INTRODUCTION:
Ligaments and tendons are well-organized fibrous connective tissues. They are mainly composed of parallel aligned collagen fibers with fibroblasts. In case of the damage or rupture in the tissues, it is one of the strategies to use cell-based therapies for their repair. We have been developing a novel tissue-engineering technique for the repair of ligaments and tendons which involves stem cell-based self-assembled tissues (scSAT) derived from synovium. As the scSAT is composed of cells with their native extracellular matrix, it is free from concern regarding long-term immunological effects. For biological reconstruction of the microstructure of the ligaments and tendons, it is required for the scSAT to have anisotropic properties. Our previous study indicated that, when the scSAT was cultured on a glass plate having microgroove structure, cells and collagen matrix were aligned parallel to the direction of the microgroove. However, the effect of microgroove depth on the tensile properties of the cultured scSAT is unknown. Therefore, the effect was determined in the present study.

MATERIALS AND METHODS:
Development of scSAT
Micro pattern-processed glass plates having an array of parallel grooves (100 µm in width and 10 or 30 µm in depth) were developed through lithographic and isotropic wet etching. Mesenchymal stem cells were obtained from the synovial membranes of human knee joint by means of collagenase treatment. After subculture, the cells were plated on the 6 well-plates (control group) or micro pattern-processed glass plates (10 or 30 µm microgroove (MG) groups) at a density of 6.0 x 10^3 cells/cm² in DMEM supplemented with 10% FBS, penicillin streptomycin, and 0.2 mM ascorbic acid 2-phosphate. After 14 days, a monolayer complex of fibroblasts. In case of the damage or rupture in the tissues, it is one of the strategies to use cell-based therapies for their repair. We have been developing a novel tissue-engineering technique for the repair of ligaments and tendons which involves stem cell-based self-assembled tissues (scSAT) derived from synovium. As the scSAT is composed of cells with their native extracellular matrix, it is free from concern regarding long-term immunological effects. For biological reconstruction of the microstructure of the ligaments and tendons, it is required for the scSAT to have anisotropic properties. Our previous study indicated that, when the scSAT was cultured on a glass plate having microgroove structure, cells and collagen matrix were aligned parallel to the direction of the microgroove. However, the effect of microgroove depth on the tensile properties of the cultured scSAT is unknown. Therefore, the effect was determined in the present study.

Histological observation and tensile test of the scSAT
Histological observation was performed for the surface structure of the scSAT using a digital microscope. Structural anisotropy was evaluated through FiberOri8Single03 (computer software). The scSAT was, then, subjected to tensile testing at a rate of 0.05 mm/s in phosphate-buffered saline at 37 degrees Celsius using a custom-made micro tensile tester. After the scSAT cut as 6 mm in width was fixed to the tensile tester’s chuck. The scSAT was subjected to a tensile testing at a rate of 0.05 mm/s. The stress-strain relationship of the scSAT during the test was obtained.

RESULTS and DISCUSSION:
Histological observation indicates that no orientation was observed in the control group, while cells and tissues were oriented along the direction of the grooves in the MG groups (Fig. 1). The scSATs exhibited anisotropic structure with the orientation intensity of 1.36 ± 0.03 and 1.26 ± 0.09 and with the angle of -5 ± 4° and -4 ± 4° in the MG10 and MG30 groups, respectively. In contrast, the control scSAT exhibited roughly isotropic structure with the orientation intensity of 1.10 ± 0.06 and with the angle of 25 ± 49°. Note that the orientation angle of 0° means parallel to the microgroove direction. Thickness of the scSAT in the MG10 group (68 ± 7.0 µm) and the MG30 group (68 ± 6.7 µm) became significantly higher as compared with in the control group (53 ± 10 µm). As compared with the control and MG10 group, the tensile test revealed that the MG30 group indicated a sudden increase of stress around 0.1 of strain. The tangent modulus of the GM10 (0.34 ± 0.29 MPa) and MG30 (1.27 ± 1.03 MPa) groups were significantly higher than that of control group (0.08 ± 0.04 MPa). The strength was significantly higher in the MG groups as compared with the control group (0.11 ± 0.02 MPa). Moreover, the tensile strength of the MG30 group (0.34 ± 0.10 MPa) was significantly higher than MG10 group (0.15 ± 0.02 MPa) (Fig. 2).