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# Early intervention of swimming exercises attenuate articular cartilage destruction in a rat model of anterior cruciate ligament and meniscus knee injuries☆

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ABSTRACT

*Aim:* The anterior cruciate ligament (ACL) and meniscus injuries often cause post-traumatic knee osteoarthritis (PTOA), which can place great limitations on patients. But to date there is no effective therapy to delay the progression of cartilage destruction in PTOA. This study aimed to compare the effects of early versus delayed swimming exercise on the chondroprotective effects in a rat PTOA model with ACL and meniscus injuries.

*Main methods:* Thirty-two adult male Sprague-Dawley rats received unilateral ACL transection and medial meniscectomy (ACLMT). These were randomly allocated to four groups: early swimming (eSW), delayed swimming (dSW), sham-operated early swimming (sham-eSW) and sham-operated delayed swimming (sham-dSW). Swimming (30 min per session) continuing for 28 days was started three days and three months after ACLMT surgery as a protocol for eSW and dSW intervention. Cartilage quality was assessed by Mankin HHGS examination (H&E, Safranin-O stain) and collagen type II (CoII) and matrix metalloproteases-13 (MMP13) immunohistochemistry.

*Key findings:* ACLMT induced the PTOA histopathological changes, inhibited CoII and enhanced MMP13 expressions in cartilage for both sham-eSW and sham-dSW groups. eSW intervention significantly enhanced CoII expression and suppressed MMP13 overexpression in superficial and transitional zones of cartilage, as well as better Mankin scores, corresponding to sham-swimming controls ($P < 0.05$). dSW intervention provided less enhancement of CoII expression and improvement of histopathological scoring, but significantly reduced MMP13 overexpression compared to animals in eSW ($P < 0.05$).

*Significance:* Early intervention by swimming at very early stages of cartilage damage provides greater benefits than delayed intervention when PTOA has already developed.

## 1. Introduction

The anterior cruciate ligament (ACL) and menisci are the two intra-articular soft tissues most often injured during sports, and both are critical for post-traumatic osteoarthritis (PTOA) development [1]. The signs and symptoms of an ACL or meniscal tears are pain, loss of sensation or stability, or stiffness and swelling, making locomotion difficult for the patient. Therefore, knee instability and restricted knee movements may play important roles in increasing the risk of articular cartilage lesions and initiating the development of PTOA [2,3].

Though surgical interventions of ACL and meniscus reconstruction have become common for athletic individuals, they do not necessarily result in better subjective or objective functional outcomes for the knee [4]. Researchers suggest that initial non-operative (conservative) rehabilitation through exercise combined with bracing and activity modification, which aims to reduce knee pain and instability, as well as restoring neuromuscular knee function after ACL and meniscus injuries, may be more effective in the general population for preventing the development of PTOA [5,6].

The prescription of exercise rehabilitation strategies seems crucial for conservative management of ACL and meniscus injuries [7]. But high-impact exercise providing mechanical load exceeding articular

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cartilage tolerance following ACL and meniscus injuries may further accelerate the progression of joint degeneration [8]. Swimming, an active exercise that has low impact on articular cartilage, may contribute to pain relief and the reduction of edema and facilitate ease of movement after cartilage injury [9,10]. But there is little consensus over chondroprotection, whether ACL and meniscus injuries should be treated early or late with swimming programs.

*Type II collagen* (CoII) is a peptide and a principle component of the extracellular matrix of articular cartilage, and damage to the fibrillary meshwork of CoII in the articular cartilage is a critical event for cartilage degeneration. Collagenase, such as the matrix metalloproteases-13 (MMP13), with a role in degradation of CoII has been considered one of main enzymes responsible for degradation of extracellular matrix (ECM) proteins [2]. However, few studies have evaluated whether swimming might have chondroprotective effects on alterations of MMP13 and CoII expressions in articular cartilage and thereby further decrease the progressive cartilage loss.

In this study, we hypothesized that early swimming intervention decreases synovial inflammation and cartilage degradation by improving histopathological features, MMP13 and CoII immunohistochemical changes as a chondroprotective effect in a rat model of ACL and meniscus injuries.

## 2. Materials and methods

### 2.1. General design

To observe the effects of different timing for swimming interventions on protection of cartilage degradation in an ACL and meniscus transection (ACLMT)-model rats, thirty-two rats that received unilateral ACLMT surgery were further randomly divided into four equal groups intervened with one of the following operations: (1) early intervention of swimming (eSW, $n = 8$), (2) delayed intervention of swimming (dSW, $n = 8$), (3) sham-operated early intervention of swimming (sham-eSW, $n = 8$) and (4) sham-operated delayed intervention of swimming (sham-dSW, n = 8) groups. Each animal received a knee joint exposure with unilateral ACLMT at Day 0. The swimming dosage used in this study was based on recommendations of previous studies for studying exercise effects on chondroprotection [10–16]. Swimming (30 min per session) continuing for 28 days was started three days and three months after ACLMT surgery as a protocol of eSW (at Days 3–30) and dSW (at Day 90–117) intervention. Sham-eSW and sham-dSW groups received sham-swimming operations along with the swimming protocol. Animals were sacrificed for histology and immunoassay studies after completing all treatments (Day 120). Histopathological and biochemical changes in the synovium and articular cartilages of the femoral and tibial condyles were assessed by histopathological staining scored by the Mankin Histological Histochemical Grading System (HHGS), as well as CoII and MMP13 immunohistochemistry. Fig. 1 is schematic diagram of the experimental design.

### 2.2. Animals

Adult male Sprague-Dawley rats (BioLASCO Co., Ltd., Taiwan) weighing 250–300 g were maintained in an animal facility under an artificial 12-h light-dark cycle. Animals had access to food and water ad libitum. The rats were individually housed and the cage numbers were used to randomly assign the animals to the experimental and control groups. All experimental procedures were performed in accordance with ethical guidelines of the International Association for Study of Pain in Animals and were approved by the China Medical University Committee on Animal Care and Use (No. 2016-067).

### 2.3. ACL transection and meniscectomy

ACLMT-induced rat PTOA model was performed as described previously [17]. Briefly, animals were anesthetized with 2.5% isoflurane (AErrane, Baxter Healthcare of Puerto Rico, PR, USA) in induction, followed by a 1.0% maintenance dose. Body temperature during anesthesia was stabilized by placing the rats on an electric warming pad. A unilateral knee selected randomly was prepared for surgery by disinfecting with iodine after the fur was shaved, and was opened via a parapatellar skin incision was made on the medial sides of the joints. The knee joint was maximally flexed to expose the ACL in the intercondylar area so that the ACL could be readily visualized. ACL and meniscus were transected with eye scissors, and a positive anterior drawer test result was used to ensure complete transection of the ligament. The patella was relocated, and the joint capsule and skin were closed separately with a 6.0 Vicryl suture. Each rat underwent an ACLMT on a randomly selected knee, with the contralateral knee used as an internal control and undergoing the same procedures consisting of opening the joint capsule, but with no ACLMT. The operated limbs were not immobilized and rats were access to food and water ad lib.

### 2.4. Swimming exercise protocols

One week prior to surgery, all rats were introduced to the custom-made swimming apparatus (round plastic housing 80 cm height 60 cm diameter) for 1–3 min per day. The apparatus was filled to an approximate depth of 65 cm with water maintained at 29 °C for each swimming session. Three days or three months after transection surgery, rats were carefully transferred into the swimming apparatus. A drop of soap was added to reduce the frequency of “floating” behavior by reducing the surface tension. In the case of such behavior rats were stimulated at the nape of the neck to enforce swimming and ensure the full session was spent exercising. Each session was at a set time daily (17:00) for 28 days. The sham-swimming control rats were also placed in the same swimming apparatus, but without water.

### 2.5. Histopathological scoring

After euthanasia with over dosage of isoflurane at Day 120, bilateral articular cartilage and joint tissues collected from the femur and tibia was fixed in 10% neutral-buffered formalin, decalcified in EDTA solution (20%, pH 7.2) for 4 weeks and then embedded in paraffin. Samples were serially cut at 5 μm thickness in the sagittal plane with a microtome. Each specimen produced approximately 20 sections. Knee joint cartilage specimens were examined under a microscope for pathologic changes by three independent and blinded observers. Hematoxylin and eosin (HE) and Safranin-O (S2255-25G, Sigma)/Fast green (FCF-Bio Basic Inc. FB0452) stainings were performed and the cartilage histopathology was then assessed using the modified Mankin HHGS scoring system to identify cartilage structure (scoring range 0–6), cell distribution (scoring range 0–3), Safranin-O staining (scoring range 0–4) and tidemark integrity (scoring range 0–1) as separate sub-items. The sum of the separate scores ranges from 0 (normal) to 14 (severe OA)

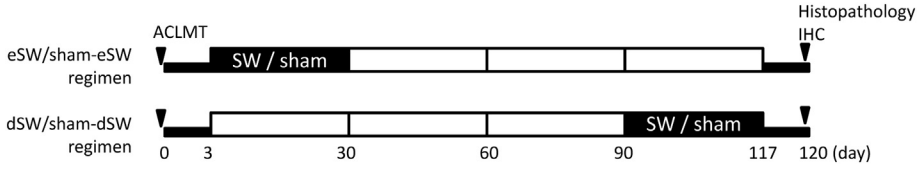


**Fig. 1.** Experimental design. ACLMT, anterior cruciate ligament and meniscus transection; dSW, delayed intervention of swimming; eSW, early intervention of swimming; IHC, immunohistochemistry; SW, swimming.