**Introduction:**
Articular cartilage has anti-adhesive properties and therefore, integration of implanted tissue to adjacent cartilage matrix has been a concern in the treatment of chondral injury. We have developed a scaffold-free three-dimensional synthetic tissue (3DST) derived from the synovium for potential application to cartilage tissue engineering. The 3DST is composed from mesenchymal stem cell (MSC)-like cells and the extracellular matrix synthesized by the cells. The 3DST is rich in adhesion molecules and adhesive property to the cartilage matrix in vitro. Moreover, the 3DST has efficient chondrogenic differentiation capacity [1]. We therefore moved to in vivo study and demonstrated that the 3DST securely repaired the incurable chondral lesion which did not bleach the subchondral bone, with good integration to the adjacent cartilage tissue, in a porcine model [2]. The purpose of this study was to quantitatively assess the quality of the repaired chondral lesions from morphological and biomechanical aspects.

**Materials and Methods:**
All animals were handled in accordance with a protocol approved by the institutional ethical committee.

*Harvest of synovial tissue and isolation of the cells:* Porcine synovial membranes were obtained from the porcine knee joints. Cells were isolated enzymatically and the adherent cells were expanded until passage 4 to 7. We previously reported that the cells exhibited the MSC-like phenotype [1].

*Development of the 3DST:* The cultured cells were plated on a culture dish at a density of 8.0x10^5/cm^2 with 0.2mM ascorbic acid 2-phosphate and cultured in monolayer. After additional culture duration, the monolayer culture was detached from the substratum by the application of shear stress at the cell-substratum interface. The detached monolayer complex was left in suspension and allowed to actively contract to develop a three dimensional form.

*Implantation of the 3DST to a partial thickness chondral defect in vivo:* Under anesthesia, the medial femoral condyle of four-month-old pig was exposed after medial parapatella incision. An 8.5 mm diameter, 1.5mm deep chondral defect which did not bleach the subchondral bone was created. The 3DST was then implanted in the defect of 8 limbs without suture. In the control groups, the defect was left empty (4 limbs). All animals were sacrificed under anesthesia at 6 months after implantation surgery. Repaired tissues were subject to morphological and mechanical compression test. The macroscopic findings in accordance with the following criteria; the score 2 was complete resurface (>90% coverage), score 1 was partial resurface (50-90% coverage), and 0 was none resurface (<50% coverage). Modified ICRS Histological Scoring of the repaired tissue: Histology from paraffin sections was evaluated using modified ICRS histological score. The defect area on the section was divided into 4 parts with 2mm width. Each area was evaluated by ICRS scoring [3]. Moreover, we created the new criterion of “Integration”. Good integration was score 3 and no integration was score 0. This integration criterion was applied to the both margins within the histology. Mechanical compression test of the repaired tissue treated with 3DST: It is accepted that biphasic viscoelastic response of articular cartilage could describe the strain-rate dependent mechanical behaviors of articular cartilage [4]. It means that the viscoelasticity of cartilage which retains interstitial water might be mainly reflected by static compression test at slow compression rate, while the matrix viscoelasticity without water could be mainly reflected at fast compression rate. A cylindrically shaped osteochondral specimen of 4 mm in diameter and 5-8 mm in depth was extracted from the divided part of repaired tissue and subjected to a quasi-static compression test at both slow (4µm/s) and fast (100 µm/s) compression rate to 70 kPa, using a compression test apparatus developed in our laboratory.

**Results:**

**Histological assessment of the repaired tissue (Fig.1):** Four of 8 limbs treated with the 3DST were completely covered with repaired tissue (A), while the rest were partially covered (B). Without the 3DST, the chondral lesions in 3 of 4 limbs had no tissue coverage and the lesion in one limb was partially covered with repaired tissue (C). The histological grading of 3DST-treated group was significantly higher than that of the control group (1.6 vs. 0.25) (D).

**Discussion:**
Without the implantation of the 3DST, chondral defect was scarcely healed and most of the lesion gave rise to osteoarthritic change. Conversely, 3DST implantation resulted in secure repair of the lesion with chondrogenic tissue. Modified ICRS scoring in this porcine model revealed that the repaired tissue with the 3DST was histologically superior to the control lesion in all aspects. Furthermore, biomechanical analysis revealed that the repaired tissue with the 3DST exhibited similar viscoelastic properties to normal articular cartilage. Finally, due to the theoretical exclusion of extrinsic (either biological or chemical) materials, cartilage repair with 3DST could have an advantage regarding safety issues after implantation, as compared with conventional scaffold-based cell therapies.

**References:**