

β -Tricalcium Phosphate As An Osteoconductive Material to Preserve Subchondral Plate for Osteochondral Repair

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INTRODUCTION: The clinical need for articular cartilage repair is paramount for patients with joint disease. Current methods for cartilage repair: chondrocyte implantation, osteochondral transplant, periosteal transplant, microfracture, and osteochondral drilling, have not been consistently successful leaving resurfacing of articular joints after acute or chronic cartilage injury still a major challenge for orthopaedic surgeons. Besides the very limited intrinsic capacity for self-regeneration, poor integration of reparative tissues or cells with existing recipient cartilage and subchondral bone tissue has been regarded as a major reason for long-term failure. In addition, we have observed that resulting changes in the subchondral plate, such as reduced bone volume and disorganized collagen, following full-thickness cartilage injury may alter biomechanical properties of the subchondral bone and result in collapse and long-term failure of implants.

MATERIALS and METHODS:

Cell culture and manufacture of scaffold-free cartilage construct.

Porcine articular cartilage was shaved aseptically from patellofemoral grooves and femoral condyles of one-week old pigs, minced and chondrocytes were isolated by enzyme digestion and afterwards seeded on tissue culture plates (100mmx20mm) at a density of 15×10^6 cells/plate in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen Corp., Grand Island, NY) supplemented with 10% fetal bovine serum (Atlanta Biologicals, Norcross, GA), 2mM L-glutamine and 1% penicillin/streptomycin (Sigma Chemical Co., St. Louis, MO) at 37 °C with 5% CO₂ supplemented with L-ascorbate-2-phosphate (50 µg/ml, Sigma Chemical Co., St. Louis, MO) to promote extracellular matrix (ECM) production. After seven days of culture, chondrocytes with ECM produced a cellular sheet which was freed by brief incubation with 0.25% trypsin-EDTA (Invitrogen Corp., Grand Island, NY) and moderate shaking. The cell/ECM membranous layer was transferred into a 50 ml conical tube and centrifuged at 250g for 20 minutes. The disc-like chondrocyte/ECM pellet was incubated for 2 weeks in the same medium until transplantation.

Transplantation for integrative repair of cartilage defect.

β -tricalcium phosphate discs (β -TCP, 5mmx3mm) were manufactured with 80% porosity. The recipients were 6-month-old female pigs (n=8). This animal protocol was approved by UTHSC Institutional Animal Care and Utility Committee. The left knee joint was aseptically accessed through a lateral parapatellar incision. On the lateral and medial femoral condyles of the knee, four full-thickness cartilage defects were created with a 5-mm biopsy punch. For chondral repair without β -TCP, the defects were limited to the cartilage tissue with no damage to the subchondral bone. For osteochondral repair with β -TCP anchors, the subchondral bone was removed and the β -TCP discs were press-fitted into the subchondral bone before cartilage construct overlay.

The animals were sacrificed 1 month after osteochondral implantation. All specimens were first scanned by microCAT II (Siemens, Knoxville, TN) at a 20µm resolution. The surface and structural congruity, maturation of cartilage implants, remodeling of subchondral bone, and degradation of β -TCP were analyzed and reconstructed by Amira 4.1 software (Visage Imaging™, Carlsbad, CA). Histological examination was conducted to determine the cartilage reparation, as well as the subchondral bone volume surrounding the biphasic implants and to monitor the bioresorption/remodeling of β -TCP.

RESULTS: Figure 1 shows the biphasic cartilage-TCP implant and the residue of β -TCP, accompanied by the in-growth of trabecular structures one month after implantation. In Figure 2, the defect sites filled with cartilage constructs alone had visible implant depressions, and microCT images confirmed radiolucent areas underlying these cartilage defects (Fig 2A). Histopathology of this area showed reduced or absent trabeculae (Fig 2B). In contrast, defect sites implanted with biphasic cartilage- β -TCP constructs were almost completely filled with white cartilaginous tissue without significant depression. Although analyses by microCT showed partial biodegradation of the bioceramic material (Fig 2C), the samples with a β -TCP disc provided better structural support and reformation of subchondral bone underneath the cartilage construct, as evidenced by the fact that there was no additional disruption of subchondral bone trabeculae (Fig 2D).

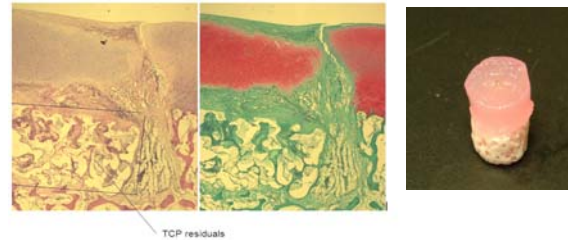


Fig 1. The microscopic and macroscopic images of biphasic cartilage- β -TCP. β -TCP was partially degraded after one-month *in vivo* while providing the support for cartilage resurfacing with the in-growth of bone trabeculae.

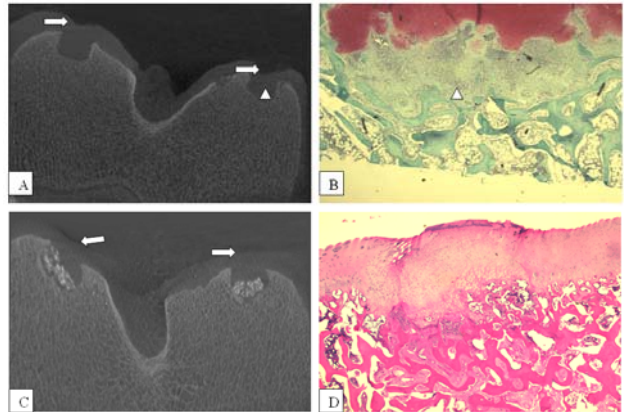


Fig 2. Cartilage repair in femoral condyle using cartilage implants only (A and B) and biphasic cartilage- β -TCP implants (C and D).

A: depression of implants (\blacktriangleright) and radiolucent area (osteolysis as shown by Δ); **B:** reduced subchondral trabeculae with connective tissue underlying implant. Safranin O-Fast green stain; **C:** cartilaginous resurfacing (\blacktriangleright); **D:** β -TCP samples at subchondral plate, indicating the maintenance of trabeculae. H.E. stain.

DISCUSSION: This study shows preliminary results for implantation of a biphasic β -TCP-cartilage construct for osteochondral repair in a porcine model. As an osteoconductive material, β -TCP can be used to provide transitional structural support for the implanted cartilaginous construct. Using cell-secreted ECM to form an allogeneic cartilaginous construct to repair cartilage defects demonstrated encouraging results with good biocompatibility. Biodegradable macroporous ceramic scaffolds have been used as engineered grafts for tissue engineering, particularly bone tissue engineering. The porous nature of these scaffolds permits the ingrowth of vascular and structural tissues and eventual replacement of the biodegradable TCP. It is critically important that a ceramic scaffold implant have a high compressive strength that is maintained as the implant is biodegraded and replaced by bone. In this study, porous β -TCP was used as an anchor to augment structural congruity and mechanically stabilize the allogeneic cartilage construct implant. As a convenient auxiliary method, the mechanical support and osteoconductivity provided by β -TCP at the subchondral plate level will contribute to stability of the engrafted tissues. The results showed better integration of this form of osteochondral implant for the short-term, which could improve long-term survival and results. With the development and optimization of the biphasic osteochondral allografts, an alternative approach for osteochondral implants and cartilage resurfacing will be feasible.

REFERENCE: Park K et al. *Artificial Organs*, 2006;30: 586-596.

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