# Comparison of three different forms of commercially available hydroxyapatite artificial bone for a hybrid implant with a tissue-engineered construct derived from synovial mesenchymal stem cells for osteochondral repair

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INTRODUCTION: For an ideal osteochondral repair, it is important to restore subchondral bone and cartilage layer-by-layer (1). Specifically, restoration of the osteochondral junction, cartilage repair with secure integration to the adjacent cartilage, and initial stabilization of subchondral bone could be the key to determining treatment outcomes. We have developed a hybrid implant made of a hydroxyapatite (HA)-based artificial bone block coupled with a scaffold-free tissue engineered construct (TEC) derived from synovial mesenchymal stem cells (MSCs). We demonstrated that the hybrid implant significantly improved osteochondral repair in a rabbit osteochondral defect model, which exhibited the formation of hyaline-like cartilage, secure good tissue integration to adjacent host tissue, and advanced repair of subchondral bone (2)(3). For less invasive surgery like arthroscopic transplantation, we prepared a granular HA and paste-like HA for the hybrid implant, as an alternative to a conventional HA block. We hypothesized that a hybrid implant of HA granules or paste coupled with synovial MSC derived TEC would show a equivalent osteochondral repair, compared with a conventional HA block-based hybrid implant, and tested this hypothesis 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. <u>Cell expansion and development of the TEC:</u> MSCs were isolated enzymatically from rabbit synovial membranes and the adherent cells were expanded until passage 3 to 5 according to our previous methods (4). The cultured cells were plated on a culture dish at a density of $4.0x10^5/cm^2$ ($9.6cm^2$) with 0.2mM ascorbic acid 2-phosphate. After additional culture duration, a complex of the cultured cells and the extracellular matrix synthesized by the cells was detached from the culture dish to develop a three-dimensional form (TEC) by active tissue contraction. <u>Implantation of the hybrid implant to an osteochondral defect in vivo:</u> Under anesthesia, 5mm diameter, 6mm deep osteochondral defect was created on the femoral groove of skeletally mature rabbits. Either HA artificial bone block (5mm diameter, 4mm deep) (NEOBONE®), HA granules (1-2 mm per piece) (NEOBONE®), or HA paste (BIOPEX®) was implanted to the lower part of the defect. The latter two types of HA filled the defect to 4mm deep from the bottom. The HA paste was hardened 24 hours after implantation. The TEC was then implanted to the upper part of the defect immediately after the implantation of each type of HA. <u>Histological evaluation:</u> Histology was stained with H&E and Safranin O staining, and then histological scores were evaluated by modified O'Driscoll score (2) at 1, 2 and 6 months after surgery (N=5 per group, each time point). <u>Biomechanical evaluation:</u> Compression testing was performed for the evaluation of repair tissue at 6 months after surgery (N=5 per group). Knees from normal rabbits were used as a control group for biomechanical testing (N= 5).

RESULTS: The osteochondral defect treated with TEC/HA block showed gradual maturation with time, and the osteochondral repair was completed at 6 months (Figure 1, 2A, 2B). The osteochondral defect treated with TEC/HA granules showed the progression of osteochondral repair until 2 months, but they showed degeneration of the repair at 6 months (Figure 1, 2A, 2B). The osteochondral defect treated with TEC/HA paste showed the progression of subchondral bone repair until 6 months, but the cartilage repair was insufficient (Figure 1, 2A, 2B). The repair tissue mediated by TEC/HA block and TEC/HA paste exhibited equivalent biomechanical properties to normal osteochondral tissue at 6 months (Figure 3). Additionally, the biomechanical property of TEC/HA granules was significantly lower than that of normal osteochondral tissue (Figure 3).

DISCUSSION: Contrary to our hypothesis, the TEC/HA block hybrid implant exhibited better histological, biomechanical findings in osteochondral repair than other groups. Less invasive surgery with arthroscopic transplantation of the hybrid implant could reduce the burden on patients, and thus further improvement is necessary.

SIGNIFICANCE: The hybrid implant of stem cell-based tissue engineered construct and hydroxyapatite-based artificial bone could be promising for osteochondral repair, but further improvement should be necessary for less invasive surgery with arthroscopic transplantation.

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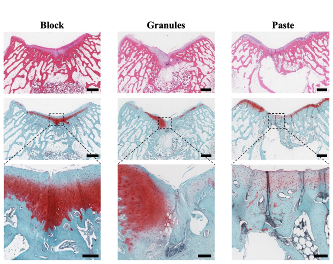


Figure 1.

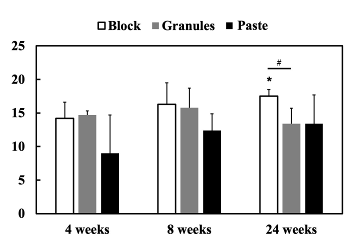


Figure 2A.

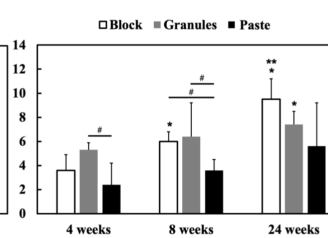


Figure 2B.

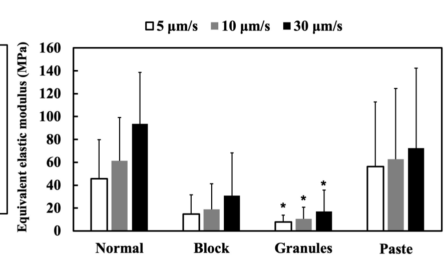


Figure 3.

Figure 1. H&E staining and Safranin O staining of repair tissues treated with the hybrid implant at 6 months.
Bar = 1 mm (Upper, middle), Bar = 200 um (Lower)
Figure 2. (A) Histological score for cartilage repair. (B) Histological score for subchondral bone repair.
*; $p < 0.05$ (vs 4 weeks), **; $p < 0.05$ (vs 8 weeks), #; $p < 0.05$, Kruskal-Wallis test
Figure 3. Compression testing for repair tissues treated with the hybrid implant at 6 months.
*; $p<0.05$ (vs normal), Kruskal-Wallis test