A hyaluronate-atelocollagen/β-TCP-hydroxyapatite Biphasic Scaffold for the Repair of Osteochondral Defects: A Porcine Study

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INTRODUCTION:
The authors had devised a novel biphasic scaffold composed of hyaluronic acid and atelocollagen for the chondral phase and hydroxyapatite and β-TCP for the osseous phase, and previously reported the in-vivo results from a rabbit study. As a rabbit model gives insufficient evidence on the potency of a cell/scaffold system to promote the healing of cartilage, a large animal model is necessary to predict the capacity of the system to promote healing of osteochondral defects in humans. The purpose of this paper was to test the hypothesis that this biphasic scaffold, with or without seeded chondrocytes, promotes cartilage repair in a porcine model.

MATERIALS AND METHODS:
We used 16 skeletally mature female minipigs (23-25 months old), weighing 50.5 ± 0.75 kg (SD). Articular cartilage was removed from the corner of the patellar groove using a scalpel and sent for cell isolation and culture. The cultured chondrocytes (passage 3) were suspended in DMEM/F-12 solution at a concentration of 2x10^6 cells/50µl and injected inside the chondral phase of the biphasic scaffold.

The in-vitro-cultured chondrocyte/biphasic scaffold composites were implanted in the critical-size osteochondral defect created in the medial and lateral condyles of the distal femur of the both knees. We made 64 osteochondral defects (diameter: 6 mm, depth: 7 mm) on the medial and lateral condyles of the right distal femora of 16 minipigs using an OATS trephine. The defects were managed using one of the following methods: the defects were filled with an autologous chondrocyte/biphasic scaffold composite (Group I, 16 defects); only the biphasic scaffold was implanted (Group II, 16 defects); the removed osteochondral fragments were placed back into the defect (Group IIIa, 8 defects); an ACI procedure was done using the fascia from quadriceps muscle and 10^7 passage 3 chondrocytes suspended in phosphate buffered saline (Group IIIb, 8 defects); the defects were left empty (Group IV, 16 defects, negative control).

Five month after the implantation, the mini pigs were euthanized, and the defects were examined grossly and microscopically using ICRS score. For biomechanical characterization, the tensile stress–relaxation property of the regenerated cartilage and the nearby native cartilage was determined by an unconfined uni-axial indentation test using a Microload system custom-designed device (RnB Co, Seoul, Korea).

RESULT:
After 2 weeks of in-vitro culture, the chondral phase became firmer, suggesting the production of extracellular matrix (Fig.1).

Surface fissures were observed in some of the defects of Groups I, II, and IV (empty defects), but these were not found in Group III. The junction to the adjacent native cartilage was less detectable in the minipigs of Groups I and III than that of the Groups II and IV. The gross grading score was similar for Group I (9.0), Group II (9.1), and Group IIIa (9.1), followed by Group IIIb (7.4) and Group IV (6.2, the negative control). Groups I, II and IIIa had significantly greater scores than that of Group IV (p<0.05) (Fig.2).

The histological findings showed that the scaffold of the chondral phase was completely degraded, and replaced by host tissue in Groups I and II (Fig.3).

ICRS Visual Histological Score was highest in Group II (13.6) followed by Group IIIb (12.8), Group I (11.6), Group IIIa (11.4) and lastly Group IV (10.1). Group II had the best scores in cell population viability, cell distribution and matrix while Group IIIa had the best score in subchondral bone reconstitution (Fig.4).

DISCUSSION:
The key-point of the biphasic scaffold lies in providing subchondral support to the implanted chondrocytes and preventing collapse and depression during the healing of osteochondral defects. Our results suggest that this biphasic osteochondral scaffold composed of hyaluronate/atelocollagen chondral phase and HA/β-TCP osseous phase is effective for repairing osteochondral defects in a large animal model.

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