CYCLIC COMPRESSIVE LOAD STIMULATE CATABOLISM OF COLLAGEN-BASED ENGINEERED-TISSUE CONTAINING HUMAN SYNOVIOUM-DERIVED STEM CELLS

+ Department of Orthopaedic Surgery, Graduate School of Medicine Osaka University
ken-nakata@umin.ac.jp

Introduction
Numerous bioactive factors, including mechanical stimuli, have been studied for regulation of matrix synthesis and degradation in cartilage explants of tissue-engineered cartilage containing chondrocytes, but few studies have examined the effects of mechanical stimuli on matrix remodeling of constructs containing mesenchymal progenitor cells. This study investigated the effects of cyclic compressive load on catabolic activity in collagen-based engineered tissue containing human synovium-derived stem cells.

Materials and Methods
Isolation of synovium-derived stem cells from human knee synovium.
Surgical specimens of human synovial membranes were obtained during arthroscopic knee surgeries. Patients gave informed consent for the use of surgical specimens for experiments under approval from Ethics Committee of the Medical Faculty. The excised synovia was cut into small pieces, washed in PBS, and digested with 0.2 % collagenase in DMEM. The liberated cells were then cultured in a monolayer at 37oC and 5% CO2.

Scaffold preparation (Porous Collagen Sponge)
Type 1 collagen extracted from bovine skin was processed for removal of telopeptide to produce Atelocollagen® gel (KOKEN, Tokyo, Japan). After Atelocollagen® gel was freeze-dried it was cross-linked and sterilized with formaldehyde to give a porous collagen sponge with adequate mechanical strength.[1] The collagen sponge was cut into a 9-mm i.d. 4-mm disk (Fig.1).

Cell seeding of scaffold and 3D culture of cell-scaffold construct
After 6 passages, cultured cells (1×10⁷ / scaffold) were suspended in 2xDMEM contained with 20% FBS and 2% antibiotics and were then mixed with 2% atelocollagen implant®. The final cell-suspension collagen solution mixture was then incorporated into a 3-D collagen scaffold by centrifugal force and made into gel at 37°C.

Cyclic loading system and loading protocols
Cyclic loading was applied to 3D constructs using a custom-made apparatus, cyclic loading stimulator (CLS). CLS consists of cylindrical loading platens, a moving stage that holds the loading platens and a linear actuator that control the motion of the moving stage (Fig.2). Using the CLS, a maximum of 12 specimens can be simultaneously subjected to dynamic compressive stimulation with a constant peak load in an ordinary CO2 culture incubator. Cyclic compression was applied to 3D tissues at 10 or 30 kPa at a frequency of 0.5 Hz for 1 hour/day. Loaded constructs and unloaded control samples were simultaneously cultured.

Analysis
Following culture, DNA content, apoptotic cells and mRNA expression levels for MMP-1, MMP-2, MMP-3, TIMP-1, IL-6 and IL-8 were determined semi-quantitatively. Protein expression of MMP-1, MMP-3, MMP-2 enzyme activity, morphology and biomechanical properties of the construct were also examined.

Data from experimental groups were examined using one-way ANOVA, and LSD test was used for multiple comparisons between individual groups (p<0.05).

Results
After loading treatment, cells in 3-D culture were uniformly embedded in collagen gel and collagen sponge without leakage of cells due to deformation of the scaffold. There was no major mechanical breakage of the collagen sponge after cyclic compression in this study. (Fig.3). Histometric analysis using digitized images demonstrated that the amount of collagen sponge of the loaded groups was significantly lower than that in controls (p<0.05) (Fig.4).

Cyclic compressive load of 10 kPa up-regulated mRNA expression levels of MMP-1, MMP-3 and TIMP-1. (Fig 6)

The tangent moduli of loaded constructs were also significantly lower than those of controls. (p<0.05) (Fig 7)

Conclusion
Cyclic compressive load reduced the collagen scaffold matrix and softened collagen-based constructs containing human synovium-derived cells by modulating the expression of MMP-1, MMP-3, TIMP-1 mRNA and MMP-2 activity. Thus, mechanical stress enhances tissue remodeling by activating catabolism of cultured tissue in the early phases of in vitro culture.

References
** Second Department of Oral and Maxillofacial Surgery, Osaka Dental University
*** Graduate School of Engineering, Kogakuin University

53rd Annual Meeting of the Orthopaedic Research Society
Poster No: 0586