INTRODUCTION:
The functional spine unit consists of two vertebrae, intervertebral disc and posterior elements (facet or zygapophysial joints and the ligaments). The facet joint is composed of the inferior articular process of one vertebra and the superior articular process of the adjacent vertebra. Lumbar facet joint syndrome is reported to have a prevalence of 15% to 40% in the population with chronic low back pain (1, 2). Facet joints are clinically important sources of chronic low back pain even after spinal fusion or artificial intervertebral disc implantation surgery. Spinal fusion operation increased stress at the adjacent levels which may accelerate facet joint degeneration (3). After artificial disc replacement, some authors reported the facet joint arthrosis was one of the reasons leading to unsatisfactory results (4). But there are few literatures reported the OA model of facet joint in small animal. In this study, our purpose was to establish a new animal model for OA of lumbar facet joint after collagenase injection in rats. We hypothesized that the OA-like changes would be induced by collagenase injection.

METHODS:
This study was approved by the Institutional Animal Care and Use Committees of the participating institutions. Three months old Sprague Dawley rats, body weight between 250 and 270 g, were used in this study. Animals were maintained in accordance with the NIH ‘Guide for the care and use of laboratory animals’. All of the surgical procedures were performed under sterile operating conditions with the rats under intraperitoneal anesthesia (50 mg/kg ketamine hydrochloride and 5 mg/kg xylazine hydrochloride). A midline incision was made on the back and the paraspinal muscle was dissected to expose bilateral lumbar facet joints. Five microliters of collagenase (type II; enzyme activity 321 U/mg, Worlington, Lakewood, NJ) was injected into the right lumbar facet joint through a 34 gauge blunt NanoFil needle (WPI, Inc., FL) at a rate of 2 μl/min controlled by a infusion pump. Five microliters of saline as a control was injected into the left lumbar facet joint with the same methods. After injection, the fascial layer of the muscle and skin were sutured. In this study, 1 U (0.2 U/μl), 10 U (2 U/μl) or 50 U (10 U/μl) collagenase or saline was injected into the facet joint. At 1, 3 and 6 weeks postoperatively the animals were euthanized with CO2 and the lumbar spines were removed upon sacrifice (N=8, each concentration of collagenase at each time point). The lumbar spines were fixed with 10% neutral buffered formalin, decalcified and then embedded in paraffin. Five micrometer sections from the facet joints were obtained for the hematoxylin-eosin and Safranin O staining.

RESULTS:
The histology of the cartilage of the control group and collagenase injection group were compared in the same section for each sample (Fig. 1A). The structure of normal articular cartilage of facet joint is shown in Figure 1B. The cartilage of the saline group was not degenerated at each time point. The surface was smooth and the matrix was densely stained red with Safranin O (Fig. 2A-C). In 1 U collagenase-injected group, superficial fibrillation of the articular surface and chondrocytes cluster in superficial zone were noted at 1 week postsurgery (Fig. 2D). Uneven articular surfaces and vertical fissure into deep zone were seen at 3 weeks (Fig. 2E). Deformed articular surface in focal area and slight reduction of Safranin O staining in the matrix domains adjacent to the fissure were seen at 6 weeks (Fig. 2F). In 10 U collagenase-injected group, articular surface irregularity, branched fissures extended into the mid zone and hypocellularity of chondrocyte in superficial and mid zone were noted. Cartilage matrix showed reduction of Safranin O stain into deep zone (Fig. 2G). At 3 weeks, irregular surface with reduction of the Safranin O stain and clustering of chondrocytes in superficial zone were observed (Fig. 2H). At 6 weeks, denudation of articular surface and fissures extended into the deep zone were noted (Fig. 2I). In 50 U collagenase-injected group, denudation with matrix loss extending to calcified cartilage interface was noted since 1 week (Fig. 2J-K). Deformation of the surface with fibrocartilaginous tissue was observed at 6 weeks postsurgery (Fig. 2L).

DISCUSSION:
We used a micro-injection method to create the OA-like changes of the facet joint. Intra-articular injection of type II collagenase was used in rabbit to induce experimental OA of knee (5). Inflammatory response in the synovium was also noted in that study. The anti-collagenase antibody was not found in the serum, this explained that the synovial reaction was not related to the immunological response. In our study, injection of collagenase into facet joint could induce hypocellularity of chondrocyte, cartilage loss and fibration which were characters of osteoarthritis. Injection of 1 U of collagenase could induce early OA-like change and 50 U of collagenase could induce severe OA-like change since 1 week postsurgery. Different severity of the osteoarthritis could be observed from the different concentration of collagenase and different time course. Using this animal model, further studies such as the effects of anti-OA reagents on facet joint cartilage or pathogenesis of collagenase on the chondrocytes in facet joint can be performed.

REFERENCES:

Fig. 1. (A) An axial section histology of the facet joint from L5/6. (Left side: control group. Right side: 50 U of collagenase-injected group at 1 week postsurgery). The facet joint is formed by the inferior articular process (IAP) of L5 and the superior articular process (SAP) of L6. (B) Normal articular cartilage of the facet joint. (Safranin O staining)

Fig. 2. Photomicrographs of facet joint cartilage in control (A-C), collagenase-injected group (1 U: D-F, 10 U: G-I, 50 U: J-L) at 1, 3 and 6 weeks postsurgery (Safranin O staining; original magnification 200x)