

Establishment of a Surgery-Induced Osteoarthritis Rat Model

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ABSTRACT

The degenerative arthritis has been a common knee joint disease in Taiwan. In mild cases of degenerative arthritis, it may just be a little uncomfortable to walk, but severe degenerative arthritis will cause difficulty in walking and even loss of basic mobility. The loss of articular cartilage in degenerative arthritis is irrecoverable. Currently, the degeneration of the knee joint can be easily divided into three levels based on its severity: primary, intermediate, and severe. The choice of treatments for degenerative arthritis will vary in according to different levels that is mainly divided into the non-surgical treatment and surgical treatment. Therefore, how to prevent knee joint degeneration is an important issue. In this study, anterior cruciate ligament transection (ACLT) plus medial meniscectomy (MMx) surgery were used to established a rat model with degenerative arthritis. According to all data, we have successfully established a rat model with degenerative arthritis via ACLT-MMx surgery. We wish this model will applied on the research of degenerative arthritis drugs and the design of therapeutic strategies for degenerative arthritis in the future.

Keywords: Anterior cruciate ligament transection, degenerative arthritis, medial meniscectomy, osteoarthritis, rat model, surgery

INTRODUCTION

Osteoarthritis (OA, osteoarthritis), commonly known as degenerative arthritis, has always been a common knee joint disease in Taiwan. The main symptom of OA is "pain", especially under the weight bearing. In mild cases of OA, it may just be a little uncomfortable to walk. Administration of medicine will be good and attenuated the mild OA, but severe OA will cause difficulty in walking and even loss of basic mobility.[1-3] The knee joints are the people's largest joint and they are responsible for the body's freedom of movement. The knee joints must bear 3-5 times the body weight every day. Therefore, the wear of the "articular cartilage" can be expected. The loss of articular cartilage is "irrecoverable" in OA, so the prevention of wear or how to prevent knee joint degeneration is an important issue.[4].

In clinical, many OA patients commonly ask what they should eat to maintain their knee joints. In fact, the real effective prevention is "reducing the burden on the knee joints", including body weight control, the unnecessary weight-bearing work reduction, the overloaded exercise avoid etc. However, there is not just one cause for the degradation of articular cartilage, and there are even many reasons that have not been explored. Therefore, when considering which treatment to choose to maintain the knee joints, don't forget that "reducing the burden on the knee joints" is definitely a more important method.[5].

Currently, the degeneration of the knee joints can be easily divided into three levels based on its severity: primary, intermediate, and severe. The choice of treatments for OA, will vary according to different levels, is mainly divided into the non-surgical treatment and surgical treatment. Because OA is still an irreversible disease so far, the goal of treatment is basically to improve the OA symptoms and cannot change the state of degeneration on the knee joints. The treatment of primary regression is based on anti-inflammatory drugs plus rest, which can be improved for the most OA patients. For OA patients with mid-stage degeneration, if the drug is not effective, further examinations are required to find out the causes, including magnetic resonance imaging (MRI) and full-length X-rays of the limbs, etc. If the main cause of OA is found, the surgical treatment can be based on the cause, including corrective surgery or half-knee artificial joint replacement. Severe OA is mostly considered total knee arthroplasty (TKA). According to the statistics in Taiwan, the proportion of Taiwanese undergoing TKA surgery each year is increasing year by year. Because of the increase in average life expectancy, the Taiwanese's sports climate is becoming more and more popular and the proportion of many elderly patients, especially those over 80 years old, undergoing surgery has increased a lot. Although the effect of artificial joint replacement is well, the useful life and some complications that may occur are still problems that cannot be completely eliminated. Therefore, the other methods for treatment will be tried for younger OA patients. [1-3; 6-8].

With the continuous progress of related research and the advancement of medical technology, we have a better understanding of the causes of knee joint degeneration and treatments have become more diversified. Through more detailed examination and evaluation, we can discuss more suitable surgical treatments with OA patients. For example, the proximal osteotomy corrective surgery is to reduce the pressure on the inside of the knee joint by correcting the O-leg to improve the OA symptoms, thereby eliminating the need to replace the artificial joints. In addition, the Taiwan's government has passed the cell therapy bill and some OA patients can even regenerate cartilage through surgery and cell therapy. These treatment methods all hope that OA patients can avoid artificial joint replacement or delay the age of artificial joint

replacement. However, OA patients can not 100% successfully grow cartilage through these treatments. This requires the professional physicians to evaluate and then fully communicate with the OA patient and family members to make an appropriate treatment plan, so as to improve the effectiveness of the OA treatment. Currently, artificial joint replacement is still the final treatment and its results are good, but there are still many options to consider before going to the last step. We are still working hard and hope that the treatment of knee degenerative arthritis can be achieved and have better results.[9-10] Taken these informations together, an optimal animal model of OA is very urgently need for the research of novel OA drugs and the design of therapeutic strategy programs.

MATERIALS AND METHODS

Experimental Animals

Adult male Sprague Dawley (SD) rats (12 weeks old) with specific pathogen-free conditions were used for this study, were purchased from BioLASCO Taiwan Co., Ltd. (Yilan, Taiwan). These SD rats were fed with standard laboratory diet (No. 5001, LabDiet®; PMI Nutrition International, St. Louis, MO, USA) and distilled water ad libitum during the experimental period. The environment was maintained room temperature (24-27°C) and 60%-70% humidity with a photoperiod of 12-hr light/12-hr dark cycle. The study will begin after a week acclimation. The Institutional Animal Care and Use Committee (IACUC) of Agricultural Technology Research Institute inspected all animal experiments and this study comply with the guidelines of protocol IACUC-110098 approved by the IACUC ethics committee.

Experimental Design

The 14 SD rats were divided into as normal control (NC) group (n = 7) and OA group (n = 7). The surgical induction in the OA group was performed at the beginning of the experiment. Body weight (BW), the widths of the knee joints, and weight-bearing symmetry were measured before the surgery as the baseline. At the end of the experiment (12th week), the knee joint section confirmed the progressive deterioration of OA joint with a reactive chondrocytes hypertrophy, and increasing cartilage erosion accompanied by chondrocytes loss.

Anterior Cruciate Ligament Transection (ACLT) Plus Medial Meniscectomy (MMx)-induced OA Rat Model

A surgery-induced OA rat model was proceeded as described previously.[11] The ACLT-MMx surgery was performed on the left knee joint of rats by a senior veterinarian. Briefly, male SD rats (350 g) were anesthetized with 5% isoflurane and then maintained with 2% isoflurane. Hair was removed around the left knee skin and sterilized with povidone-iodine solution and 70% ethanol. Later, the left knee joint skin was cut with a sterilized scalpel. An incision was made in the medial aspect of the left joint capsule. The anterior cruciate ligament was transected using a sterilized scalpel, and the medial meniscus was removed completely using a sterilized tenotomy scissor. Following ACLT-MMx surgery, the incision joint was irrigated with normal saline/antibiotics. The incision joint capsule was sutured with 4-0 vicryl. The 4-0 monofilament nylon was used for the incision skin closure. Finally, the wound area was sterilized with povidone-iodine solution. Analgesics (3 mg/kg/day ketoprofen) and antibiotics (100 mg/kg/day cefazolin) were administered intramuscularly for 3 days to prevent pain and infection (Figure 1).



Figure 1. The process of anterior cruciate ligament transection plus medial meniscectomy-induced OA rat model

Measurement Values of Physiological Items

All rats were detected the changes in BW, water intake, and food intake during the experiment. Regular measurement and comparison of BW of rats in the NC group and OA group. In addition, the changes in water intake and food intake in the NC group and OA group were also recorded daily.

Measurement of the Width of Knee Joints

The width of the knee joint was measured with an electronic digital caliper (Mitutoyo America Corporation) once a week after ACLT-MMx surgery until the end of the experiment. The level of actual joint swelling induced by ACLT + MMx surgery was evaluated according the width (mm) of the surgical left knee joints of OA rats minus the width (mm) of the right knee joints of OA rats.

Measurement of the Static Hindlimb Weight-Bearing Forces Test

Static hindlimb weight-bearing forces was measured using an incapitance tester (Linton Instrumentation, Norfolk, UK) to detect OA-induced changes in postural equilibrium every two weeks from 0, 2, 4, 6, 8, 10, and 12 weeks of the experimental period. Briefly, the rats were placed on their hind paws in an acrylic box containing an inclined plane (65° from horizontal) that was placed above the apparatus. The rats were first trained to stand on their hind limbs in an acrylic box equipped with a 65° inclined plate. In the weight-bearing test, the acrylic box will be placed on the bipedal balance tester, allowing the rats to stand on their hind limbs by themselves. After a brief accommodation period, the weight that the rats applied to each hind limb was measured independently by the apparatus. Five times measurements were taken and averaged for each rat.[11]

Histopathological Examination of Knee Joint

After the rats were sacrificed, the knee joints of rats were taken out and fixed with 10% formalin for 48 hours, and then the knee joint was immersed in decalcification solution for 28 days under the decalcification step. After the decalcification was completed, the knee joints were embed the tissue in paraffin, make a paraffin section (paraffin section), and cut into three sections each with a thickness of 5 μ m. Then, the sections were stained for tissues (including HE staining and toluidine blue/fast green etc), and the average morphological changes of the keen joint tissues were observed under a microscope by a senior pathologic veterinarian. Some items of the pathologic examination as the cartilage matrix loss width, tibia cartilage degeneration score, total and significant cartilage degeneration widths, and zonal depth ratio of the lesions and synovial reaction were evaluated following as:

a. Loss width of cartilage matrix

The loss of cartilage matrix starts from the destruction of the surface cartilage into fibrous and matrix loss, and it gradually deteriorates to deeper and more extensive matrix loss, eventually leading to the loss of the full-thickness cartilage matrix. This assessment covers the magnitude of the loss of cartilage thickness. The severity of cartilage matrix loss is divided into the surface width (0% depth zone, the extent of any matrix loss across the intact cartilage area on both sides), and the middle layer width (In the 50% depth zone, the cartilage matrix loss has reached the middle layer), and the tidal line layer width (100% depth zone, the matrix has been severely lost to the tide line), the sum of the widths (μm) of the above three parts is the loss amplitude of cartilage matrix. The severity of the disease ranges from 1 to 5 score according to the severity: 1 score = minimal (< 1%); 2 score = mild (1-25%); 3 score = moderate (26-50%); 4 score = moderate/severe (51-75%); 5 score = severe/extremely severe (76-100%).

b. Cartilage degeneration score

The main evaluation criterion is the damage and death of chondrocyte. Use the knee joint tibial medial load-bearing platform to delineate three-level zones, each of which is the outer zone (from the medial edge of the joint), the middle zone and the inner zone (adjacent to the center of the cruciate ligament knee joint), and the degree of damage is assessed on a scale of 0-5. The severity of the disease ranges from 1 to 5 score according to the severity: 1 score = minimal (< 1%); 2 score = mild (1-25%); 3 score = moderate (26-50%); 4 score = moderate/severe (51-75%); 5 score = severe/extremely severe (76-100%). The damage and death of chondrocyte in each zone was between 0-5 score and the total score of the three zones was 15 scores.

Statistical Analysis

The data were expressed as mean $\pm$ SD. All graphical representations and statistical calculations were aided by GraphPad Prism version 8.0. All comparisons were made by two-way ANOVA and all significant differences are reported at $*p < 0.05$.

RESULTS**Measurement of BW, water intake, and food intake of rats**

During the experiment, the changes in BW, water intake, and food intake of rats in each group are shown in Table 1. During the entire experimental period, the rats in OA group showed that the ACLT + MMx surgery would slow the growth of the rats before 4th week ACLT + MMx surgery, but the BW of each group maintained a steady increase with time. From the 4th week to the end of the experiment (12th week), there was no significant difference on the BW between each group. In addition, the average daily food intake in NC and OA groups was 31.2 ± 1.4 g/rat/day and 31.2 ± 1.1 g/rat/day, respectively. The average daily water intake in NC and OA groups was 49.1 ± 3.2 mL/rat/day and 48.9 ± 4.8 mL/rat/day, respectively. The average daily food intake and water consumption of each group during the four-week experimental period showed that there was no significant difference between two groups (Table 1).

Table 1. During the experiment, the changes in body weight, water intake, and food intake of rats in each group. Different words (a or b) are reported the significant differences at $p < 0.05$

Experimental period	NC	OA
	BW (g)	
Before ACLT + MMx surgery	417.3 ± 11.3 ^a	411.6 ± 9.9 ^a
ACLT + MMx surgery		
1 st week	441.9 ± 11.6 ^a	421.1 ± 15.0 ^b
2 nd week	468.8 ± 14.5 ^a	449.9 ± 23.9 ^b
3 rd week	493.4 ± 18.7 ^a	469.8 ± 16.7 ^b
4 th week	522.8 ± 25.1 ^a	496.6 ± 22.8 ^a
5 th week	539.1 ± 25.6 ^a	517.9 ± 24.0 ^a
6 th week	550.9 ± 29.1 ^a	552.3 ± 25.0 ^a
7 th week	567.7 ± 31.3 ^a	570.7 ± 26.5 ^a
8 th week	579.5 ± 33.3 ^a	581.5 ± 27.1 ^a
9 th week	587.4 ± 37.8 ^a	590.8 ± 26.9 ^a
10 th week	595.3 ± 37.0 ^a	599.9 ± 29.7 ^a
11 th week	608.6 ± 38.0 ^a	618.5 ± 31.3 ^a
12 th week	617.0 ± 40.3 ^a	623.8 ± 29.0 ^a

Measurement of static hindlimb weight-bearing forces test of rats

Using of the incapacitance tester to assess whether the rat's knee joints is painful or discomfort. To measure the weight distribution of the rat's hind limbs when standing, and understand the discomfort of the rat's joints. Under normal conditions, the weight distribution of the hind limbs of the rats will be more even, and the pressure difference between the two feet will be close to 0. If the rat has a unilateral knee joint injury, the uninjured hind limbs will bear more weight, and the pressure difference between the two feet will be increase.

As shown in Table 2, the pressure difference between the two legs before the ACLT + MMx surgery was close to 0 between NC and OA groups. There was no significant difference between NC and OA groups. After the ACLT + MMx surgery, the pressure between the feet of the OA group was significantly higher than that of the NC group ($p < 0.05$). It was observed that the pain value increased significantly after the ACLT + MMx surgery in OA group.

Table 2. During the experiment, measurement of static hindlimb weight-bearing forces of Rats in each group. Different words (a or b) are reported the significant differences at $p < 0.05$

Experimental period	NC	OA
	Hindlimb forces (g)	weight-bearing
Before ACLT + MMx surgery	0.3 ± 0.2^a	0.3 ± 0.2^a
ACLT + MMx surgery		
0 th week	0.3 ± 0.2^a	100.7 ± 6.0^b
2 nd week	0.5 ± 0.3^a	108.9 ± 6.5^b
4 th week	0.3 ± 0.2^a	101.6 ± 2.4^b
6 th week	0.3 ± 0.4^a	94.1 ± 3.3^b
8 th week	0.3 ± 0.3^a	93.1 ± 4.7^b
10 th week	0.3 ± 0.2^a	86.3 ± 6.9^b
12 th week	0.3 ± 0.4^a	78.9 ± 7.6^b

Measurement of the width of knee joints of rats

Using of an electronic digital caliper to measure the width of the knee joint of the rat's hind limbs and measure it once a week to understand the degree of swelling of the rat's knee joint. As shown in Table 3, there was no significant difference in the width of the knee joint between each group before ACLT + MMx surgery. After ACLT + MMx surgery, the width of rat's knee joint in the OA group was significantly wider than that in the NC group ($p < 0.05$). A significant increase in the rat's knee joint swelling was observed after ACLT + MMx surgery. Under the gross examination, the OA rat's knee joints were wider than the NC rats (Figure 2).

Table 3. During the experiment, measurement of the width of knee joints of rats in each group. Different words (a or b) are reported the significant differences at $p < 0.05$

Experimental period	NC	OA
	Width of knee joints (mm)	
Before ACLT + MMx surgery	7.7 ± 0.5^a	7.6 ± 0.5^a
ACLT + MMx surgery		
0 th week	7.7 ± 0.5^a	10.5 ± 0.4^b
1 st week	7.8 ± 0.3^a	11.4 ± 0.4^b
2 nd week	7.8 ± 0.2^a	10.8 ± 0.4^b
3 rd week	7.8 ± 0.3^a	11.5 ± 0.3^b
4 th week	7.9 ± 0.1^a	11.3 ± 0.4^b
5 th week	7.9 ± 0.3^a	11.6 ± 0.1^b
6 th week	7.9 ± 0.3^a	11.6 ± 0.4^b

7 th week	8.0 ± 0.3 ^a	11.8 ± 0.6 ^b
8 th week	7.9 ± 0.3 ^a	11.6 ± 0.5 ^b
9 th week	7.8 ± 0.3 ^a	11.7 ± 0.4 ^b
10 th week	7.9 ± 0.2 ^a	10.9 ± 0.4 ^b
11 th week	7.9 ± 0.2 ^a	10.6 ± 0.3 ^b
12 th week	7.9 ± 0.1 ^a	10.7 ± 0.1 ^b

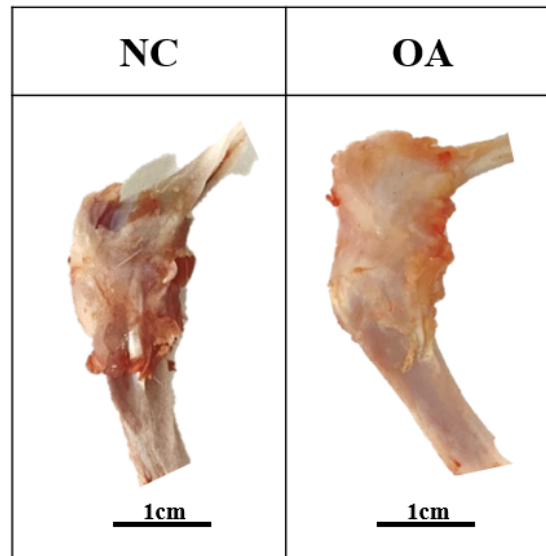


Figure 2. The gross of the left knee joint of rats in the NC and OA group

Pathologic examination on knee joints of rats

The rats in each group were performed with or without ACLT + MMx surgery. After ACLT + MMx surgery for 12 weeks, all rats were euthanized. The rat's knee joints were dissected and fixed in 10% neutral formalin. After 48 hours of fixation, the rat's knee joints were moved to the decalcification solution. After decalcification was completed, to embed in paraffin, section and stained with H&E, toluidine blue, Safranin O/fast green, Masson's trichrome. Safranin O/fast green and toluidine blue staining were used to observe the loss of proteoglycans; Masson's trichrome staining was used to assess the degree of fibrosis; the severity of articular cartilage damage was assessed through histopathological scores, and the histopathology was evaluated. The score is graded and the evaluation items were cell necrosis, osteophytes, cartilage loss and degeneration, subchondral sclerosis, and fibrocartilage formation. The severity of the disease ranges from 1 to 5 score according to the severity: 1 score = minimal (< 1%); 2 score = mild (1-25%); 3 score = moderate (26-50%); 4 score = moderate/severe (51-75%); 5 score = severe/extremely severe (76-100%).

H&E staining was used to observe cartilage damage in joint tissues, and histopathological scores were performed to assess the severity of articular cartilage damage. The average scores of lesion degree were 1.00 ± 0.00 score and 2.14 ± 0.83 score, respectively. Therefore, the average scores of pathological changes in the OA group were significantly higher than those in the NC ($p < 0.05$) (Figure 3).

Toluidine blue staining was used to observe the fibrous tissue filling the cartilage defect and evaluated the degree of fibrosis. The average scores of the rat's knee joints of the NC group and the OA group were 1.00 ± 0.00 score and 1.29 ± 0.49 score, respectively, and there was no significant difference in the average score between the groups (Figure 4).

Safranin O & fast green staining was used to observe the expression level of glycosaminoglycan in proteoglycans. The average scores of lesions in the NC group and OA group were 1.0 ± 0.0 score and 2.6 ± 0.8 score, respectively. The average scores of lesions in the OA group were significantly higher than that in the NC group ($p < 0.05$) (Figure 5).

The condition of collagen and bone was observed by Masson's trichrome staining. The average scores of the disease degree of the NC group and the OA group were 1.0 ± 0.0 score and 2.1 ± 0.4 score, respectively. The average score of lesion severity in OA group was significantly higher than that of the NC group ($p < 0.05$) (Figure 6).

The average scores of the total lesions of each group stained by H&E, toluidine blue, Safranin O/fast green, and Masson's trichrome. The average scores of the total lesions of the NC group and the OA group were 4.0 ± 0.0 score and 8.1 ± 1.5 score, respectively. The average score of the total lesion degree of the OA group was significantly higher than that of the NC group ($p < 0.05$) (Figure 7).

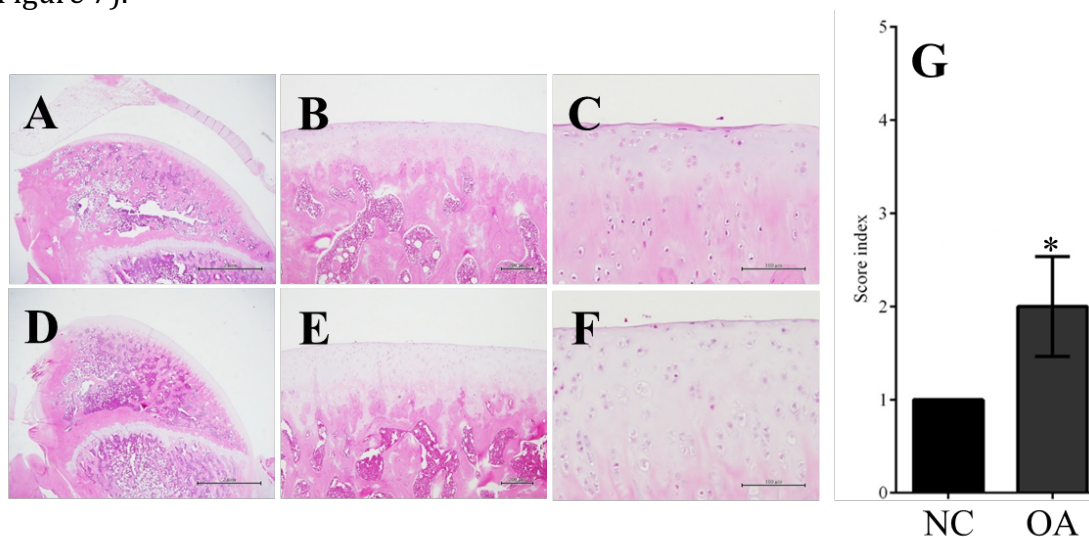


Figure 3. There are two groups of the experimental rats ($n = 7/\text{group}$). ACLT + MMx surgery was used to induce knee joint injury in rats and the injury level of knee joint by using H&E staining. (A-C) Normal control group (NC); (D-F) Osteoarthritis group (OA). (G) The lesion score of knee joint injury in NC and OA. The field of view magnification is $\times 20$ (A & D), $\times 100$ (B & E), and $\times 400$ (C & F). * $p < 0.05$ (NC vs. OA)

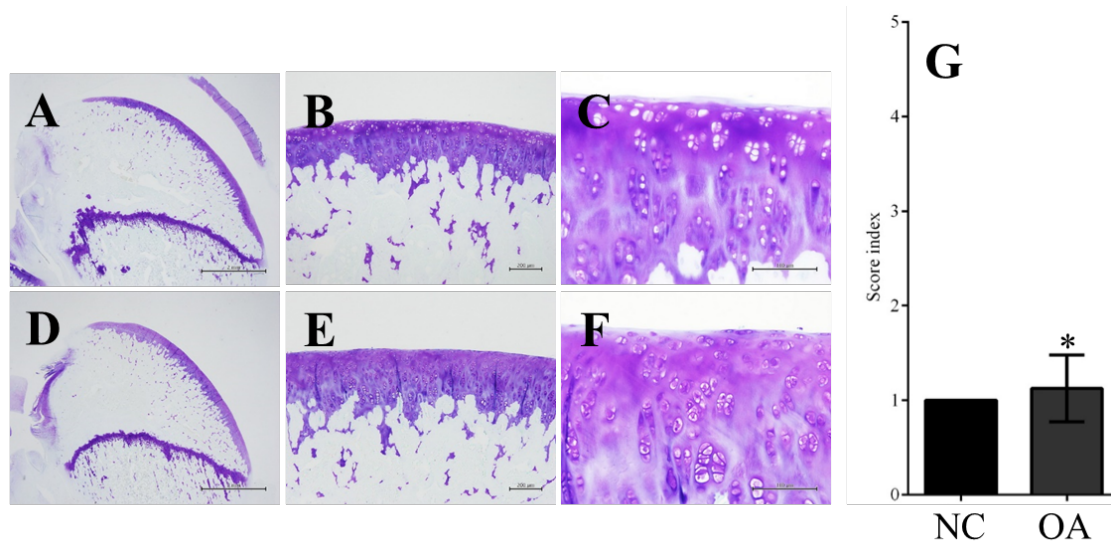


Figure 4. There are two groups of the experimental rats ($n = 7/\text{group}$). ACLT + MMx surgery was used to induce knee joint injury in rats and the injury level of knee joint by using toluidine blue staining. (A-C) Normal control group (NC); (D-F) Osteoarthritis group (OA). (G) The lesion score of knee joint injury in NC and OA. The field of view magnification is $\times 20$ (A & D), $\times 100$ (B & E), and $\times 400$ (C & F). * $p < 0.05$ (NC vs. OA)

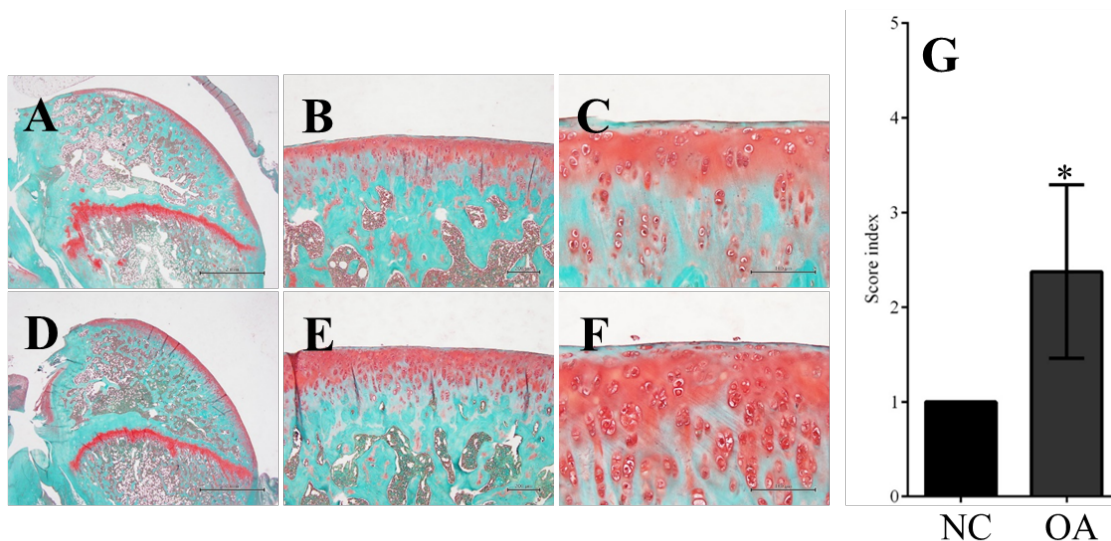


Figure 5. There are two groups of the experimental rats ($n = 7/\text{group}$). ACLT + MMx surgery was used to induce knee joint injury in rats and the injury level of knee joint by using fast green staining. (A-C) Normal control group (NC); (D-F) Osteoarthritis group (OA). (G) The lesion score of knee joint injury in NC and OA. The field of view magnification is $\times 20$ (A & D), $\times 100$ (B & E), and $\times 400$ (C & F). * $p < 0.05$ (NC vs. OA)

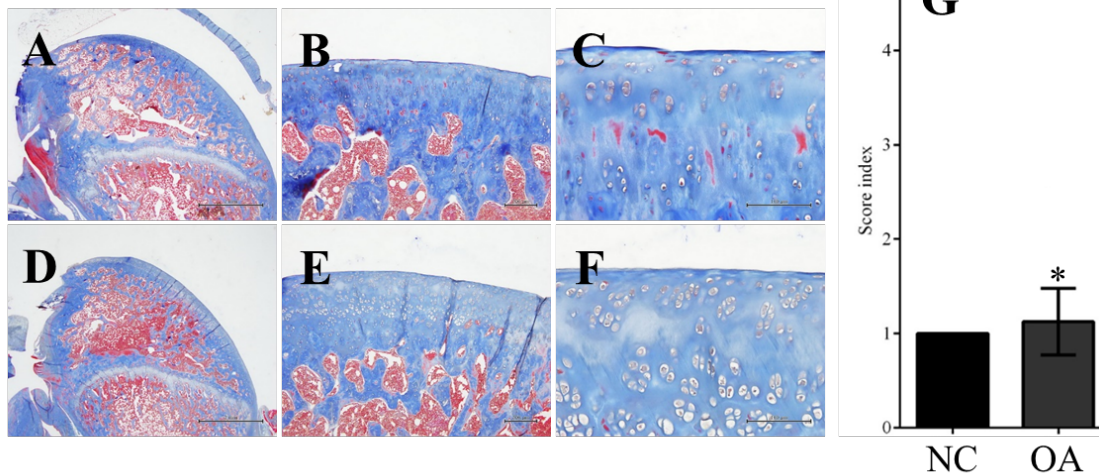


Figure 6. There are two groups of the experimental rats ($n = 7/\text{group}$). ACLT + MMx surgery was used to induce knee joint injury in rats and the injury level of knee joint by using Masson's trichrome staining. (A-C) Normal control group (NC); (D-F) Osteoarthritis group (OA). (G) The lesion score of knee joint injury in NC and OA. The field of view magnification is $\times 20$ (A & D), $\times 100$ (B & E), and $\times 400$ (C & F). * $p < 0.05$ (NC vs. OA)

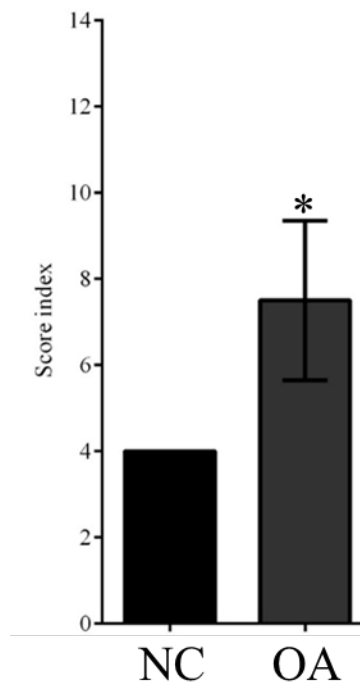


Figure 7. There are two groups of the experimental rats ($n = 7/\text{group}$). ACLT + MMx surgery was used to induce knee joint injury in rats and the injury level of knee joint by using H&E staining, toluidine blue staining, fast green staining, and Masson's trichrome staining. The total of injury scores in the left knee joint of rats in NC and OA groups. * $p < 0.05$ (NC vs. OA)

DISCUSSION

According to the literatures, [12-16] ACLT + MMx surgery induced a constant and gradually increasing knee width as a result of progressive knee joint inflammation. The static hindlimb

weight-bearing forces test to assess pain behavior during OA progression. Similar to the previous report,[15-16] ACLT + MMx surgery-induced OA elicited a constant change in weight-bearing asymmetry compared to that of the NC group, which only presented acute pain in the first few weeks as a result of the surgical procedure. In this study, using of the incapitance tester to assess whether the rat's knee joints was painful or discomfort. It was observed that the pain value increased significantly after the ACLT + MMx surgery in OA group. The pressure between the hindlimbs of the OA group was significantly higher than that of the NC group. Using of an electronic digital caliper to measure the width of the knee joint of the rat's hind limbs.

After ACLT + MMx surgery, the width of rat's knee joint in the OA group was significantly wider than that in the NC group and a significant increase in the rat's knee joint swelling was observed. Pathologic examination on knee joints of rats in each group were performed. After ACLT + MMx surgery for 12 weeks, knee joints of rats were sectioned and stained with H&E, toluidine blue, Safranin O/fast green, Masson's trichrome. H&E staining was used to observe cartilage damage in joint tissues, and histopathological scores were performed to assess the severity of articular cartilage damage. The average scores of pathological changes in the OA group were significantly higher than those in the NC. Toluidine blue staining was used to observe the fibrous tissue filling the cartilage defect and evaluated the degree of fibrosis. There was no significant difference in the average score between the groups. Safranin O & fast green staining was used to observe the expression level of glycosaminoglycan in proteoglycans. The average scores of lesions in the OA group were significantly higher than that in the NC group. The condition of collagen and bone was observed by Masson's trichrome staining. The average score of lesion severity in OA group was significantly higher than that of the NC group. Finally, the average score of the total lesion degree of the OA group was significantly higher than that of the NC group based on H&E staining, toluidine blue staining, Safranin O/fast green staining, and Masson's trichrome staining. According to the above results, an ACLT + MMx surgery-induced OA rat model has been successfully established.

In addition, according to the literatures, [17-21] no significant difference of BW between NC group and OA group. In this study, during the entire experimental period, the rats in OA group showed that the ACLT + MMx surgery would slow the growth of the rats before 4th week ACLT + MMx surgery, but the BW of each group maintained a steady increase with time. From the 4th week to the end of the experiment (12th week), there was no significant difference on the BW between each group. In addition, the average daily food intake and the average daily water intake in NC and OA groups during the four-week experimental period showed that there was no significant difference between two groups.

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CONCLUSIONS

The degenerative arthritis has been a common knee joint disease in Taiwan. In mild cases, it may just be a little uncomfortable to walk, but severe OA will cause difficulty in walking and even loss of basic mobility. The loss of articular cartilage is irrecoverable. Therefore, how to prevent knee joint degeneration is an important issue. In this study, ACLT + MMx surgery were used to established an OA rat model. According to all data, we have successfully established an

OA rat model. We wish this model will applied on the research of OA drugs and the design of therapeutic strategies in the future.

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