INTRODUCTION

Biomechanical stimuli have fundamental roles in the maintenance and remodeling of ligament tissues. We have reported the relationships between stress-induced collagen expressions and integrin-mediated cellular behaviors in anterior cruciate ligament (ACL)-derived cells cultured on two-dimensional chambers. Several studies have reported that three-dimensional collagen scaffolds stimulate cellular activities using in-vitro culture models. However, collagen scaffolds purified from animals have several problems for a clinical use.

To achieve a new technique for regeneration therapy after the ACL injury, we investigated the effects of uni-axial mechanical stretch on human ACL-derived cells cultured in a chemically synthesized self-assembling peptide gel.

MATERIALS AND METHODS

Cells and cell culture: Human ACL fibroblasts were isolated from intact ACL samples obtained at total knee arthroplasties in patients suffering osteoarthritis (mean age, 70 years). Surrounding synovial tissues were carefully removed from ACL samples. The remaining samples were separately cut into 5-mm pieces, and maintained in Dulbecco’s modified Eagle’s medium (DMEM; Wako, Osaka, Japan) containing fetal bovine serum (HyClone, South Logan, UT, USA) and penicillin/streptomycin (Sigma, St Louis, MO, USA). After 2 weeks of organ culture, the migrated out-growth cells were subcultured.

Self-assembling peptide gels: The self-assembling peptide, CH(CONH)-RLDLRLALRLDLRL-[CONH], was synthesized by a solid-phase method using standard Fmoc strategy. The peptide powder was dissolved in 10% sucrose solution. Then, the solution was sterilized by filtration through a 0.22 µm filter. The pH of the solution was adjusted to approximately pH = 6.5 by adding aliquots of 0.5% sodium hydrogen carbonate solution, which was also sterilized by the filtration. The final concentration of the peptide was 2.4 mM (0.4 w%).

Three-dimensional (3-D) gels and stretching experiments: Human ACL fibroblasts suspended in serum-free DMEM were mixed with the peptide gel. The final concentration of cells and peptide was 7 x 10^6 cells/mL and 1.6 mM, respectively. Then, the cells were incubated on a PDMS bridge of the chamber for 1 week before stretching experiments. Uni-axial cyclic tensile strain (CTS: 0.5 Hz, 10% strain) was applied to the peptide gel placed on a PDMS bridge using a STB-140 devise (STREX, Osaka, Japan) for 4 days (4 hours/day).

RT-PCR and quantitative real-time PCR analyses: The expression of COL1A1 and glyceraldehyde-3-phosphate dehydrogenase (G3PDH) was assessed in 3-D-cultured ACL cells. Relative COL1A1 expression levels were normalized with each G3PDH level.

Immunohistochemistry: 3-D gels were fixed with 4% paraformaldehyde/PBS. Frozen sections (5-µm thickness) were prepared, and stained with DAPI and an anti-type I collagen antibody. The amount of type I collagen secreted from 3-D-cultured ACL cells was quantified using Image J software (National Institute of Health) as described.

RESULTS

RT-PCR analyses revealed that mechanical stretch (16-h-CTS) stimulated COL1A1 expression in ACL-derived cells cultured in a self-assembling peptide gel (Fig. 2A). Relative COL1A1 expression was increased up to a 6.7-fold level of non-stretched cells by mechanical stretch (Fig. 2B).

Immunohistochemical analyses revealed that mechanical stretch increased type I collagen synthesis in 3D-cultured ACL cells (Fig. 3E, F). Relative amount of type I collagen secreted from ACL-derived cells was increased up to a 4.4-fold level of non-stretched cells by 16-h-CTS (Fig. 4).

DISCUSSION

This newly developed self-assembling peptide gel, which does not contain type I collagen, enabled us to evaluate the amount of type I collagen production in 3-D-cultured ACL cells. The present study demonstrated that mechanical stretch increased type I collagen synthesis in 3-D-cultured ACL cells. Our results suggest that this self-assembling peptide gel may have an advantage in creating a tissue-engineered ACL.

SIGNIFICANCE:

Our results may contribute to achieve a new regeneration therapy against the ACL injury.

REFERENCES


Fig. 1. (A) Self assembling peptide gel (2.4 mM). (B) Cells with peptide gel (7 x 10^6 cells/mL, 1.6 mM). (C) 3-D gel stretch chamber. A PDMS bridge was attached on the chamber (red arrow).

Fig. 2. (A) COL1A1 and G3PDH mRNA expression after mechanical stretch for 16 hours. (B) COL1A1 expression levels were normalized with G3PDH level.

Fig. 3. (A, D) DAPI (blue). (B, E) Type I collagen (green). (C, F) Merged images.

Fig. 4. Relative COL1A1 levels were normalized with G3PDH level.

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