INTRODUCTION:
Joint immobilization is a useful treatment modality in orthopedics, but it also causes unfavorable outcomes such as joint contracture, periarthritis osteoporosis, and osteoarthritis (OA). Type II collagen is a principal component of the extracellular matrix of the articular cartilage. Damage to the fibrillar meshwork of type II collagen is critical and irreversible event. Collagenases such as matrix metalloproteinase (MMP)-8 and 13 degrade type II collagen and are thought to develop OA. Though type I collagen is a minor component of normal articular cartilage, some researchers detected them in OA cartilage. The purpose of this study was to investigate the expression patterns of collagen types I and II, MMP-8 and MMP-13 in the articular cartilage after joint immobilization by multite.

METHODS:
Animals: The protocol for the experiments was approved by the Animal Research Committee of Tohoku University. Unilateral knee joints of adult male Sprague-Dawley rats (body weight 380-400g) were immobilized at 150° of flexion with a plastic plate and metal screws for various periods (3 days, 1, 2, 4, 8, and 16 weeks). Sham operated rats had only screws inserted [1].

Tissue preparation: After fixed with 4% paraformaldehyde and decalcified, specimens were embedded in paraffin. The paraffin embedded tissue was cut into 5-μm thick sagittal sections of the medial midcondylar region of the knee. We chose three areas (non-contact, transitional and contact areas) from the articular cartilage of the femur and tibia (Figure 1A).

In Situ Hybridization (ISH): Digoxigenin (DIG)-labeled single-strand RNA probes were prepared. Fragments encoding mouse pro-alpha 1 (II)-collagen [2] and rat MMP-8 [3] were obtained from the total RNA of embryonic rat limbs using RT-PCR and subcloned into the PCR II TOPO.

Immunohistochemistry (IHC): The sections were deparaffinized and endogenous immunoglobulins were blocked by BSA. The slides were incubated with a goat anti-rabbit antibodies (1:200, Santa Cruz Biotechnology) and rabbit anti-rat MMP-13 antibody (sc-30073, Santa Cruz Biotechnology). For collagen type II, the slides were incubated with Alexa Fluor 488 donkey anti-rabbit IgG (A21206, Invitrogen).

Quantitative RT-PCR: The articular cartilage in the contact area of the femur and tibia was obtained. The samples were placed in a vessel containing 1 ml QIAzol (Qiagen) and homogenized. The total RNA of the homogenate was purified using RNeasy Lipid Tissue Mini Kit (Qiagen). Complementary DNA was synthesized using Cloned AMV First-strand cDNA Synthesis Kit (Invitrogen). PCR efficiencies and relative expression levels of collagen type II, MMP-8 and MMP-13 as a function of EF1α1 were calculated as previously described [2, 3].

Statistical Analysis: Differences between the immobilized and control groups were compared at each time point by Mann-Whitney’s U test. A value of P < 0.05 was accepted as statistically significant.

RESULTS:
Expression of type II collagen and MMP-8 mRNA was decreased after 3 days in the three areas, but increased after 2 weeks at hypertrophic differentiated chondrocytes in the transitional area (Figure 1B and C). Immunostaining of type II collagen at the transitional and contact areas was decreased (Figure 2A). Immunostaining of type I collagen was increased at hypertrophic differentiated chondrocytes in the transitional area and superficial chondrocytes in the non-contact area (Figure 2B). Immunostaining of MMP-13 was observed at the hypertrophic differentiated chondrocytes in the transitional area (Figure 2C). Expression levels of type II collagen mRNA was decreased, however, MMP-8 and -13 mRNA was increased by quantitative PCR (Figure 3).

CONCLUSION:
The mechanism of the articular cartilage degeneration after immobilization differs at the three specific areas.

REFERENCES: