Osteochondral repair using a novel biphasic implant made of scaffold-free tissue engineered construct derived from synovial mesenchymal stem cells and hydroxyapatite-based artificial bone

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INTRODUCTION:
For ideal osteochondral repair, it is important to facilitate zonal restoration of subchondral bone and cartilage layer-by-layer [1]. Specifically, restoration of osteochondral junction and cartilage repair with secure integration with adjacent cartilage could be the key to determine the treatment outcome. We previously reported the feasibility of a novel fully interconnected hydroxyapatite (HA) artificial bone to repair subchondral bone [2]. Furthermore, we have developed a scaffold-free tissue engineered construct (TEC) derived from synovial mesenchymal stem cells (MSCs) to repair articular cartilage [3,4]. The hybridization of the TEC with HA could be potentially a biphasic implant to meet the requirements as described above. The purpose of this study was to investigate the feasibility of this hybrid material to repair osteochondral defect using a rabbit osteochondral defect model.

METHODS:
All animals (skeletally mature New Zealand White rabbits) were handled in accordance with a protocol approved by the institutional ethical committee. Cell expansion and development of the TEC: MSCs were isolated enzymatically from synovial membranes and the adherent cells were expanded until passage 4 to 7 according to our previous methods [3]. The cultured cells were plated on a culture dish at a density of 4.0×10⁴/cm² (9.6cm²) with 10% fetal bovine serum. After additional culture duration, a complex of the cultured cells and the extracellular matrix synthesized by the cells was detached from the substratum to develop a three dimensional form (TEC) by active tissue contraction. Implantation of the hybrid material to a osteochondral defect in vivo: TEC was detached from culture dishes just before implanted surgery, and bind to HA (NEOBONE®, 5 mm diameter, 4mm deep) without any glue to create the osteochondral hybrid. Under anesthesia, 5 mm diameter, 6mm deep osteochondral defect was created on the femoral groove of skeletally mature rabbits. The hybridization of the TEC and HA was done just before implantation and the biphasic implant was then implanted in the defect without suture for 17 right knees (12 specimens for histology, 5 for biomechanical test). In the control group, HA was implanted for 9 right knees (all specimens for histology). We also prepared 5 untreated normal knees as a control group for biomechanical testing. Histological evaluation and biomechanical test: Histology was stained with H&E and Toluidine Blue staining, and then histological scores were evaluated by modified ICRS score (cartilage part) [4,5] and O’Driscoll score (subchondral bone part) [6] at 2 and 6 months after surgery. In addition, micro-indentation testing was performed for untreated normal control and repaired tissues with the hybrid to measure stiffness at 6 months after surgery.

RESULTS:
Hybridization of the TEC with HA
The TEC was immediately attached on HA block to developed the complex with strength enough for surgical implantation. Histological assessment: The osteochondral defect treated with the hybrid material showed more advanced repair response of subchondral bone and cartilage with good tissue integration to the adjacent cartilage. Notably, repair of subchondral bone was completed at 2 months after surgery, while repaired cartilage was subject to further maturation hereafter. At 6 months, osteochondral repair was completed with osteochondral tissue, and the repaired tissue maintained good tissue integration with surrounding tissue (Fig.1). On the other hand, the defect treated with HA alone showed delayed repair of subchondral bone followed by incomplete osteochondral repair with poor integration to adjacent cartilage (Fig.1). Modified ICRS score at 6 months after surgery was significantly higher in hybrid group than that in HA group. Especially, the category “integration” was significant better in hybrid group at both 2 and 6 months after surgery (Fig.2). O’Driscoll score was significantly higher in hybrid group at 2 months after surgery (Fig.3). Mechanical property: The repaired osteochondral tissue treated with the hybrid material restored the stiffness equal to normal osteochondral tissue (Fig.4).

DISCUSSION:
We demonstrated that the hybrid implant significantly improved osteochondral repair histologically and biomechanically. Especially, the advanced repair of subchondral bone as well as secure good tissue integration to adjacent host tissue by 6 months after surgery could warrant longer-term durability and furthermore could be beneficial in the introduction of accelerated rehabilitation program at early stage post surgery. As scaffold-free, the TEC contained no animal or chemical derived materials, and furthermore HA has been widely clinically used. Therefore, our hybrid material could be feasible to repair osteochondral damages with effectiveness and safety.

SIGNIFICANCE:
The present biphasic implant was proved to be feasible to osteochondral repair layer-by-layer with good tissue integration to adjacent host tissue. As a clinical relevance, the hybrid of scaffold-free material and clinically used ceramics could be advantageous in terms of safety and cost effectiveness. Further, early restoration of subchondral bone could be beneficial for the introduction of accelerated rehabilitation.

REFERENCES:

Fig.1 H&E and Toluidine Blue (TB) staining of repaired tissues. Upper: 2 months after surgery. Lower: 6 months. Bar=1mm.

Fig.2 Modified ICRS score of repaired tissue. *; p<0.05

Fig.3 (Left) O’Driscoll score of repaired tissues. *; p<0.05

Fig.4 (Right) Mechanical properties of repaired tissues.