless, regenerative outcomes vary substantially across anatomical locations. Differences in vascular supply, embryological origin, biomechanical loading, and local remodeling dynamics may influence the biological performance of implanted biomaterials [6]. The canine model offers a valuable translational platform for evaluating skeletal biomaterials because

its bone remodeling characteristics and healing patterns are similar to those in humans. Furthermore, canine long bones and mandibular bone provide an opportunity to investigate site-dependent variations in bone regeneration within the same biological system [7-9]. Recent preclinical studies have further demonstrated bone-regenerative performance of macroporous CPCs and hydroxyapatite/beta-tricalcium phosphate scaffolds (HA/ β -TCP) scaffolds in animal defect models [10, 11]. This study aims to evaluate the histological response to β -TCP implantation in experimentally created canine skeletal defects and compare patterns of bone regeneration between mandibular and long-bone environments. We hypothesized that β -TCP would support effective bone regeneration across all defect sites while showing differences in bone maturation by anatomical location.

Materials and Methods

Study design and β -TCP granule preparation

This is a prospective, controlled experimental study. A total of 10 healthy adult dogs were allocated into two experimental groups of 5: Group 1 with standardized osseous defects surgically created bilaterally in the body of the mandible; group 2 with standardized osseous defects surgically created bilaterally in the proximal femoral metaphysis. To eliminate systemic and inter-subject biological variations, an internal control model was implemented for each animal. At treatment sites, the defects created on the right side of the mandible or right proximal femur were packed and augmented with pure β -TCP granules. At control sites, the identical contralateral defects created on the left side were left unfilled/empty to serve as negative controls, allowing natural physiological healing to occur. All animals were maintained under standardized postoperative conditions for a predefined healing period of four months (16 weeks). Following this period, the animals were humanely euthanized, and en bloc bone specimens comprising the defect sites and adjacent host bone were harvested for comprehensive histological, histomorphometric, and qualitative tissue response analyses.

The porous β -TCP granules were synthesized via a solid-state reaction combined with the polymeric sponge replica technique [12]. High-purity analytical-grade calcium carbonate (CaCO_3) and calcium hydrogen phosphate (CaHPO_4) (Merck, Germany) were mixed in an equimolar ratio and subjected to thermal treatment at 1000 °C in an electric furnace to yield phase-pure β -TCP powder. A ceramic slurry was subsequently prepared and impregnated into open-cell polyurethane foam templates

to replicate an interconnected porous architecture. Following drying, burnout of the polymeric template, and high-temperature sintering, the resulting porous ceramic blocks were crushed and mechanically sieved using standard ASTM test sieves. Porous β -TCP granules with a uniform particle size distribution of 1-2 mm were collected, ultrasonically cleaned, dried, and sterilized prior to surgical implantation. The processing, sizing, and final packaging procedures were conducted in an ISO-7 cleanroom facility. Finally, the packaged β -TCP granules were sterilized via gamma irradiation at a standard dose of 25 kGy prior to surgical implantation.

Surgical procedure

Preoperative preparation and anesthesia

All surgical interventions were performed under strict aseptic conditions in a dedicated veterinary surgical suite. Before surgery, the animals underwent a 12-hour fasting regimen with free access to water. General anesthesia was induced via intravenous injection of ketamine hydrochloride (10 mg/kg) and diazepam (0.5 mg/kg) through a cephalic vein catheter. After endotracheal intubation, anesthesia was maintained with 1.5–2.0% isoflurane in 100% oxygen using a semi-closed rebreathing circuit. Continuous intraoperative monitoring included electrocardiography (ECG), pulse oximetry (SpO_2), end-tidal carbon dioxide (EtCO_2), and rectal temperature. Prophylactic antibiotic therapy (cefazolin sodium, 20 mg/kg, with intravenous injection [IV]) was administered 30 minutes prior to skin incision. The surgical regions (bilateral mandibular bodies or bilateral proximal hindlimbs) were clipped, shaved, scrubbed with 4% chlorhexidine gluconate, and draped with sterile surgical towels.

Mandibular extraction socket defect creation and implantation

The mandibular defect model was established using a standardized bilateral premolar tooth extraction approach. With the animal positioned in dorsal/lateral recumbency, the oral cavity was irrigated with a 0.12% chlorhexidine gluconate solution. Local infiltration anesthesia (lidocaine 2% with 1:100,000 epinephrine) was administered into the buccal and lingual vestibules adjacent to the target mandibular premolars (third mandibular premolar) bilaterally. A sulcular incision was made around the gingival margin (Figure 1), and an atraumatic, flapless/mucoperiosteal flap extraction was carried out. Multi-rooted teeth were hemisected buccolingually using a high-speed diamond fissure bur under copious



Figure 1. Macroscopic images of defect creation and biomaterial grafting in the two experimental groups

Note: Group 1: Premolar tooth extraction socket left empty as an untreated control (upper left), and grafted with β -TCP granules (upper right). Group 2: Surgically created circular cortical/cancellous bone defect left unfilled (lower left), and implanted with the β -TCP granules (lower right).

sterile saline cooling to preserve the cortical alveolar plates (especially the thin buccal plate). The roots were carefully elevated and extracted using fine veterinary dental elevators and extraction forceps. Following root removal, the extraction sockets were thoroughly curetted using a Lucas curette to debride any residual periodontal ligament fibers or granulation tissue, and irrigated copiously with sterile 0.9% physiological saline. Sockets were inspected to ensure the integrity of the buccal, lingual, and interradicular osseous walls. The fresh extraction sockets on the right side were filled and incrementally packed with sterile β -TCP granules up to the level of the crestal bone margin. Care was taken to maintain

granular integrity and inter-particle porosity without excessive compaction.

The identical contralateral extraction sockets on the left side received no graft material and served as untreated negative controls, allowed to heal spontaneously via blood clot formation. Primary, tension-free soft tissue closure was achieved over the extraction sites using periosteal releasing incisions where necessary. The mucoperiosteal edges were approximated and sutured using interrupted absorbable monofilament sutures (Vicryl suture 4-0). Animals in group 1 were maintained on a soft diet for the first two weeks post-surgery to minimize mechanical disturbance to the healing oral sites.

Femoral defect creation and implantation

With the animal positioned in lateral recumbency, each proximal femur was operated on in sequence: (i) Exposure: A craniolateral skin incision was made over the greater trochanter and proximal femoral diaphysis. The superficial gluteal muscle and fascia lata were incised, and the vastus lateralis muscle was elevated to expose the proximal lateral aspect of the femur below the trochanteric ridge, (ii) Defect preparation: A standardized cylindrical unicortical bone cavity (6 mm in diameter and 20 mm in depth) was drilled perpendicular to the longitudinal bone axis using sequential trephines/drills under constant chilled sterile saline irrigation (Figure 1). The right proximal cavity was packed with β -TCP granules, ensuring intimate contact between the biomaterial and the bleeding cancellous trabecular host bed. The left contralateral defect was thoroughly flushed and left vacant to fill spontaneously with a blood clot as an untreated control. Muscular and fascial envelopes were repositioned anatomically and sutured with Vicryl 2-0. Subcutaneous tissue was approximated with Vicryl 3-0, and the cutaneous layer was closed with interrupted nylon sutures.

Postoperative care and euthanasia

Analgesic coverage was maintained post-operation with tramadol hydrochloride (2–4 mg/kg, subcutaneous/intramuscular injection [SC/IM], twice daily) and meloxicam (0.2 mg/kg on day 1, followed by 0.1 mg/kg once daily, SC) for 5 consecutive days. Antibiotic therapy (amoxicillin/clavulanic acid, 20 mg/kg, orally) was continued for 7 days. Surgical wounds were inspected daily for signs of inflammation, wound dehiscence, seroma, hematoma, or infection. Cutaneous sutures were removed on postoperative day 14.

Animals were housed in individual kennels, provided with commercial standard canine diets (soft/wet food during the initial two weeks for group 1) and potable water *ad libitum*, and allowed unrestricted weight-bearing and movement. At the completion of the four-month (16-week) healing period, all animals were humanely euthanized via an intravenous overdose of sodium pentobarbital (100 mg/kg). The intact defect sites and surrounding host bone tissue were harvested *en bloc* and immediately fixed in 10% neutral buffered formalin (NBF) for histological preparation.

Clinical and macroscopic assessments

General *in vivo* clinical monitoring

Throughout the 4-month postoperative period, a veterinarian blinded to the study design monitored all animals daily. The veterinarian systematically recorded clinical parameters to assess overall physiological health, wound healing, and potential adverse reactions. General activity, demeanor, rectal body temperature, hydration status, appetite, body weight progression (measured weekly), and bowel/urinary functions were evaluated. For femoral defect animals, gait pattern, posture, weight-bearing capacity on the operated hindlimbs, and signs of lameness or joint stiffness were scored longitudinally using a semi-quantitative grading scale. In group 1 (mandibular defect), oral cavity and mucosal barrier above the extraction sockets were inspected for soft-tissue healing, mucosal color, swelling, surgical wound dehiscence, loss of primary closure, graft particle extrusion/exposure, mucosal ulceration, and signs of infection or suppuration. In group 2 (femoral defect), the surgical incisions were examined for erythema, localized edema, wound discharge (seroma/hematoma), induration, signs of surgical site infection (SSI), and palpated for local tenderness or soft-tissue fluctuance.

Macroscopic (gross) examination

At the end of the four-month healing period and immediately following euthanasia, a gross pathological examination of the retrieved anatomical sites was performed prior to tissue fixation. The defect areas (right treatment sides and left untreated control sides) in both the mandible and femur were exposed via careful dissection of overlying soft tissues. Defect margins and the extent of cortical bridging, surface continuity, contour regularity, and restoration of anatomical architecture were visually inspected. For the β -TCP-treated defects, the presence of visible residual biomaterial granules, integration of

granules into the newly formed osseous matrix, and the stability/anchorage of the graft mass were documented.

Tissues were thoroughly scrutinized for gross markers of chronic inflammation, fibrous encapsulation around the granules, soft-tissue invagination, granuloma formation, osteolysis, cortical expansion, or tissue necrosis. Standardized gross photographs of each site (with a calibrated metric ruler) were obtained under uniform studio illumination for documentation and semi-quantitative macroscopic scoring.

Histopathological and histomorphometric evaluations

After euthanasia, the retrieved *en bloc* bone specimens containing the defect sites and at least 5 mm of adjacent native host bone were immediately fixed in 10% NBF (pH=7.4) for 48 hours at room temperature. Specimens were thoroughly rinsed in running tap water and transferred to a decalcifying solution consisting of 10% ethylenediaminetetraacetic acid (EDTA, pH=7.2–7.4) at 4 °C. The endpoint of decalcification was confirmed radiographically and via the chemical oxalate precipitation test. The decalcification solution was refreshed every 48 hours over an interval of 4–6 weeks. Following complete decalcification, specimens were systematically dehydrated through an ascending graded series of ethanol (70%, 80%, 95%, and 100%), cleared in xylene, and embedded in paraffin wax. Serial longitudinal and coronal sections of 5 μ m thickness were cut along the central long axis of each defect using a precision rotary microtome (Leica Biosystems). To assess general tissue morphology, cellular architecture, inflammatory infiltration, and bone matrix organization.

Quantitative histomorphometric evaluation was performed in Stereo Investigator software (Version 9, MicroBrightField, USA). The newly formed trabecular bone volume fraction within the predefined defect region of interest (ROI) was estimated using a stereological point-counting method with an M42 test system, in accordance with the Cavalieri principle. The serial histological sections were prepared and stained according to the study protocol, and the ROI was consistently defined across all specimens prior to analysis. Sections were examined under light microscopy, and the M42 test system was superimposed on randomly selected microscopic fields within the ROI. The number of grid points intersecting trabecular bone and the total number of points falling on the entire bone section were recorded for each field. The trabecular bone volume fraction (BV/TV) was subsequently calculated as the ratio of the number of

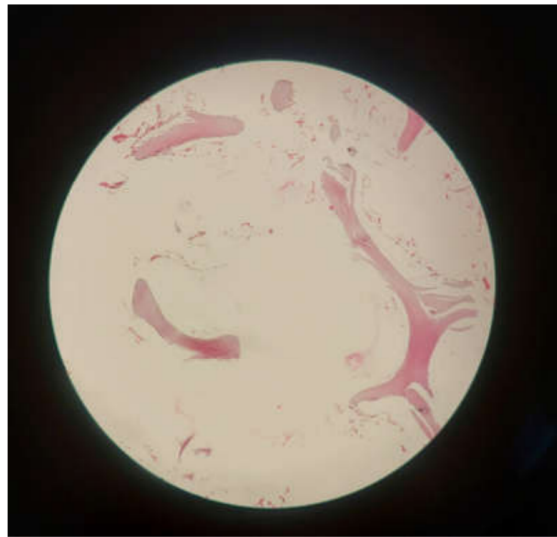


Figure 2. Image of the untreated mandibular extraction socket in a control site showing incomplete socket bridging and a minimal presence of immature woven bone trabeculae within the defect area (H&E staining, $\times 100$)

test-grid points within the trabeculae to the total number of test-grid points within the ROI, multiplied by 100. To minimize sampling bias, a minimum of five randomly selected, non-overlapping microscopic fields were analyzed per slide at a constant magnification. Fields with artifacts, tissue folds, tearing, or sectioning defects were excluded from the analysis. A single observer performed all histomorphometric assessments while blinded to the experimental groups to eliminate measurement bias, and the mean value for each specimen was used for subsequent statistical analysis.

Statistical analysis

Statistical analyses were conducted in SPSS software, version 26, R software version 4.3.1, and GraphPad Prism software version 9. Continuous variables were assessed for distributional characteristics and were reported as Mean $\pm$ SD). Because the two anatomical models comprised small independent groups and treatment was assigned consistently to the right side with the contralateral side serving as control, quantitative findings were presented and interpreted primarily descriptively in this

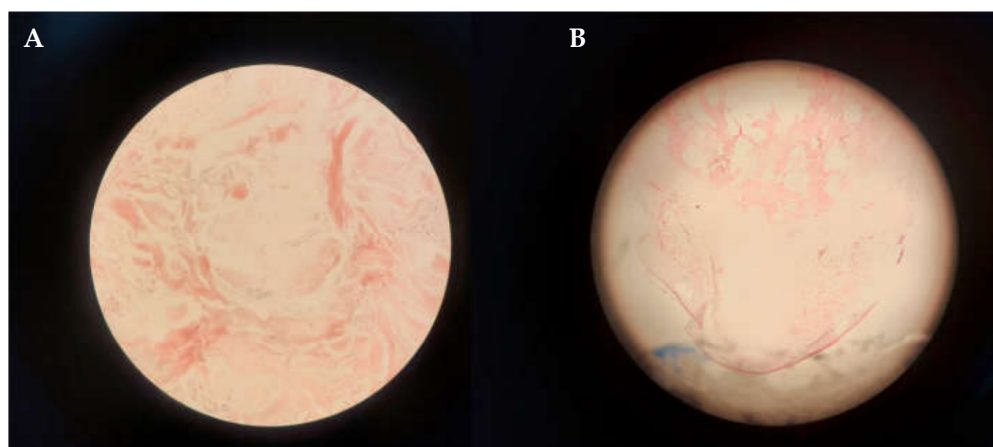


Figure 3. Image of the canine mandible showing the healing pattern of the treated site in group 1 (A: H&E staining, $\times 40$; B: H&E staining, $\times 100$)

Note: The defect is predominantly filled with abundant connective tissue, which has replaced the osteogenic material. No well-formed bony trabeculae are evident. In these areas, immature bone formation is markedly delayed, and the newly formed bone may remain immature and fail to progress to mature lamellar bone during the observation period.

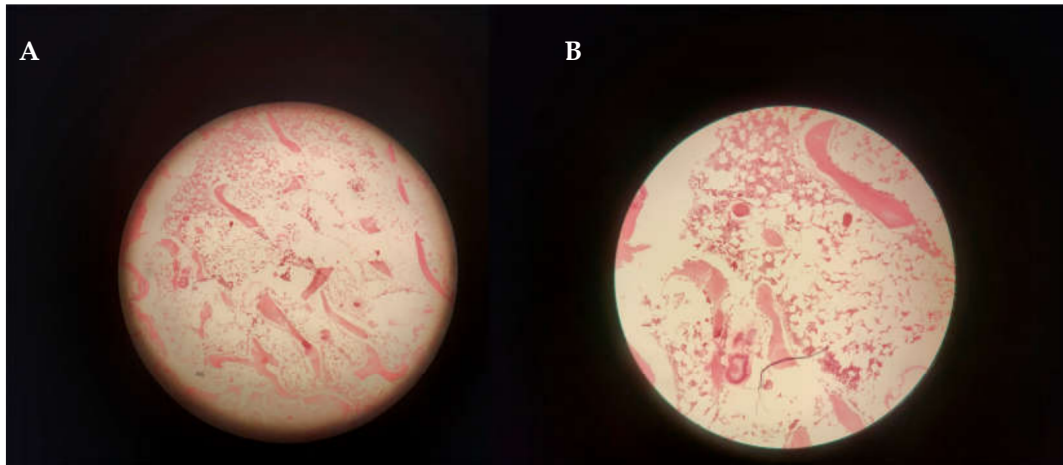


Figure 4. Histological image of the untreated control defect in the proximal femur (A: H&E staining, $\times 40$; B: H&E staining, $\times 100$)

Note: As evident, the surgically created cavity is incompletely bridged, showing only sparse, disorganized trabeculae of immature woven bone. Nevertheless, active osteoblasts and osteoprogenitor cells are recruited along the defect margins, attempting to repair the osseous cavity.

report. Within-group differences (treated versus controls) and between-model comparisons were evaluated in the context of the study design, without overinterpreting inferential significance. An independent observer evaluated each set of measurements in a blinded manner to minimize observational bias.

Results

General in vivo clinical findings

All 10 animals survived the surgical procedures and completed the entire 4-month postoperative period without systemic complications or casualties. Postoperative recovery was smooth across all animals, with vital signs, body temperature, and appetite returning to baseline levels within 24-48 hours after surgery. Weekly weight

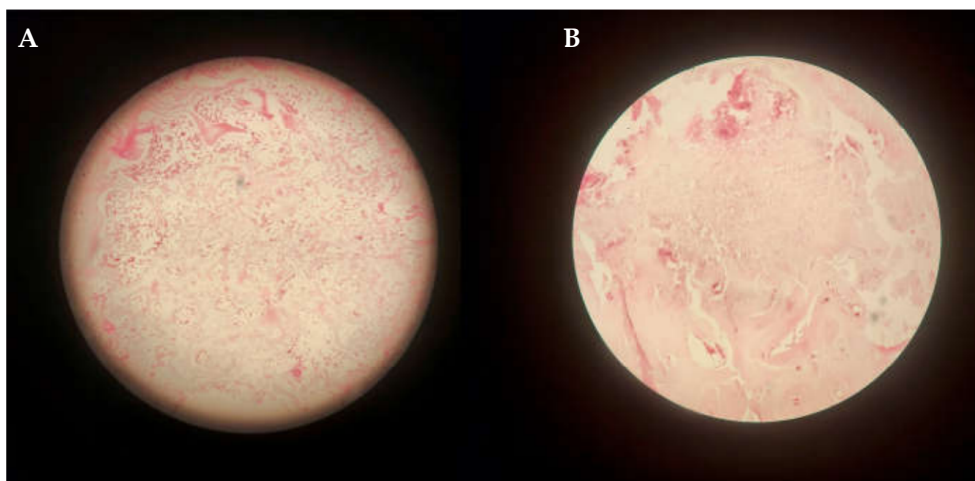


Figure 5. Histological image of the treated defect in the proximal femur (A: H&E staining, $\times 40$; B: H&E staining, $\times 100$)

Note: Complete defect bridging is observed, characterized by abundant immature woven bone trabeculae together with mature lamellar bone. These findings support the marked osteoconductive performance of the β -TCP scaffold.

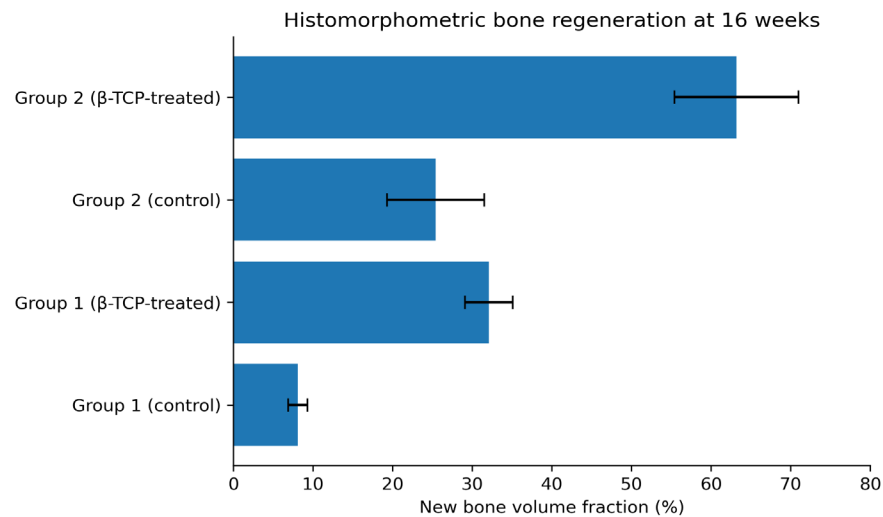


Figure 6. The BV/TV (%) in the study models over the 4-month post-surgery period

Note: Data are presented as Mean±SD.

measurements showed progressive, normal body weight gain throughout the study, indicating adequate nutritional intake and no systemic toxicity. In group 1 with mandibular defects, soft-tissue healing progressed unevenly; primary mucosal closure was maintained over both the β -TCP-grafted (right side) and control (left side) sites, with no evidence of wound dehiscence, graft particle exfoliation/exposure, persistent gingival erythema, mucosal ulceration, or chronic infection. In group 2 with proximal femoral defects, surgical skin incisions healed by primary intention without seroma, hematoma, or SSI. Animals in this group demonstrated normal limb function and full weight-bearing capacity on both hindlimbs within 7-10 days post-surgery, with no detectable lameness, gait asymmetry, or localized pain upon physical palpation of the operated sites at the time of euthanasia.

Macroscopic (gross) findings

Macroscopic inspection of the harvested en bloc bone specimens at 4 months post-surgery revealed distinct structural differences between the β -TCP-treated defects and the untreated controls across both anatomical sites. All surgical sites showed complete soft-tissue coverage without evidence of fibrous encapsulation, abscess formation, granulomatous reaction, or osteolysis. In both the mandibular and femoral models (groups), the β -TCP-treated defects were completely filled with dense, well-integrated tissue that exhibited seamless continuity with the adjacent native host bone. The implanted β -TCP granules were no longer individually distinguishable as loose particles; instead, they were macroscopically anchored and incorporated within a firm,

bone-like hard tissue matrix. In contrast, the untreated control defects (left side) showed incomplete spontaneous healing, with central concavities/depressions filled predominantly with soft fibrous tissue, particularly in the femoral defects. In comparing the two anatomical sites, the regenerated tissue in the femoral sites demonstrated superior macroscopic structural hardness and cortical alignment resembling the surrounding host bone architecture, whereas the mandibular sites exhibited smooth, fully restored alveolar contours with less prominent cortical density at the macroscopic surface.

Histopathological findings in group 1

Histological analysis of the control site (with left-side mandibular defects and without β -TCP) revealed a markedly different healing pattern compared to the treatment site. In the absence of a bone graft material, the extraction sockets showed significant dimensional changes and incomplete osseous regeneration (Figure 2). Although the surface was covered by a thin layer of stratified squamous epithelium, the underlying defect volume was primarily occupied by loosely arranged fibrous connective tissue rather than mineralized bone. The core defect in the control site contained only sparse, thin, disorganized trabeculae of immature woven bone, largely confined to the periphery and the apical third of the socket. Centrally, the defect remained devoid of substantial bone formation, suggesting that natural healing without a scaffold was insufficient to bridge the gap within the 4-month period. Furthermore, the control site showed noticeable alveolar ridge collapse and crestal bone resorption, whereas the β -TCP-treated site (right

side of the mandible) showed better preservation of the alveolar contour. At the end of the 4-month period, the newly formed tissue in these control sites was predominantly non-mineralized. Without an osteoconductive scaffold, the healing appeared “hollow,” as the fibrovascular tissue failed to undergo the osteogenic transformation seen in the grafted sites. No significant inflammatory response was noted, indicating that the lack of healing resulted from insufficient structural support rather than biological rejection.

Histological examination of the mandibular defect sites (group 1) at four months post-surgery revealed significant tissue repair characterized by a distinct pattern of bone regeneration and soft-tissue coverage compared to the femoral defects. In the β -TCP-treated site (right mandible), dense, vascularized fibrocollagenous connective tissue and early primitive bone matrices (osteoid seams) extensively filled the defect cavities and had largely replaced the resorbed β -TCP granules (Figure 3).

Fully organized, mature lamellar bone trabeculae had not yet completely developed in the mandibular specimens by the end of the 4-month period. Instead, the newly regenerated osseous architecture in the mandible predominantly consisted of immature woven bone trabeculae embedded within active osteogenic connective tissue. The bone-forming activity was confirmed by the presence of active osteoblasts along the margins of the newly deposited osteoid matrix. This observation is consistent with established physiological patterns of mandibular wound healing in veterinary and human literature, where differences in remodeling patterns may result in the persistence of a substantial woven bone component rather than complete lamellar transformation.

Importantly, most specimens achieved complete defect filling and soft-tissue integrity. The crestal surface of the grafted socket defects was entirely bridged and sealed by a thick, mature, and well-stratified non-keratinized/keratinized squamous epithelium with underlying lamina propria, restoring the natural anatomical barrier of the oral mucosa. No soft-tissue dehiscence, inflammatory cell infiltration, epithelial invagination into the graft space, foreign-body reactions, or necrotic tissue were identified.

Histopathological findings in group 2

The second experimental group, with an empty osseous cavity surgically created in the proximal region of the femur without the placement of any bone grafting material, was used as a negative control to evaluate natu-

ral spontaneous bone healing in the absence of osteogenic stimulation. As shown in Figure 4, the control site was not completely filled at the end of the 4-month healing period, leaving a discernible residual void within the host bone. Only a sparse number of delicate, immature trabeculae of woven bone, characterized by randomly arranged collagenous matrix and entrapped osteocytes, were observed extending from the defect margins into the cavity. Nevertheless, abundant osteoblastic activity was evident near the defect site; numerous cuboidal, active osteoblasts lined the newly formed trabecular surfaces, accompanied by resident osteoprogenitor/stem cells in the surrounding perivascular connective tissue. This cellular response reflects an active attempt by the host reparative mechanism to populate and bridge the surgical cavity with newly synthesized bone matrix. However, despite this pronounced osteogenic effort at the defect periphery, the reparative process remained incomplete and largely failed to achieve full osseous consolidation within the observation period. Notably, no direct contact between host bone and the defect centroid was observed, and the central portion of the cavity was largely occupied by loose areolar connective tissue and bone marrow elements, with no signs of inflammatory infiltrate, foreign-body reaction, or necrotic debris.

At the treatment site (with right-side proximal femoral defects treated with β -TCP granules), osseous healing of the created cavity was successfully accomplished by the end of the 4-month period. As illustrated in Figure 5, the surgically created defect was completely filled with newly regenerated bone, and the original cavity outline was no longer discernible at the microscopic level. The regenerated tissue comprised a dense network of numerous trabeculae, encompassing a mixed population of immature woven bone and mature lamellar bone, with well-organized osteons and Haversian systems characteristic of mature bone architecture. The implanted β -TCP provided a 3D osteoconductive framework that supported cellular ingrowth, bone apposition, and progressive bridging of the defect. Osteoprogenitor and osteoblastic cells were observed at the defect margins and along newly formed trabeculae, while the granular scaffold maintained its space and contact with the bleeding cancellous host bed. These findings are consistent with osteoconduction and progressive scaffold replacement by newly formed bone; they do not, by themselves, establish intrinsic osteoinductive activity of β -TCP.

Biocompatibility of the grafted material was corroborated by the high degree of integration; the majority of the implanted β -TCP granules was progressively resorbed and replaced by newly formed bone trabeculae,

with only minimal residual material embedded within the regenerated matrix. Active osteoblasts exhibiting prominent cuboidal morphology were consistently observed along the periphery of the newly formed bone trabeculae, confirming ongoing osteogenic activity at the time of tissue harvesting. Importantly, no histological evidence of purulent (pyogenic) infection, granulomatous inflammation, foreign-body giant cell reaction, or tissue necrosis was detected in any of the β -TCP-treated specimens, underscoring the excellent biocompatibility and safety profile of the tested biomaterial.

Bone histomorphometric findings

Quantitative histomorphometric analysis of newly formed bone trabeculae (BV/TV) at 4 months post-implantation corroborated the histological observations and demonstrated clear site- and defect-model-associated differences in osteogenic outcomes (Figure 6). Among all evaluated cohorts, the highest mean BV/TV was observed in the β -TCP-treated defects in group 2 ($63.2 \pm 7.8\%$), compared with untreated femoral defects ($25.4 \pm 6.1\%$). In Group 1, β -TCP implantation was associated with a higher mean BV/TV ($32.1 \pm 3\%$) compared to the untreated controls ($8.1 \pm 1.2\%$). The mean BV/TV in the β -TCP-treated proximal femur was approximately twofold that observed in the β -TCP-treated mandibular model over the 4-month period. Overall, these histomorphometric findings confirm that while β -TCP granules effectively promote osseous bridging and scaffold osteoconduction in both skeletal compartments relative to their respective untreated controls, the regenerative outcome at the end of the 4-month period differed substantially between the two anatomical/defect models, with a higher volume of mineralized trabecular bone in the femoral model.

Discussion

The present study evaluated the osteogenic performance and biocompatibility of β -TCP granules in reconstructing standardized osseous defects in two anatomically distinct skeletal/defect models, the proximal femur model (representing appendicular long bones) and the mandibular model (representing alveolar jawbone) in a canine model over a 4-month healing period. The histological and histomorphometric findings unequivocally showed that, while β -TCP served as an effective, biocompatible, osteoconductive scaffold that enhanced bone regeneration compared with untreated controls in both skeletal models, the structural maturity and quantitative volume of newly formed bone over 4 months differed between the two models.

Beta-TCP is widely recognized as a classic synthetic bone graft substitute due to its chemical similarity to the mineral phase of natural bone and its predictable degradation kinetics driven by both cell-mediated resorption and chemical dissolution. In our canine model, histopathological evaluation over the 4-month post-implantation period confirmed excellent local tissue tolerance for β -TCP across all grafted sites. The complete absence of pyogenic infection, foreign-body giant cell reactions, fibrous encapsulation, or necrotic zones supports favorable local biocompatibility of the biomaterial in this canine model. The particulate granular architecture of β -TCP provided an interconnected porous environment that can support vascular and cellular ingrowth and new-bone apposition. As the material progressively degraded, residual granules became integrated within newly formed trabecular bone. The active cuboidal osteoblasts lined newly deposited bone surfaces, consistent with ongoing bone apposition at the tissue-biomaterial interface. Recent *in vivo* studies have also indicated that macroporosity and calcium-phosphate scaffold composition can influence biomaterial resorption, host-bone replacement, and osseous regeneration [10, 13].

A key finding of this study was the difference in BV/TV between the proximal femoral and mandibular models. Quantitative histomorphometric analysis showed a higher mean BV/TV in β -TCP-treated femoral defects than in β -TCP-treated mandibular defects. This difference should be interpreted in the context of the distinct defect models and may reflect anatomical, embryological, biomechanical, and physiological factors inherent to the canine skeleton. The canine femur originates from the mesoderm and develops via endochondral ossification, characterized by a highly vascularized trabecular meshwork and a rich hematopoietic bone marrow niche. This environment hosts an abundant population of bone marrow-derived mesenchymal stem cells capable of rapid recruitment and robust osteogenic differentiation. In contrast, the canine mandible develops from the neural crest via intramembranous ossification. The dense cortical architecture of the jawbone, coupled with a different intrinsic stem cell population (ectomesenchymal origin), inherently exhibits different metabolic turnover and slower remodeling kinetics. The proximal femoral metaphysis in dogs benefits from a rich, highly anastomosed capillary network that can rapidly re-establish blood supply to the defect interior. Rapid vascularization is a prerequisite for scaffold-mediated bone repair, delivering oxygen, nutrients, and systemic osteoinductive signals. Conversely, alveolar extraction sockets in the canine mandible rely predominantly on endosteal blood supply from the surrounding alveolar walls, which may

contribute to a different angiogenic response within the central portion of the socket. Bone remodeling in dogs is strongly modulated by mechanical forces (Wolff's law). The proximal femur is subjected to continuous axial dynamic loading and strain during locomotion, which mechanically stimulates osteocytes and enhances local bone remodeling toward mature lamellar bone architecture. Conversely, while the canine mandible experiences intense masticatory forces, stress distribution within an isolated tooth extraction socket reflects a different remodeling environment, resulting in prolonged retention of immature woven bone trabeculae and intertrabecular fibrous tissue, as observed in the study group 1.

Regarding the clinical and histological implications of canine socket healing in group 1, despite the lower percentage of mature bone trabeculae in the mandibular defects compared to the femoral defects, β -TCP grafting in canine extraction sockets demonstrated greater mean BV/TV than untreated controls. Untreated control sockets in dogs exhibited considerable crestal bone resorption and collapse of the alveolar ridge contour, with the defect core filled primarily with non-mineralized fibrovascular tissue, a classic feature of unassisted socket healing in large animal models. In contrast, β -TCP placement preserved the 3D envelope of the alveolar ridge and provided a stable substrate for soft-tissue closure. Histological examination of the grafted sockets demonstrated complete mucosal re-epithelialization, characterized by a thick, well-stratified, mature squamous epithelium sealing the crestal surface. This barrier function is clinically vital in canine oral surgery, as it prevents epithelial down growth into the underlying bone defect and protects the regenerating matrix from bacterial contamination from the oral cavity. The presence of primitive osteoid seams and woven bone in the deep socket layers indicates ongoing remodeling, suggesting that additional remodeling beyond 4 months may be required for full lamellar conversion in canine alveolar ridge defects.

This study had some limitations. There were only five animals in each anatomical model, which limits statistical power and the precision of effect estimates. The mandibular and femoral experiments used different defect types and were performed in separate animal groups; therefore, differences observed between these models cannot be attributed to anatomical site alone. Moreover, the animals were assessed over a single 4-month period, precluding direct inference about healing kinetics over time. Finally, treatment was consistently assigned to the right side while the contralateral (left) side served as control site; consequently, side-allocation bias cannot be excluded. These considerations should be taken into ac-

count when interpreting the between-model comparison and planning future confirmatory studies.

Conclusion

Beta-TCP is an osteoconductive and highly biocompatible bone graft substitute that can enhance bone regeneration in both mandibular and femoral defects in dogs. However, regenerative capacity and tissue maturation rate are highly site-dependent, since femoral defects showed a higher new-bone volume fraction and more advanced structural maturation than mandibular defects at the end of the 4-month post-surgery period. These site- and defect model-based differences should be considered when translating biomaterial performance across different skeletal models and from preclinical to clinical models.

Ethical Considerations

Compliance with ethical guidelines

All experimental procedures were conducted in accordance with relevant institutional and national guidelines for animal research and were approved by the RESEARCH Ethics Committee of [AJA University of Medical Science](#) (Code: [IR.AJAUMS.REC.1405.161](#)).

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Authors' contributions

Conceptualization and writing the original draft: Saeed Hesaraki; Methodology, investigation, and supervision: Mostafa Shahrezaee; Project administration and animal surgery: Davoud Sharifi; Pathological evaluations: Javad Ashrafi-Helan; Formal analysis: Ehsan Fallah and Mohammad Hossein Shahrezaee; Review and editing: Mostafa Shahrezaee, Seyed Alireza Parhiz, Seyed Shahab Ghazi Mirsaeid, Ehsan Fallah and Mohammad Hossein Shahrezaee; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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