Meniscus reconstruction by a tendon autograft with recombinant human bone morphogenetic protein-2 in a rabbit model

+1Naka, Y; 1Hashimoto, Y; 1Takaoka, K
+1Department of Orthopaedic Surgery, Osaka City University Graduate School of Medicine, Osaka, JAPAN.
ynaka1114@med.osaka-cu.ac.jp

ABSTRACT INTRODUCTION
Meniscus has an important role in load transmission, shock absorption, knee stability and lubrication of the knee joint. Meniscus is a fibrocartilaginous tissue composed primarily of water and an interlacing network of collagen fibers interposed with fibrochondrocyte. Composition of the meniscus is mainly type I collagen, but in the central area of the inner, nonvascularized region of the meniscus, type II collagen is synthesized. Because the vast majority of the meniscus is avascular especially in inner zone, healing potential of the meniscus is poor. Thus, meniscus injury may lead to long-term degenerative joint changes. Recombinant human bone morphogenetic protein-2 (rhBMP-2) is an important factor that induces chondrogenesis and osteogenesis in vitro. Previously, we presented a new technique using rhBMP-2 injection to a tendon, and successfully regenerated fibrocartilage in the tendon-bone junction. In this process, we showed that rhBMP-2 induced chondrogenesis before the enchondral ossification in the tendon. We hypothesized that rhBMP-2 injection to a tendon grafted within knee joint would induce chondrogenesis without osteogenesis under the intra-articular condition. In the present study, we attempted meniscus regeneration by a tendon autograft injected by rhBMP-2.

METHODS
RhBMP-2 was dissolved into neutral buffer at a concentration of 1 µg/µL. To check a leakage of the solution out of the tendon at injection, we added 0.1% indocyanine green (ICG) solution to the rhBMP-2. Final concentration of rhBMP-2 solution was 0.1 µg rhBMP-2/µL. Healthy, adult female New Zealand White rabbits were utilized in this study. The animals in the experimental group (n=10) were anesthetized and a longitudinal skin incision 5 cm in length and medial parapatellar approach was made to expose the knee joint. The medial meniscus was detached from medial collateral ligament and resected and replaced by the tendon of the semitendinosus. Ten µl of rhBMP-2 solution was injected with a micro-syringe and 28G needle (Ito Corp., Shizuoka, Japan) into peripheral half of the grafted tendon. For the control group, the right hind limb was treated in the same fashion but only buffer solution (10µL) was injected into the tendon. Five animals were sacrificed from experimental and control group respectively with an overdose of pentobarbital at 4 and 8 weeks after surgery, respectively. In radiological examination, all of the harvested tissues were radiographed with a soft X-ray apparatus (SOFRON; Sofron Co., Ltd., Tokyo, Japan). Calcification was graded as follows: no calcification, calcification at basal region, or diffuse calcification. After radiological examination, all tissues were decalcified by using 0.5 M ethylenediamine tetracetic acid for 2 weeks and embedded in paraffin. Sections 5µm in thickness were obtained using a microtome (Leica Microsystems, Wetzlar, Germany) and stained with hematoxylin-eosin and toluidine blue and examined under light microscopy. In addition, localization of type I, II and III collagen was identified using a monoclonal antibody to Type I, II and III collagen (Daichi Fine Chemical Co., Ltd., Takaoka, Japan).

RESULTS SECTION
On radiography, all samples were graded to no calcification at 4 weeks after surgery. At 8 weeks in the experimental group, 3 of 5 were graded to no calcification, and 2 were graded to calcification at basal region, but none was graded to diffuse calcification. In control group, all samples were graded to no calcification. Histologically, metachromasia by toluidine blue staining indicating proteoglycan appeared in the inner zone of the reconstructed meniscus at 4 and 8 weeks in the experimental group. But, no metachromasia in toluidine blue staining was seen in control group. At 8 weeks, small ossicles was seen in the basal zone of the meniscus from the experimental group. On immunohistochemistry, type II collagen was detected in the same area of metachromasia in toluidine blue staining in the experimental group (Fig. 1b). But, it was not detected in control group (Fig. 1c). Type I and III collagen were detected in the whole area of the experimental meniscus. The distribution of Type I and III collagen in control group was similar staining in experimental group.

DISCUSSION
In the present study, we demonstrated that it is possible to induce chondrogenesis within a tendon graft by rhBMP-2 injection under the intra-articular milieu. They expressed Type II collagen and proteoglycan in inner zone of a tendon graft. The reconstructed meniscus by a tendon autograft injected with rhBMP-2 resembled to intact meniscus. In previous study, clinical trial of meniscus reconstruction using only semitendinosus tendon was reported, but its result was not satisfactory. Cartilage matrices might work as shock absorber and protect articular cartilage of the joint. Under extra-articular condition, rhBMP-2 induces ectopic ossification by injection to a tendon after 4 weeks. In contrast, a tendon graft placed within joint did not ossify after 4 weeks. This result suggested a possibility that ossification might be blocked under intra-articular condition. Although ectopic ossification presented after 8 weeks, it was small and located in basal region, which it might not affect adversely to articular cartilage of the joint. This study was a short term study, and a long term study will need to investigate persistent function of the regenerated meniscus.

Figure 1: Immunohistochemistry using anti-type II collagen antibody
(a) Intact medial meniscus of a rabbit.
(b) Reconstructed meniscus by a tendon graft injected by rhBMP-2.
(c) Reconstructed meniscus by a tendon graft only. (control)