In vivo Gap Repair between Bone and Articular Cartilage with Co-Cultured Human Articular Chondrocyte and Human Osteoblast on PLGA Mesh Scaffold

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INTRODUCTION: The occurrence of a gap between subchondral bone and articular cartilage is fundamental in the progression of the osteonecrosis of the femoral head (ONFH). This is because continuous weight-bearing force hinders regeneration of the gap and leads to degeneration of articular cartilage. Thus, enhancing gap healing would slow the degenerative changes of the joint. Mesh-type PLGA (poly lactic-co-glycolic acid), a synthetic polymer, is a commercially available material, with excellent property as a cell carrier. We hypothesized that human chondrocytes and osteoblasts would maintain their cellular phenotype after co-cultured on PLGA mesh. Then, these cell-scaffold constructs can be used for gap repair in the necrotic femoral head. In this study, we evaluated the regeneration potential of these cell-scaffold constructs *in vivo*.

METHODS: Co-culture of chondrocyte and osteoblast & proliferation analysis: Human chondrocyte cells were harvested from the resected femoral head of ONFH patients during total hip arthroplasty with the approval of Institutional Review Board of authors' hospital. We used commercially available hFOB1.19 cell line for osteoblast. We performed co-culture technique with two cell lines. For proliferation analysis and phenotype expressions, we counted cell numbers and used RT-PCR with specific primers representing chondrocytes and osteoblasts: type II collagen, aggrecan, alkaline phosphatase (ALP), and osteocalcin (OC). **Producing a gap-mimic construct and *in vivo* experiment:** We produced a gap-mimic construct comprising three layers: cartilage, scaffold, and bone (Figure 1). We used mesh-type PLGA (Ethicon) with dimensions of 10x8 mm as a scaffold. Gap-mimic constructs comprised two groups: Acellular scaffold (n=10) and Co-cultured cells on scaffold (n=10). The constructs were implanted into subcutaneous pouches of nude mice and harvested after 4 and 8 weeks for gross and microscopic analysis. **Gross evaluation and microscopic analysis:** The vertically divided constructs were stained with hematoxylin and eosin (H&E) for histological analysis. To evaluate the gap healing, we assessed the degree of attachment and the presence of cell invasion at the interface. We determined the proportion of the length of contact between bone and cartilage within the construct, and categorized it into ranges of 0 to 25%, 25 to 50%, 50 to 75%, or 75 to 100%. Cell invasion was inspected at the interface.

RESULTS: Chondrocytes and osteoblasts successfully adhered and proliferated on the mesh-type PLGA. Proliferation assays conducted seven days post-seeding showed that the number of chondrocytes increased by approximately 4.3 times (4.3 x 104 ± 0.5 x 104 cells) and osteoblasts (1.2 x 104 ± 0.1 x 104 cells, 120% of seeded cells) by about 1.2 times (Figure 2). The RT-PCR affirmed that both chondrocytes and osteoblasts were proliferating while maintaining their respective phenotypes. The results of the gross and microscopic analysis of the harvested construct are as follows. In the case of the gap-mimic construct without cells, all constructs harvested at 4 weeks showed no attachment or cellular invasion. Some of the constructs harvested at eight weeks showed attachment (4 out of 6 constructs with 0-25%, and 2 with 75-100%), but no cellular invasion was observed. In the case of the gap-mimic construct with co-cultured cells, 2 case with 0-25%, 1 case with 50-75%, and 1 case with 75-100% attachment was observed at four weeks. Cellular invasion was observed in 1 out of 4 cases. At eight weeks, 1 case with 0-25%, 2 cases with 50-75% and 3 cases with 75-100% attachment were observed. Cellular invasion was detected in 4 cases out of 6 (Figure 3).

DISCUSSION: Human chondrocytes and osteoblasts can be co-cultured on PLGA mesh with their own phenotype. Cell-carrier complex showed healing potential between the bone and cartilage *in vivo*.

SIGNIFICANCE/CLINICAL RELEVANCE: Cell transfer technique can be a possible option for repair the gap between subchondral bone and articular cartilage in ONFH patients.

IMAGES AND TABLES:

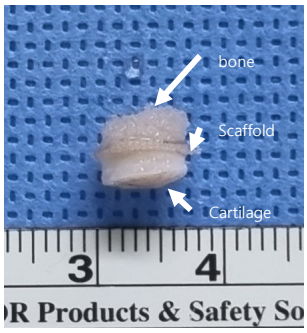

Fig 1. A gap-mimic construct

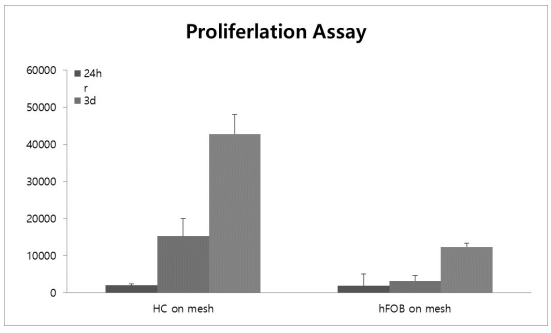

Fig 2. Numbers of cell count on PLGA after seeding

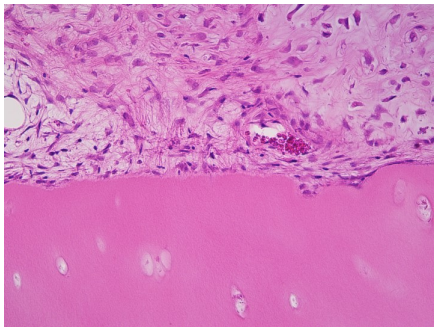

Fig 3. Interface with cellular invasion between bone and cartilage of the harvested construct