Follow us @BoneJointRes

Freely available online OPEN ACCESS

BJR

CARTILAGE

Modelling osteoarthritis in mice via surgical destabilization of the medial meniscus with or without a stereomicroscope

W. Hu,
J. Lin,
J. Wei,
Y. Yang,
K. Fu,
T. Zhu,
H. Zhu,
X. Zheng

From Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China

Aims

To evaluate inducing osteoarthritis (OA) by surgical destabilization of the medial meniscus (DMM) in mice with and without a stereomicroscope.

Methods

Based on sample size calculation, 70 male C57BL/6 mice were randomly assigned to three surgery groups: DMM aided by a stereomicroscope; DMM by naked eye; or sham surgery. The group information was blinded to researchers. Mice underwent static weightbearing, von Frey test, and gait analysis at two-week intervals from eight to 16 weeks after surgery. Histological grade of OA was determined with the Osteoarthritis Research Society International (OARSI) scoring system.

Results

Surgical DMM with or without stereomicroscope led to decrease in the mean of weight-bearing percentages (-20.64% vs -21.44%, $p = 0.792$) and paw withdrawal response thresholds (-21.35% vs -24.65%, $p = 0.327$) of the hind limbs. However, the coefficient of variation (CV) of weight-bearing percentages and paw withdrawal response thresholds in naked-eye group were significantly greater than that in the microscope group (19.82% vs 6.94%, $p < 0.001$; 21.85% vs 9.86%, $p < 0.001$). The gait analysis showed a similar pattern. Cartilage degeneration was observed in both DMM-surgery groups, evidenced by increased OARSI scores (summed score: 11.23 vs 11.43, $p = 0.842$), but the microscope group showed less variation in OARSI score than the naked-eye group (CV: 21.03% vs 32.44%; $p = 0.032$).

Conclusion

Although surgical DMM aided by stereomicroscope is technically difficult, it produces a relatively more homogeneous OA model in terms of the discrete degree of pain behaviours and histopathological grading when compared with surgical DMM without stereomicroscope.

Cite this article: *Bone Joint Res* 2022;11(8):518–527.

Keywords: Osteoarthritis, DMM, Microscopy, Cartilage degeneration, Joint pain

Article focus

- Can microsurgical techniques help to optimize osteoarthritis (OA) model in mice during surgery by destabilization of the medial meniscus (DMM)?
- Is the application of stereomicroscope during surgical DMM associated with the stability of OA model in mice?

Key messages

- Modelling OA via surgical DMM with or without a stereomicroscope can both successfully induce mild-to-moderate OA.
- Performing surgical DMM under stereomicroscope is technically difficult, but it produces a more homogeneous OA

Correspondence should be sent to Xianyou Zheng; email: zhengxianyou@126.com

doi: 10.1302/2046-3758.118.BJR-2021-0575.R1

Bone Joint Res 2022;11(8):518–527.

model in terms of the discrete degree of pain-related behaviours and histopathological grading.

Strengths and limitations

- This is the first study to systematically compare the effectiveness of microscope in OA establishment via surgical DMM.
- We used only the C57/BL6 strain of mice; other strains like the 129S6/SvEv mouse, or larger animals like rats, may be less susceptible to the variability we observed when DMM surgery was conducted with or without a stereomicroscope.

Introduction

Osteoarthritis (OA) is one of the most prevalent joint diseases worldwide and is a leading source of chronic pain, loss of quality of life, and disability.[1,2] There are no effective curative therapies available to date, and despite intensive research, the exact pathogenesis of OA has not yet been uncovered. The murine OA model has become one of the most commonly used models in preclinical OA research, largely because the musculoskeletal system of mice develops quickly and exhibits many of the same pathological features as humans.[3,4] Surgical OA models that mimic OA in humans caused by cruciate ligament ruptures and meniscal tears have proved especially useful.

Surgical destabilization of the medial meniscus (DMM) in the mouse OA model has become the gold standard for studying the onset and progression of post-traumatic OA.[5,6] In DMM surgery, transection of the medial meniscotibial ligament (MMTL) mimics OA histopathology and produces an extremely reproducible OA with a slower progression.[7-9] Compared with the anterior cruciate ligament transection (ACLT) model,[10] the DMM model produces fewer trauma and cartilage defects that are mostly confined to the medial tibial plateau and the central load-bearing area of the femoral condyle.[7] The DMM model also shows many of the characteristics of OA seen in patients, such as cartilage damage, osteosclerosis, osteophyte formation, ligament calcification, and mild synovitis.[11] Surgical DMM model has been proven to be one of the most common and cost-effective ways to induce mild-to-moderate OA in mice.

One drawback of the surgically induced OA murine model, however, is that the surgical procedures are especially difficult to perform, because mouse joints are extremely small, as is the surgical field. Anatomical structures of the mouse knee joint, in particular the MMTL, are very difficult to identify without magnification, potentially adding further obstacles to achieving successful DMM surgery.[5] These obstacles may also cause unnecessary trauma to other critical structures nearby such as tibial cartilage, which may lead to inconsistent results on behavioural and histological evaluation of OA model.

Over the last decades, microsurgical techniques were used to perform the DMM surgery which successfully induced experimental OA.[3,12] Surprisingly, the relative effectiveness of inducing OA in the surgical DMM model using an operating stereomicroscope has not yet been demonstrated. In this study, we conducted a randomized trial in mice to compare two ways of inducing OA through surgical DMM: without the aid of a stereomicroscope (i.e. naked-eye) and with the aid of a stereomicroscope. The effectiveness of OA model inducement was evaluated and analyzed using behavioural and histological measures.[13,14] The motivation for our study was to explore the advantages of microsurgical techniques in DMM murine models, to improve the stability of modelling, and decrease experimental cost, laying a strong foundation for follow-up OA-related basic research.

Methods

Ethical approval, experimental animals, and maintenance. All animal experiments were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital. These protocols followed national regulations on the use of laboratory animals, which are consistent with the ARRIVE guidelines.[15] We have included an ARRIVE checklist to show that we have conformed to the ARRIVE guidelines. Male C57BL/6 mice (eight to ten weeks old) were obtained from the Laboratory Animal Centre of Shanghai Jiao Tong University and were housed in groups of five in plastic cages containing wood shavings. The mice were maintained in a specific pathogen-free grade facility, which was in a temperature- and humidity-controlled environment (23°C ± 2°C; 50% ± 10%) in a 12-hour light/dark cycle. Food and running water were freely available.

Study design. The general scheme of the experiment is shown in Figure 1a.

Sample size calculation. Our sample size was based on a type I error set to 0.05, with a power of 80%. The superiority margin was set to 0. We performed a pilot DMM study on ten mice to determine the number of animals required; to detect a 1.00 difference of absolute deviation (AD) of Osteoarthritis Research Society International (OARSI) score between microscope (2.75) and naked-eye groups (3.75), 26 mice would be required in each group. Considering the possible loss of mice, we overpowered the sample size to 30 each for the microscope and naked-eye groups, and ten for the sham-operated group.

Randomization. A total of 70 mice were assigned consecutive numbers from 1 to 70 by one operator (WH). Then, the independent surgeon (JL) remotely divided the mice into three groups based on block randomization: naked-eye group; microscope group; and sham-operated group. The surgeon saw the mice on the day of operation and performed the operation according to the grouping. In total, 30 mice were allocated to the naked-eye group and were operated for surgical DMM without a stereomicroscope, while another 30 mice were allocated to the microscope group and experienced surgical DMM with an operating stereomicroscope. Ten mice were allocated

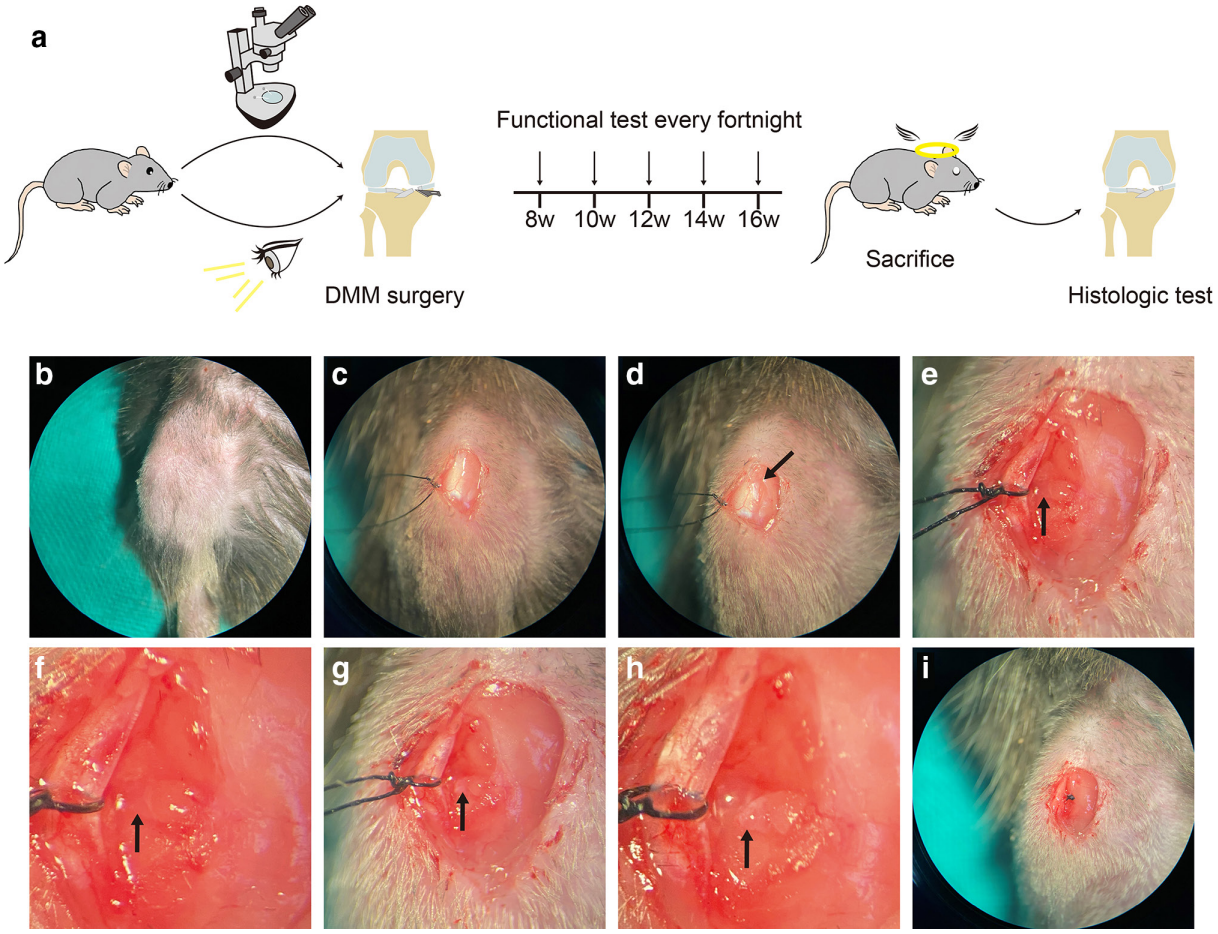

Fig. 1

a) General scheme of the experimental design. Mice received destabilization of the medial meniscus (DMM) surgery either with or without the aid of a stereomicroscope. Eight weeks postoperatively, static weightbearing testing and von Frey testing for hypersensitivity to pain were administered every two weeks. Mice were processed for histological evaluation at 16 weeks postoperatively. b) to i) Images of the surgical field viewed through the stereomicroscope show representative magnified views from a mouse of the microscope group (n = 30). b) Preparation of surgical site, shaved knee (right knee). c) Exposure of knee joint. d) Incision on the medial side of the patellar ligament (black arrow). e) Exposure of the medial meniscotibial ligament (MMTL, black arrow, 4× magnification). f) MMTL under high-power magnification (black arrow, 16×). g) Exposure of the articular cartilage after severing MMTL (incisal margin of MMTL, black arrow, 4×). h) Incisal margin of MMTL under high-power magnification (black arrow, 16×). i) Sutured joint capsule.

to the sham-operated group. The order the animals were assessed on behavioural tests was also random.

Blinding. Only the surgeon knew the group assignments. Researchers (WH, JW) who conducted functional behavioural tests and histological processing were blinded to group assignments, as were the researchers (YY, KF, TZ) who performed outcome evaluation and data analysis.

Surgical DMM procedures. Mice were transferred from the Laboratory Animal Centre to the Shanghai Jiao Tong University Affiliated Sixth People's Hospital one week prior to DMM surgery. The operating area was thoroughly wiped down with 75% (v/v) ethanol. Microsurgical instruments were sterilized and all surgical procedures were aseptic. All the surgeries were performed by the same surgeon (JL), who had been trained systemically and had five years of experience of inducing surgical DMM model in mice.

The mice were anaesthetized with isoflurane throughout surgery. The hair around the right knee of the mouse was shaved with clippers (Figure 1b). For the microscope group, we used a stereomicroscope (SMZ168 series, Motic, China). The anaesthetized mouse was positioned in a supine position on the heating pad with right hind limb exposed. A 5 mm longitudinal incision was made with a #11 scalpel blade (JinZhong, China) to expose the distal patellar tendon and the proximal tibial plateau (Figure 1c). A small incision was made along the medial side of the patellar ligament to gain access to the joint capsule (Figure 1d). The fat pad over the cranial horn of the medial meniscus was dissected away with micro-scissors (JinZhong). Any mild haemorrhage was controlled.

With microscopic magnification, the MMTL was clearly identified, running from the cranial horn of the medial meniscus laterally onto the anterior tibial plateau (Figures 1e and 1f). The MMTL was carefully severed with micro-scissors without damaging any articular cartilage or other soft-tissue. DMM was accomplished by exposing

the cut margin of the MMTL (Figures 1g and 1h), freeing the medial meniscus to move medially. The knee joint capsule was closed with a single 6-0 poliglecaprone resorbable suture (Ethicon, USA) (Figure 1i). The skin wound was closed with two to three 5-0 non-absorbable sutures (Ethicon). In between mice, instruments were cleaned, rinsed, and sterilized.

The naked-eye group mice underwent the same procedures, except they were not aided by a stereomicroscope.[7,16] For the sham-operated group, only their joint capsules were opened surgically, with all other procedures being identical to the microscope group.

Fluids were replaced by intraperitoneal injection of 0.5 ml warm sterile saline. Operated mice were placed in a disinfected recovery box until they regained full consciousness. They were then transferred to a clean cage with fresh wood shavings. Signs of pain or distress were monitored for 72 hours postoperatively. Sutures were removed 14 days post-surgery, and mice received routine health checks every two to three days until end point (16 weeks after surgery).

Outcome measures. The OARSI score in each group was regarded as the primary outcome. Secondary outcome measures included static weightbearing distribution (SWB), von Frey test, gait analysis, synovitis score, and immunohistochemistry of collagen type II. Detailed information on pain-related behavioural tests and histological evaluation are described in the Supplementary Material.

Statistical analysis. Continuous data were expressed as means and 95% confidence intervals (CIs). Independent-samples *t*-tests were used for two sample comparisons. The F-test was used to compare the homogeneity of variance. Welch's *t*-test was used for correction if the data dispersion had unequal standard deviations. Tests were considered statistically significant at $p < 0.05$. GraphPad Prism software (version 8.0, GraphPad Software, USA) was used for statistical analyses and to construct graphs.

Results

DMM surgery performed with a stereomicroscope produces more homogeneous behavioural performance on OA pain tests. The mean percentage weightbearing asymmetry (Right/Left Hind (R/L)) in the naked-eye and microscope groups was statistically indistinguishable across all testing periods. Importantly, the percentage weightbearing asymmetry in both DMM groups was apparently lower than that in the sham-operated group (Figure 2a), showing that DMM surgery conducted with or without a stereomicroscope produces functional deficits in SWB distribution.

Table I presents detailed results on the variability of SWB performance. While mean percentage weightbearing asymmetry was not different between the two DMM surgical groups, statistical dispersion in each group was different, especially in the first month of testing (Figures 2b and 2c; Table I). The absolute deviation (AD) and the coefficient of variation (CV) reflect the group-specific dispersion of percentage weightbearing asymmetry, thus indirectly reflecting the stability of OA models. The AD of percentage weightbearing asymmetry (Figure 2b) in the microscope group tended to be lower than that in the naked-eye group at eight weeks

(microscope: 95% CI 3.37 to 5.65; naked-eye: 95% CI 8.80 to 15.75; $p < 0.001$), ten weeks (microscope: 95% CI 5.39 to 7.94; naked-eye: 95% CI 7.02 to 12.54; $p = 0.042$), 12 weeks (microscope: 95% CI 3.00 to 4.95; naked-eye: 95% CI 6.18 to 9.82; $p < 0.001$), 14 weeks (microscope: 95% CI 2.73 to 4.61; naked-eye: 95% CI 5.48 to 8.28; $p < 0.001$), and 16 weeks (microscope: 95% CI 2.98 to 4.99; naked-eye: 95% CI 5.22 to 7.61; $p = 0.002$, all independent-samples *t*-test) after surgery. The CV of percentage weightbearing asymmetry (Figure 2c) in the naked-eye group tended to decrease over time. By contrast, the CV of percentage weightbearing asymmetry in the microscope group remained stable and lower than that in the naked-eye group ($p = 0.015$). These dispersion measures suggest that DMM surgery aided by a stereomicroscope is superior compared to DMM surgery done under the naked eye in terms of SWB.

Table II presents detailed results on the variability of percentage withdrawal threshold (R/L) between the two DMM surgical groups. Like the weightbearing asymmetry, the mean percentage withdrawal threshold (Figure 2d) in the naked-eye and microscope groups showed no significant difference. Interestingly, the AD of the percentage withdrawal threshold (Figure 2e) in the microscope group was significantly lower than that in the naked-eye group at most timepoints after surgery (8 weeks: (95% CI 4.93 to 8.00) vs (95% CI 10.07 to 16.88); $p = 0.004$; 10 weeks: (95% CI 5.73 to 8.70) vs (95% CI 9.97 to 15.91); $p = 0.001$; 12 weeks: (95% CI 4.44 to 7.44) vs (95% CI 7.05 to 11.67); $p = 0.015$; 14 weeks: (95% CI 2.99 to 5.34) vs (95% CI 4.83 to 8.78); $p = 0.023$; 16 weeks: (95% CI 3.10 to 5.33) vs (95% CI 4.01 to 7.13); $p = 0.154$). Additionally, the CV of percentage withdrawal threshold (Figure 2f) in the naked-eye group showed a downward trend over time. But in the microscope group, the CV of percentage withdrawal threshold was stable and lower than those in the naked-eye group ($p = 0.030$). These results indicated that DMM surgery performed with the aid of a stereomicroscope produced more reproducible OA pain in von Frey test.

DMM surgery performed with a stereomicroscope produces more homogeneous functional performance of gait. Schematic illustration of gait analysis was shown in Figure 3a. Gait analysis revealed obvious gait abnormalities in both naked-eye and microscope groups (Figures 3b and 3c). This is consistent with the findings of previous studies that mice with DMM-induced OA exhibit severe changes in gait.[17,18] Analysis of the timing of gait showed that DMM mice spent less time standing on their right hind limb than on their left hind limb (Figure 3d). Quantitative analysis of gait data showed that the R/L percentage of print area, mean intensity, swing speed, and duty cycle were not statistically different between naked-eye and microscope groups at each timepoint,

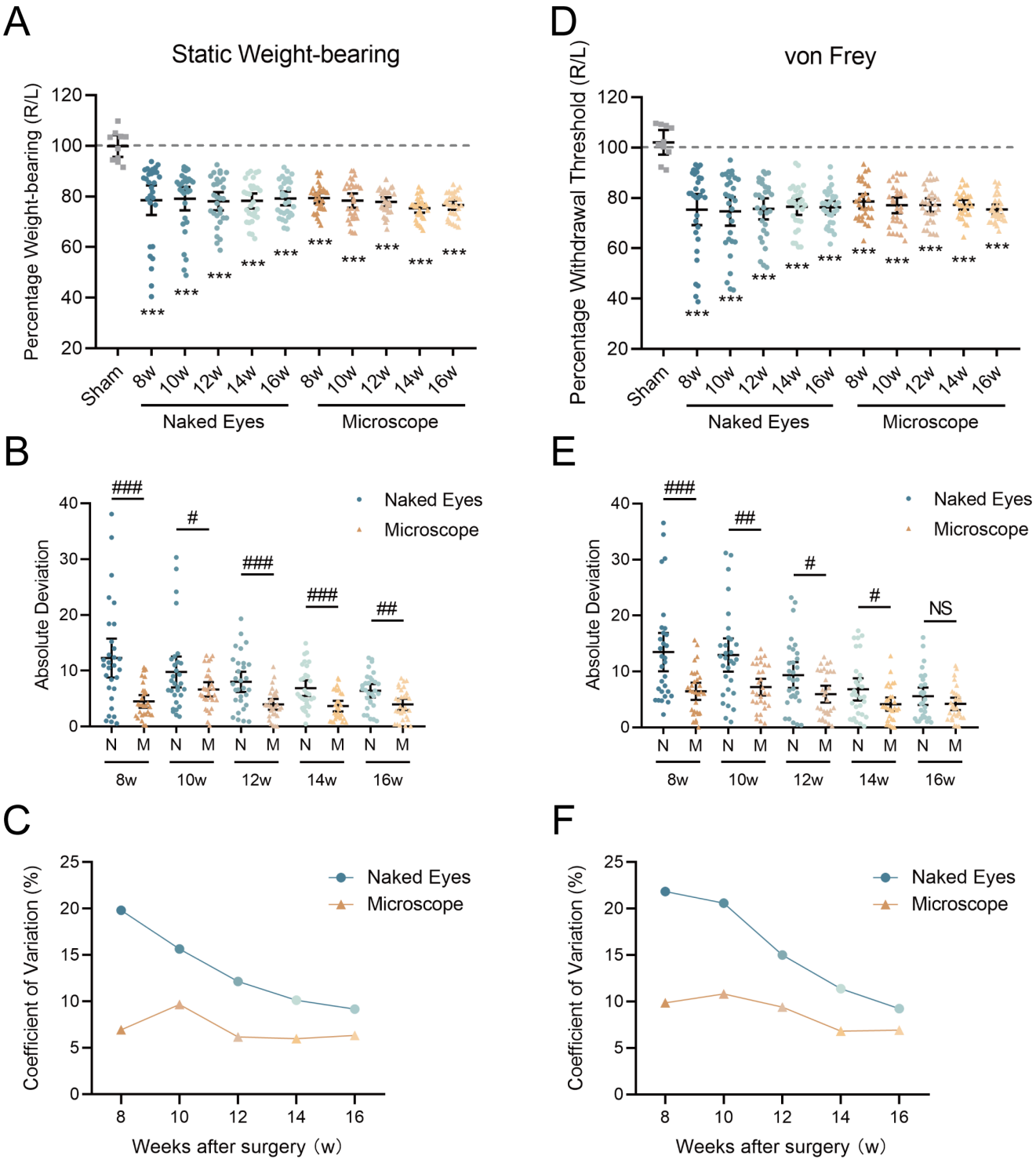

Fig. 2

Destabilization of the medial meniscus (DMM) surgery performed with a stereomicroscope produces more consistent behavioural performance on osteoarthritis (OA) pain tests. a) Percentage weightbearing asymmetry (Right/Left Hind (R/L)) in three groups at eight, ten, 12, 14, and 16 weeks post-surgery. b) Absolute deviation (AD) of percentage weightbearing asymmetry (R/L) in the microscope (M; n = 30) group differed to that of the naked-eye (N; n = 30) group at eight, ten, 12, 14, and 16 weeks post-surgery. c) Coefficient of variation (CV) trend over time. d) Percentage withdrawal threshold (R/L) in the N and M groups at eight, ten, 12, 14, and 16 weeks post-surgery. e) AD of percentage withdrawal threshold (R/L) results of the M group differed to those of the N group at eight, ten, 12, 14, and 16 weeks post-surgery. f) CV trend over time for the percentage withdrawal threshold. Data are expressed as means with 95% confidence intervals. Statistical analysis was performed using independent-samples *t*-test. ***p < 0.001 in comparison to the sham-operated group; #p < 0.05, ##p < 0.01, and ###p < 0.001 in comparison to the N group. NS, no significance.

Table I. Percentage of weightbearing asymmetry (Right/Left Hind) showed different statistical dispersion between the naked-eye group and the microscope group.

Weeks after surgery	8		10		12		14		16	
Group	**N**	**M**	**N**	**M**	**N**	**M**	**N**	**M**	**N**	**M**
Mean	78.56	79.36	79.17	78.37	78.12	77.85	78.28	75.34	79.23	76.61
95% CI of mean	72.75 to 84.37	77.30 to 81.42	74.54 to 83.79	75.54 to 81.20	74.58 to 81.67	76.05 to 79.64	75.32 to 81.25	73.66 to 77.02	76.52 to 81.94	74.80 to 78.42
Standard deviation	15.57	5.51	12.39	7.59	9.49	4.81	7.94	4.50	7.26	4.86
CV, %	19.82	6.94	15.65	9.68	12.15	6.18	10.14	5.98	9.17	6.34
95% CI of CV	15.47 to 24.17	5.42 to 8.46	12.22 to 19.08	7.56 to 11.80	9.48 to 14.81	4.82 to 7.53	7.92 to 12.36	4.66 to 72.9	7.16 to 11.18	4.95 to 7.73
Homogeneity of variance (F-test)	p < 0.001		p = 0.010		p < 0.001		p = 0.003		p = 0.034	

CI, confidence interval; CV, coefficient of variation; M, microscope group; N, naked-eye group.

Table II. Statistical dispersion of percentage withdrawal threshold (Right/Left Hind) differed from the naked-eye group to the microscope group.

Weeks after surgery	8		10		12		14		16	
Group	**N**	**M**	**N**	**M**	**N**	**M**	**N**	**M**	**N**	**M**
Mean	75.35	78.65	74.67	77.09	75.67	77.17	76.53	77.31	76.26	75.45
95% CI of mean	69.20 to 81.50	75.75 to 81.54	68.93 to 80.41	73.98 to 80.21	71.43 to 79.92	74.46 to 79.88	73.28 to 79.79	75.35 to 79.28	73.63 to 78.89	73.50 to 77.39
Standard deviation	16.47	7.75	15.38	8.34	11.36	7.26	8.72	5.27	7.04	5.22
CV, %	21.86	9.86	20.60	10.82	15.01	9.41	11.39	6.82	9.23	6.92
95% CI of CV	17.06 to 26.65	7.69 to 12.02	16.08 to 25.12	8.45 to 13.20	11.72 to 18.31	7.34 to 11.47	8.89 to 13.89	5.33 to 8.32	7.21 to 11.25	5.40 to 8.43
Homogeneity of variance (F-test)	p < 0.001		p = 0.002		p = 0.019		p = 0.009		p = 0.113	

CI, confidence interval; CV, coefficient of variation; M, microscope group; N, naked-eye group.

but the R/L percentages were lower than "100" in both DMM-surgery groups, indicating that these mice experienced more severe pain in their right hind limbs than left hind limbs (Supplementary Figure a). Further analysis of those results showed that the ADs of these four parameters in the naked-eye group were significantly greater than those in the microscope group (Figures 3e to 3h). These results also indicate that performing surgical DMM with or without a stereomicroscope can successfully induce OA pain, but performing surgery with stereomicroscope produces more consistent pain-related behavioural performance.

Performing DMM surgery with a stereomicroscope leads to a more consistent histological profile. Successful inducement of OA model in medial knee joint was observed at 16 weeks after DMM surgery, evidenced by degradation and calcification of articular cartilage. Loss of collagen type II immunostaining colocalized with cartilage lesions (Figure 4a). Fibrillation, osteophytes, narrowing of the joint space, and subchondral bone thickening were also found in the medial knee joint. However, the lateral aspect of the joint remained intact and normal (Figure 4b).

The summed OARSI scores (Figure 4c) for quantifying OA damage were significantly higher in both DMM groups compared to the sham-operated group ($p < 0.001$, independent-samples *t*-test); scores between naked-eye and microscope groups showed no difference. Surprisingly, we observed a similar pattern of variability of the summed OARSI scores as we did for the behavioural assessments; scores in the naked-eye group were more dispersed. The mean ADs of the summed medial tibial plateau (MTP) and medial femoral condyle (MFC) OARSI scores (Figure 4d) were significantly higher in the naked-eye group than in the microscope group (MTP: 95% CI 3.23 to 4.67 vs 95% CI 2.00 to 3.25, $p = 0.006$; MFC: 95% CI 1.92 to 3.20 vs 95% CI 1.92 to 3.20, $p = 0.023$, both independent-samples *t*-test), suggesting that DMM surgery aided by a stereomicroscope also produced a more consistent histological profile. The mean synovitis scores (Figure 4e) in the naked-eye and microscope groups were statistically indistinguishable (1.23 vs 1.18, $p = 0.619$, independent-samples *t*-test).

Finally, average optical density quantification (Figure 4f) revealed a significant loss of collagen type II in the DMM groups compared to the sham-operated group ($p < 0.001$, independent-samples *t*-test). The collagen loss was restricted to the MTP and MFC quadrants, consistent with cartilage lesions, indicating successful inducement of OA in the medial knee joint.

The maximum histological scores for MTP, MFC, lateral tibial plateau (LTP), and lateral femoral condyle (LFC) quadrants were statistically indistinguishable between the naked-eye and microscope groups (Supplementary Figure b), but both were significantly higher compared

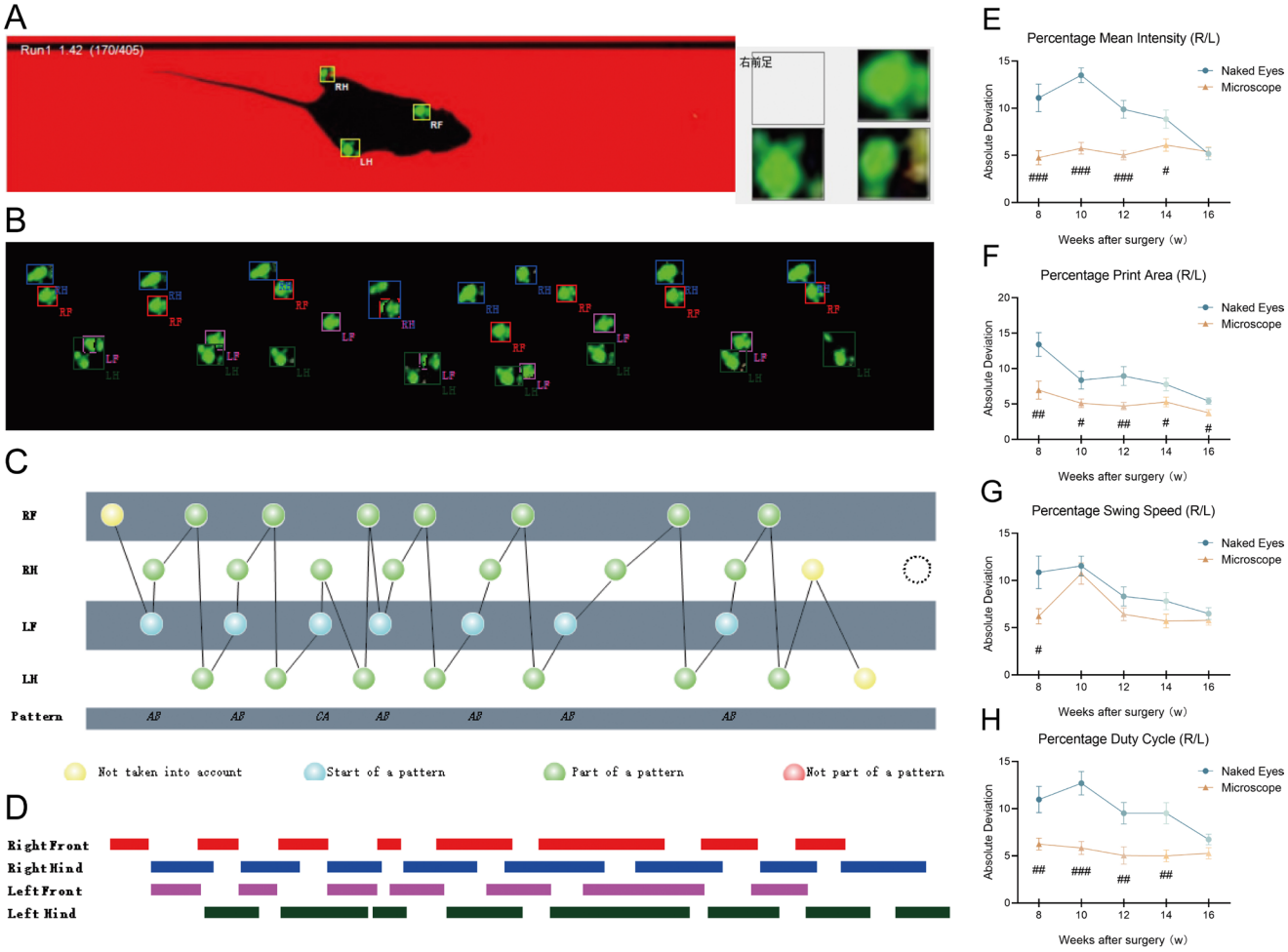

Fig. 3

Destabilization of the medial meniscus (DMM) surgery performed with a stereomicroscope produces more homogeneous functional performance of gait. a) Schematic illustration of gait analysis. b) Representative digital images of hind-limb footprints during gait analysis. c) Illustration showing representative foot-placement pattern during gait analysis. d) Representative timing view of gait. e) to h) Trends of absolute deviation for percentage print area, mean intensity, swing speed, and duty cycle (Right/Left Hind (R/L)) over time. Data are expressed as means with 95% confidence intervals (CIs). Statistical analysis was performed using independent-samples *t*-test. #p < 0.05, ##p < 0.01, and ###p < 0.001 in comparison to the naked-eye group. LF, left front; LH, left hind; RF, right front; RH, right hind; CA, cruciate type-A (foot-placement sequence: RF-LF-RH-LH); AB, alternate type-B (foot-placement sequence: LF-RH-RF-LH).

to the sham-operated group (p < 0.001, independent-samples *t*-test).

Discussion

In the present study, we systematically compared two different ways to surgically induce OA in a mouse model via DMM: surgery done with the aid of a stereomicroscope versus surgery done using only the naked eye. We have two main findings. First, during our observations, both groups (with and without a stereomicroscope) expressed similar levels of OA pain, as well as cartilage erosion and denudation. This indicates that OA can be successfully induced via DMM either with or without an operating stereomicroscope.

Our second main finding suggests, however, that the two methods of inducing OA via DMM surgery are not equivalent. DMM surgery aided by a stereomicroscope is superior to that conducted without a microscope in terms of variability of the outcome measures. More specifically, the greater variability in the naked-eye surgery group indicated that several animals experienced more pain than those in the microscope surgery group, but considering histopathological evaluation such as the "summed OARSI score" (Figure 4c), the greater variability suggested that some mice in the naked-eye group showed healthier articular cartilage. This might indicate that conducting DMM surgery with the naked eye caused less consistent meniscal destabilization. To the best of our knowledge, these results are the first to objectively demonstrate that DMM-OA model is optimized in mice through microsurgery, in terms of histological and functional performance.

We attribute the increased stability of OA performance to application of microsurgery technology and equipment.[19,20] Small anatomical structures inside the knee joint of mice are hard to identify. One way to increase the visual area in surgery is to use magnification, practically

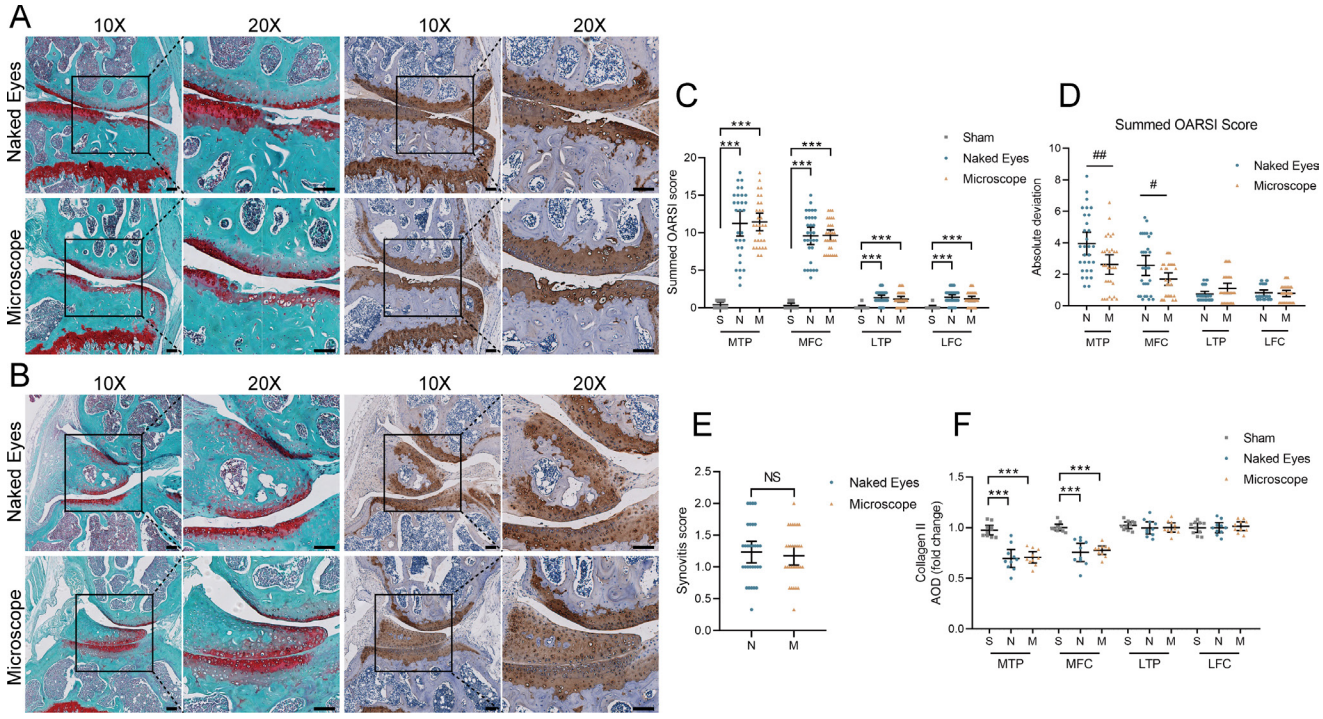

Fig. 4

Histological evaluation of surgical destabilization of the medial meniscus (DMM)-induced osteoarthritis (OA). a) and b) Representative images of Safranin O/Fast green staining (left panels) and diaminobenzidine (DAB)-visualized collagen type II immunohistochemical staining (right panels) of right knee frontal sections from naked-eye (upper rows) and microscope (lower rows) groups at 16 weeks after DMM surgery (A: MTP and MFC; B: LTP and LFC; scale bar, 100 μm). c) Quantitation of summed Osteoarthritis Research Society International (OARSI) scoring for histological evaluation at 16 weeks. Mean OARSI scores of four analysis quadrants for microscope (M; n = 30), naked-eye (N; n = 30), and sham-operated (S; n = 10) groups are plotted. d) ADs of the summed OARSI scores for M and N groups. e) Mean scores for synovial tissue inflammation of joint capsule. f) Average optical density (AOD) quantification of collagen type II immunostaining (n = 10). Data are expressed as means with 95% confidence intervals (CIs). Statistical analysis was performed using independent-samples *t*-test. ***p < 0.001 in comparison to the S group; #p < 0.05 and ##p < 0.001 in comparison to the N group. LFC, lateral femoral condyle; LTP, lateral tibial plateau; MFC, medial femoral condyle; MTP, medial tibial plateau; NS, no significance.

accomplished either by operating loupes or surgical stereomicroscopes. The quality and depth of field afforded by a stereomicroscope markedly aided visualization of the MMTL. During our study, we anecdotally observed more soft-tissue erosion in mice operated without the aid of a stereomicroscope. This extra damage beyond MMTL is likely due to surgical trauma caused by unaided vision. The stability of the knee relies partially on many soft-tissues, including the anterolateral structures, the lateral collateral ligament, and the posterior cruciate ligament.[21,22] In other words, extra-MMTL damage caused by poor vision likely explains the decreased consistency in the DMM-OA model induced without the aid of a stereomicroscope.

Our results have several practical implications. Increased variability in establishing an OA model affects study design and research costs. Sample size and power calculations help to determine if a study is feasible based on a priori assumptions about the study results and available resources.[23,24] When data dispersion is great, as in DMM surgery done without a microscope, a larger sample size will be required to detect the same difference based on the same power considerations. Thus, reproducible OA models in terms of functional and histopathological outcomes can help researchers to decrease the sample size, avoid unnecessary repeats, and save time. Also, for OA research related to valuable animals, such as transgenic mice, it can be difficult to afford these types of animals,[25,26] and it is even more expensive if some have to be 'wasted' because of an inconsistent OA model. OA induced by DMM surgery aided by a stereomicroscope can help to reduce the number of valuable animals and reduce research costs.

There are a few limitations in our study. First, we only used eight- to ten-week-old male C57BL/6 mice. Studies with OA models are subject to a multitude of variables, and outcome will depend on many factors such as species and strain, age, and sex.[27] Mice of other strains (129S6/SvEv) or sex (female), or different ages (six to eight weeks) have not been considered, which may affect the outcome of our research. Second, sample size calculation was based on the OARSI score in our pilot study. Higher statistical efficiency may be achieved when multiple parameters are taken into consideration.[23] Third, the operator (JL) who performed the surgeries of DMM was unblinded to the group assignments, which may have had an impact on the results. For example, the surgeon may have been more careful

and cautious when he could not directly visualize the meniscotibial ligament, or the surgery may have taken longer without a microscope, so the tissue may have dried out. These subjective factors derived from the operator himself may have affected our results. To minimize the measurement bias during operations, the surgeon was blinded to the design of this study until he finished all surgeries, and was requested to conduct them in strict accordance with the guidelines. Eventually, the OA histopathology was only evaluated at a single timepoint because of the limitation of research cost. Considering our pilot study of OA histopathology, it is possible that the trajectories of histopathological changes showed a similar pattern with the pain-related tests from eight to 16 weeks post-surgery.

In conclusion, our study sheds new light on the application of microsurgery for surgical DMM in mice. Although surgical stereomicroscope-aided DMM aided is technically difficult, it produces a relatively more homogeneous OA model in terms of the pain assessments and histopathological grading when compared with surgical DMM without stereomicroscope. However, since both ways can successfully induce mild-to-moderate OA, researchers can produce useful outcomes when performing surgical DMM under the naked eye. An individualized approach can be taken by researchers based on the design of their study. For novice researchers, we recommend that they perform surgical DMM in mice with an operating stereomicroscope, which can produce more robust results and avoid unnecessary waste.

Supplementary material

Specific information on pain-related behavioural tests and histological evaluation. Figures showing quantitative data of gait analysis and the maximal Osteoarthritis Research Society International scores at 16 weeks after surgery.

References

1. **Murray CJ**, **Atkinson C**, **Bhalla K**, **et al**. The state of US health, 1990-2010: burden of diseases, injuries, and risk factors. *JAMA*. 2013;310(6):591–608.
2. **He CP**, **Jiang XC**, **Chen C**, **et al**. The function of lncRNAs in the pathogenesis of osteoarthritis. *Bone Joint Res*. 2021;10(2):122–133.
3. **Bapat S**, **Hubbard D**, **Munjal A**, **Hunter M**, **Fulzele S**. Pros and cons of mouse models for studying osteoarthritis. *Clin Transl Med*. 2018;7(1):36.
4. **Glasson SS**, **Chambers MG**, **Van Den Berg WB**, **Little CB**. The OARSI histopathology initiative - recommendations for histological assessments of osteoarthritis in the mouse. *Osteoarthr Cartil*. 2010;18 Suppl 3:S17-23.
5. **Culley KL**, **Dragomir CL**, **Chang J**, **et al**. Mouse models of osteoarthritis: surgical model of posttraumatic osteoarthritis induced by destabilization of the medial meniscus. *Methods Mol Biol*. 2015;1226:143–173.
6. **Tao Z**, **Zhou Y**, **Zeng B**, **Yang X**, **Su M**. *MicroRNA-183* attenuates osteoarthritic pain by inhibiting the TGFα-mediated *CCL2/CCR2* signalling axis. *Bone Joint Res*. 2021;10(8):548–557.
7. **Glasson SS**, **Blanchet TJ**, **Morris EA**. The surgical destabilization of the medial meniscus (DMM) model of osteoarthritis in the 129/SvEv mouse. *Osteoarthritis Cartilage*. 2007;15(9):1061–1069.
8. **Li J**, **Zhang B**, **Liu W-X**, **et al**. Metformin limits osteoarthritis development and progression through activation of AMPK signalling. *Ann Rheum Dis*. 2020;79(5):635–645.
9. **David MA**, **Smith MK**, **Pilachowski RN**, **White AT**, **Locke RC**, **Price C**. Early, focal changes in cartilage cellularity and structure following surgically induced meniscal destabilization in the mouse. *J Orthop Res*. 2017;35(3):537–547.
10. **Hayami T**, **Pickarski M**, **Zhuo Y**, **Wesolowski GA**, **Rodan GA**, **Duong LT**. Characterization of articular cartilage and subchondral bone changes in the rat anterior cruciate ligament transection and meniscectomized models of osteoarthritis. *Bone*. 2006;38(2):234–243.
11. **Huesa C**, **Ortiz AC**, **Dunning L**, **et al**. Proteinase-activated receptor 2 modulates OA-related pain, cartilage and bone pathology. *Ann Rheum Dis*. 2016;75(11):1989–1997.
12. **Fang H**, **Beier F**. Mouse models of osteoarthritis: modelling risk factors and assessing outcomes. *Nat Rev Rheumatol*. 2014;10(7):413–421.
13. **He Z**, **Nie P**, **Lu J**, **et al**. Less mechanical loading attenuates osteoarthritis by reducing cartilage degeneration, subchondral bone remodelling, secondary inflammation, and activation of NLRP3 inflammasome. *Bone Joint Res*. 2020;9(10):731–741.
14. **Lin J**, **Hu W**, **Gao T**, **et al**. ε-Poly-l-lysine as an efficient cartilage penetrating and residing drug carrier with high intraarticular injection safety for treating osteoarthritis. *Chemical Engineering Journal*. 2022;430:133018.
15. **Percie du Sert N**, **Hurst V**, **Ahluwalia A**, **et al**. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biol*. 2020;18(7):e3000410.
16. **Zhang H**, **Li J**, **Xiang X**, **et al**. Tert-butylhydroquinone attenuates osteoarthritis by protecting chondrocytes and inhibiting macrophage polarization. *Bone Joint Res*. 2021;10(11):704–713.
17. **Zhu S**, **Zhu J**, **Zhen G**, **et al**. Subchondral bone osteoclasts induce sensory innervation and osteoarthritis pain. *J Clin Invest*. 2019;129(3):1076–1093.
18. **Muramatsu Y**, **Sasho T**, **Saito M**, **et al**. Preventive effects of hyaluronan from deterioration of gait parameters in surgically induced mice osteoarthritic knee model. *Osteoarthritis Cartilage*. 2014;22(6):831–835.
19. **Pons G**, **Tang JB**. Major changes in lymphatic microsurgery and microvascular surgery in past 20 years. *Clin Plast Surg*. 2020;47(4):679–683.
20. **Bonde CT**, **Jensen LT**, **Tos T**, **et al**. Microsurgery in reanimation, lymphoedema and hand surgery. *Ugeskr Laeger*. 2016;178(23):V01160072. (Article in Danish)
21. **Athwal KK**, **El Daou H**, **Lord B**, **et al**. Lateral soft-tissue structures contribute to cruciate-retaining total knee arthroplasty stability. *J Orthop Res*. 2017;35(9):1902–1909.
22. **Tavlo M**, **Eljaja S**, **Jensen JT**, **Siersma VD**, **Krogsgaard MR**. The role of the anterolateral ligament in ACL insufficient and reconstructed knees on rotatory stability: A biomechanical study on human cadavers. *Scand J Med Sci Sports*. 2016;26(8):960–966.
23. **Schmidt SAJ**, **Lo S**, **Hollestein LM**. Research techniques made simple: sample size estimation and power calculation. *J Invest Dermatol*. 2018;138(8):1678–1682.
24. **Rodríguez Del Águila M**, **González-Ramírez A**. Sample size calculation. *Allergol Immunopathol (Madr)*. 2014;42(5):485–492.
25. **Mobley CB**, **Vechetti IJ**, **Valentino TR**, **McCarthy JJ**. CORP: Using transgenic mice to study skeletal muscle physiology. *J Appl Physiol (1985)*. 2020;128(5):1227–1239.
26. **Lorenz J**, **Grässel S**. Experimental osteoarthritis models in mice. *Methods Mol Biol*. 2014;1194:401–419.
27. **van der Kraan PM**. Factors that influence outcome in experimental osteoarthritis. *Osteoarthritis Cartilag*. 2017;25(3):369–375.

Author information:
- W. Hu, MD, Orthopaedic Surgeon
- J. Lin, MD, Orthopaedic Surgeon
- J. Wei, MD, Orthopaedic Surgeon
- K. Fu, MD, PhD, Orthopaedic Surgeon
- T. Zhu, MD, Orthopaedic Surgeon
  Department of Orthopaedic Surgery, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China.
- Y. Yang, MD, PhD, Researcher, Institute of Microsurgery on Extremities, Department of Orthopedic Surgery, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China.
- H. Zhu, MD, PhD, Orthopaedic Surgeon, Researcher
- X. Zheng, MD, PhD, Orthopaedic Surgeon, Researcher
  Department of Orthopaedic Surgery, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China; Institute of Microsurgery on Extremities, Department of Orthopedic Surgery, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China.

Author contributions:
- W. Hu: Investigation, Writing – original draft.
- J. Lin: Investigation, Writing – original draft.
- J. Wei: Investigation, Writing – original draft.
- Y. Yang: Formal analysis, Writing – review & editing.

- K. Fu: Formal analysis, Writing – review & editing.
- T. Zhu: Formal analysis, Writing – review & editing.
- H. Zhu: Conceptualization, Writing – review & editing.
- X. Zheng: Conceptualization, Writing – review & editing.

- W. Hu, J. Lin, and J. Wei contributed equally to this work.

- H. Zhu and X. Zheng contributed equally to this work.

- W. Hu, J. Lin, and J. Wei are joint first authors.

- H. Zhu and X. Zheng are joint senior authors.

Funding statement:
- The authors disclose receipt of the following financial or material support for the research, authorship, and/or publication of this article: financial support from the National Natural Science Foundation of China (Project No. 81974331, No. 82172421 to Xainyou Zheng; No. 82002324 to Hongyi Zhu) and from Shanghai Jiao Tong University Affiliated Sixth People's Hospital (No. ynms202107 to Yunlong Yang).

ICMJE COI statement:
- The authors declare no conflicts of interest.

Ethical review statement:
- Each author certifies that his or her institution approved the animal protocol for this investigation and that all investigations were conducted in conformity with ethical principles of research.

Open access funding
- The authors report that the open access funding for their manuscript was self-funded.