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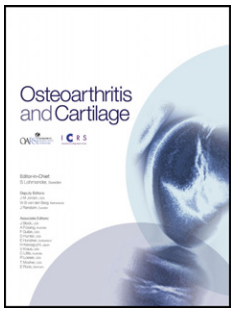

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REVISION 2

Comparison of gait and pathology outcomes of three meniscal procedures for induction of knee osteoarthritis in sheep

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Running title: Sheep meniscectomy vs meniscal release

Summary

Objective(s): Meniscectomy of sheep induces a well-established animal model of human osteoarthritis (OA). This study compared the clinical (lameness) and pathological outcomes of unilateral, complete medial meniscectomy versus two less traumatic and more easily performed meniscal destabilization procedures.

Methods: Four-year old wethers (n=6/group) underwent sham operation, cranial pole release (CPR), mid-body transection (MBT) or total meniscectomy (MX) of the medial meniscus. Joints were assessed for gross pathology (cartilage erosion and osteophytes), histomorphometry, two histopathology scoring methods (modified Mankin-type and Pritzker score), and immunohistology for ADAMTS- and MMP-cleaved neopepitopes, at 12 weeks post-op. Ground reaction forces (GRFs) were determined by forceplate in a subset (n=4/group) at baseline, 2.5, 8, and 12 weeks post-op.

Results: Gross pathology scores of operated groups differed significantly from sham animals ($P<0.05$) but not from each other, though qualitative differences were noted: CPR sheep developed more cranial and focal lesions, while MBT and MX joints showed more widespread lesions and osteophyte formation. Similarly, histopathology scores were significantly elevated versus sham but did not differ between operated groups at

P<0.05, except for a trend for lower tibial cartilage histopathology in MBT consistent with the immunohistologic pattern of reduced aggrecanase-cleavage neopepitope in that model. CPR sheep developed less femoral subchondral sclerosis, suggesting some residual biomechanical effect from the destabilised but intact meniscus. Few significant differences were noted between operated groups in forceplate analyses, though gait abnormalities appeared to be least in CPR sheep, and most persistent (>12 weeks) in MBT animals.

Conclusion: The well-validated ovine meniscectomy model and the simpler meniscal destabilization procedures resulted in broadly similar joint pathology and lameness. Meniscal CPR or MBT, as easier and more clinically relevant procedures, may represent preferred models for the induction of OA and evaluation of potential disease-modifying therapies.

Introduction

The menisci perform many important roles within the knee joint complex, such as improving congruity and stability of the femoro-tibial contact, mechanical shock absorption and load-sharing, facilitating limited rotation via menisco-tibial translation, and generating proprioceptive feedback via internal mechanoreceptors[1,2]. Menisci consist of approximately 75% collagen by dry weight, with collagen fibrils predominantly oriented in a circumferential fashion to resist tensile hoop stresses during loading. A complex of meniscotibial, meniscofemoral, and peripheral capsular attachments restrain meniscal movement, particularly outward 'extrusion' under loading[2,3].

Meniscal injuries are among the most frequent injuries dealt with by orthopedic surgeons, and are commonly treated by arthroscopic meniscal debridement or meniscectomy[3]. Surgical meniscectomy is known to be a risk factor for knee osteoarthritis (OA), increasing relative risk approximately 6-fold[4,5]. However long term outcomes, particularly for clinical measures, are more favourable where only partial meniscectomy occurs – typically removal of less than one third of the meniscus, leaving the peripheral rim intact[6,7]. This points to the importance of the deep circumferential fibres in the peripheral rim in resisting hoop stresses induced by joint loading[3].

By contrast, meniscotomy or 'meniscal release' (meniscal ligament desmotomy) procedures have been advocated as adjunct procedures in dogs to reduce symptomatic meniscal injury secondary to cruciate ligament rupture, especially after tibial plateau levelling osteotomy (TPLO)[8,9]. In this context, hemimeniscectomy has been proposed to have fewer degenerative sequelae than complete removal[10,11]. However there are relatively few studies directly comparing outcomes of complete meniscectomy versus various partial procedures advocated as salvage surgeries, in either animals or man. Robinson (2006) found no difference between the effect of meniscectomy versus meniscal release on vertical ground reaction forces (GRFs) after TPLO in dogs[12], whilst hemimeniscectomy had no additive effect after meniscal release on tibial translation or pressure distribution in canine cadaver studies[13,14]. Johnson (2004) found no clinically important differences in outcomes (including gait analysis, gross or microscopic pathology, subchondral bone density) 16 weeks after complete medial meniscectomy or caudal pole hemimeniscectomy, though a lesser increase in synovial fluid 7D4 neoepitope (a marker of chondroitin sulphate change) suggested a lower degree of chondrocyte disturbance in the less radical procedure, that might potentially be significant in longer term studies[15]. A comparison of the same two procedures in sheep, again including gait analyses, also found no significant difference between surgeries[16]. Further, meniscal release alone in the cruciate-intact canine stifle has been shown to cause lameness and degenerative joint disease[17]. Together these studies suggest that any surgery impairing the ability of the meniscus to resist hoop stresses will likely have similar outcomes, regardless of the amount of meniscal tissue removed.

Variations of meniscectomy or meniscotomy surgery have been used for the surgical induction of OA in mice, rabbits, rats, guinea pigs, dogs, and sheep, either alone, or in combination with other insults such as anterior cruciate ligament transection[18,19]. Total medial or lateral

menisectomy in sheep reliably induces focal cartilage, bone and synovium disturbances closely resembling human OA, and represents a useful model for evaluation of OA therapeutics[18,20,21]. Gait analyses in this model have demonstrated a similar pattern of hindlimb loading to humans[22], and post-surgical GRF changes comparable to OA patients[23,24]. One drawback of these models is that the surgical access required to sever the ligament attachments at each meniscal pole is difficult, necessitating a large incision, prolonged operative time, and careful haemostasis. The objective of this study was therefore to investigate whether more easily performed procedures, namely mid-body meniscotomy or cranial pole meniscal release, would produce similar clinical (gait analysis) and pathological (gross and histologic pathology scoring) outcomes compared to the well-established total meniscectomy model.

Methods

EXPERIMENTAL DESIGN

Twenty-four, 4-year old Merino wethers, selected for uniformity of size and conformation, were subjected (unilaterally, in the right knee) to one of four variants of meniscal surgery to induce osteoarthritis (n=6 per treatment): (1) total medial meniscectomy (MX), using a combination of scalpel and meniscotome to sever capsular and ligamentous attachments as described previously[25]; (2) mid-body meniscal transection (MBT) of the medial meniscus using a No. 11 scalpel blade, after partial stripping of peripheral capsular attachments; (3) cranial pole meniscal release (CPR), by isolation and transection of the cranial meniscotibial ligament; or (4) a sham procedure replicating the common surgical approach via medial parapatellar arthrotomy, but without meniscal damage (SHAM). Surgery was performed under halothane anaesthesia after intravenous diazepam/ketamine induction, with electrocautery used to maintain careful intraoperative haemostasis. After a brief indoor recovery period, all sheep were allowed free range on irrigated pasture for the remainder of the 12 week trial period. Animal use in this study was approved by the Murdoch University Animal Ethics Committee.

GAIT ANALYSIS

Sheep were acclimatised to being lead by a halter and leadrope over several sessions prior to surgery, and a subset of four of the best-adapted animals was selected from each group, except the sham group. Ground reaction forces were determined pre-operatively and at 2.5, 8 and 12 weeks post-operatively, using an in-ground piezoelectric forceplate [Kistler® 9281CA, Kistler Instrumente, Switzerland] capturing at 300Hz [BioWare v3.21, Kistler Instrumente, Switzerland]. Each animal was halter-lead in circles so as to repeatedly cross the region of the forceplate; results are the mean of five 'clean' traces per leg.

Analysis of forceplate data was performed as previously described[24] after normalising to percent of total contact (stance) time (CT). Normalised traces were used to determine peak vertical (F_z) GRF, normally equivalent to the higher of the two peaks of the typical two-peaked curve (PVF). CT was multiplied by average vertical force across the contact period (AVF) to yield vertical impulse (IMP). Loading slope (LS) was determined as the average slope of the initial 20% of normalised contact period. All data were normalised to body weight (BW) in Newtons as recorded prior to each session.

To allow graphical summary of results, normalised data from all 4 animals in each group were pooled to generate a mean composite, normalised trace at each timepoint. This was then recorrected to mean contact time to yield a simulated force versus time plot representing the mean of all four animals, compared to baseline pre-operative data (as shown in Figure 1).

PATHOLOGY AND HISTOPATHOLOGY

All sheep were euthanased at 12 weeks post-surgery, when both knee joints were opened and photographed for later scoring by a single observer (DB) blinded to treatment. Cartilage damage (0-4) and osteophyte development (0-3) were scored as previously described[26]. Mid-coronal osteochondral sections (3-4mm thick) of the medial femoral condyle (MFC) and medial tibial plateau (MTP) were processed histologically as previously described[26], before scoring of toluidine blue-stained sections using two published scoring systems: (1) a modified Mankin-type scoring system[27] (briefly, sections were scored for structural damage (0-10), chondrocyte density (0-4), cell cloning (0-4), loss of pericellular (0-4) and interterritorial (0-4) loss of toluidine blue, and tidemark changes (0-3); the maximum possible score was 29); and (2) the Pritzker *et al.* (2006) histopathology grading system[28], which scores the product of 6 grades (depth of lesion) and 4 stages (extent of involvement) to derive a maximum score of 24. After application of the published method failed to detect significant differences between groups, a slightly modified method was applied such that in all specimens the region of the tibial condyle normally covered by the meniscus (MTP-C) was scored separately from the uncovered region (MTP-U). For each method, the scores of 2 independent, blinded observers (CL and MS) were pooled and a mean score determined for each group. Addtionally, mean cartilage thickness and subchondral bone plate thickness was determined in the inner (axial), middle, and outer zones of each condyle (*ie.* each representing 1/3rd of the articular surface) by image analysis as previously described[26].

Serial sections of the MTP from 3 animals in each of the four surgical procedure groups were immunostained with antibodies recognising (i) the ADAMTS-cleaved aggrecan interglobular domain neoepitope NITEGE (mAb Agg-C1 0.55µg/ml; provided by Dr. Carl Flannery, Pfizer Inc, Cambridge, MA), (ii) the MMP-cleaved aggrecan interglobular domain neoepitope DIPEN (affinity-purified polyclonal antisera 0.17µg/ml; provided by Assoc. Prof. Amanda Fosang, University of Melbourne, Melbourne, Victoria, Australia), and (iii) MMP-13 (LS-B3168 Lifespan Biosciences polyclonal antisera 2µg/ml). Sections were rehydrated, and digested with either 0.1 units/ml protease-free chondroitinase ABC, (Sigma-Aldrich, Castle Hill, New South Wales, Australia) plus 0.1 units/ml of keratanase I (Sapphire Biosciences, Alexandria, New South Wales, Australia) for NITEGE and DIPEN, or 500U/ml bovine testicular hyaluronidase (Sigma-Aldrich, Castle Hill, New South Wales, Australia) for MMP-13, prior to overnight incubation with primary antibodies or equivalent concentrations of species-matched immunoglobulins on identically treated sections as negative controls and color development as previously described[29].

STATISTICAL COMPARISONS

Gross pathology and histopathological scores were analysed using the Kruskal-Wallis test for multiple groups and if significance was found, Mann Whitney U-tests for between group comparisons were used. GRF data were compared as body-weight corrected data, with data from 2.5, 8, and 12 weeks post-surgery compared with baseline (Week 0) data from the same leg by paired t-test. In addition, data from the right (operated) leg and the contralateral left leg

were also compared by paired t-test. As within-animal (paired) comparisons were used for all gait analysis, results are presented as both group weight-corrected means, and mean percentage of the appropriate baseline value. Significance level of $P<0.05$ was used for all comparisons; to aid interpretation of data differences at a significance level of $P<0.10$ are also indicated in Table 1 and 2.

Results

GAIT ANALYSIS

When considered together, all three meniscal surgeries temporarily caused moderate unloading of the limb of the operated limb, and flattening of the normal two-peaked vertical GRF versus time curve *(Figure 1)*. In the operated right leg, reduction in AVF, PVF, and IMP were greatest at 2.5 weeks post-surgery (to approximately 85% of baseline) *(Figure 2)*, with a compensatory increase in CT on the unoperated left leg. At 8 weeks post-op, PVF and AVF were reduced for both legs (approximately 87% and 93% of baseline for the right and left legs respectively), though an increase in CT compensated such that vertical impulse was normal. At 12 weeks post-op, GRFs had mostly recovered with only a slight reduction in AVF (right leg) and increased CT (both legs) persisting. Loading slope was more severely and persistently depressed after surgery, though only significantly reduced relative to baseline ($P<0.05$) in the MBT group at 2.5 and 8 weeks post-op. Though GRFs were not tested in the sham group, subjective assessment failed to detect any gait disturbance beyond the immediate post-surgical period.

Few significant differences were noted between operated groups in ground reaction forces *(Table 1)*, though gait abnormalities appeared to be least in the CPR group, and most persistent in MBT animals. The MBT group was notable for persistent significant abnormality of both left

leg (CT, IMP) and right leg (AVF, PVF) GRFs relative to baseline at 12 weeks. Composite GRF plots suggested that CPR sheep retained a typical two-peaked trace pattern throughout the trial period, while MX and MBT sheep showed a more 'plateau-like' GRF trace at 2.5 and 8 weeks, with partial recovery of the two-peaked pattern at 12 weeks *(Figure 1)*.

[Figure 1 and 2, and Table 1 near here]

JOINT PATHOLOGY

All meniscal surgeries resulted in fissuring or focal erosion of cartilage in the operated medial compartment, with few or mild changes in cartilage of the lateral condyles. Moderate-marked osteophyte development was typically observed. As is typical in mature sheep, mild cartilage changes (slight softening or fibrillation) were also commonly observed in the medial compartment of sham-operated sheep. Partial regrowth of soft, jelly-like meniscoid tissue was frequently observed filling the distracted meniscal defect of MBT sheep, and encroaching into the joint of MX sheep *(Figure 3)*.

Gross pathology scores of all operated groups differed significantly from sham animals ($P<0.05$) but not from each other, though qualitative differences were noted *(Figure 4)*: CPR sheep

developed focal lesions mostly in the cranial area of the tibial condyles and corresponding femoral contact areas, MBT showed more centrally-located lesions, while cartilage lesions in MX joints appeared to be more widespread. MBT joints showed a trend towards greater osteophyte formation that was not statistically significant (*P*=0.054).

Modified Mankin-type histopathology scores were significantly elevated versus sham, but did not differ between operated groups, with the exception of the medial tibial condyle of MBT animals, which showed a trend towards lower scores versus MX that was not statistically significant (*P*=0.055), and did not differ statistically from sham-operated sheep *(Table 2)*. The Pritzker histopathology scores were significantly elevated versus sham for meniscus-covered tibial cartilage (MTP-C) only, with femoral cartilage showing a significant increase in stage but not grade of histopathology versus sham *(Table 2)*. The histopathology scores derived by the two methods were significantly correlated (*P*<0.001, *R*=0.768) but showed considerable variation of Mankin-type score within some mid-level Pritzker scores *(Figure 5)*. Examples of typical cartilage histopathology are in Figure 6.

[Table 2, Figures 3 - 6 near here]

In sham-operated joints the ADAMTS-generated aggrecan neoepitope NITEGE was localized to the inter-territorial matrix of the calcified cartilage (Figure 7A). Following MX and CPR, NITEGE was also present in the inter-territorial matrix of the superficial third of the non-calcified cartilage (Figure 7B&C). In contrast, the MMP-generated aggrecan neoepitope DIPEN was present in the

non-calcified inter-territorial matrix in SHAMs, with decreased staining observed in MX and CPR joints (Figure 7E-G). Chondrocytes in the deep and calcified cartilage zones stained positive for MMP-13 in SHAMs (Figure 7K). Following MX, CPR and to a lesser extent MBT, chondrocytes throughout the depth of the non-calcified cartilage were immunopositive for MMP-13 (Figure 7L-N). Equivalent concentrations of species-matched IgG showed no positive staining.

[Figure 7 near here]

Histologically-determined mean cartilage thickness *(Table 3)* was not significantly affected in any surgical group, except for a thinning of cartilage in the inner (unprotected) zone of the MTP of the CPR group (P=0.044). Subchondral bone plate thickness was substantially increased in the middle zone of the MFC in MX (P=0.012) and MBT (P=0.022) sheep, but not CPR sheep; the CPR group therefore showed significantly less central subchondral sclerosis in the MFC compared to MX (P=0.038).

[Table 3 near here]

Discussion

Results from this comparative study confirm that all tested surgical interventions have similar clinical and pathological outcomes, regardless of whether the meniscus is removed or destabilised. Only a few differences were noted: namely, forceplate data suggested that mid-body meniscal transection resulted in more severe and prolonged GRF deficit, compared to milder changes following cranial pole release. This difference suggests that the nature of the meniscal lesion may influence the post-surgical gait deficits in this model, though the temporary nature of these deficits suggests they are not correlated to progressive cartilage degradation, and instead may be primarily related to synovial or subchondral bone pain[21,24,30]. Pain from the meniscus itself cannot be discounted since it is richly innervated, particularly at its poles, a property usually attributed to joint-sparing proprioception[1,31]. Subchondral sclerosis was reduced in the CPR model - a significant outcome which suggests that the destabilised meniscus may have some residual function in spreading contact forces – and it is possible that the milder gait deficit may be attributable to reduced subchondral pathology. However it is also possible that the focal point of bone sclerosis was merely shifted out of the plane of the histological sections.

The degree of lameness observed from all surgeries (except sham) was in line with other studies of sheep meniscectomy[23,24], and showed many of the key changes observed in human OA patients, namely modest reduction in peak GRFs, delayed early loading, greater minimal mid-stance force (thus loss of distinctive two-peak GRF), and increased stance time[32-34]. The effects of meniscectomy in humans have only been studied kinematically, showing reduced range-of-motion, increased knee adduction moment, reduced mid-stance knee flexion, and

increased external rotation[35,36,37]. Sheep may therefore represent a more useful functional model of meniscectomy than smaller species, given that after comparable surgery rabbits show little change in knee kinematics[38], whilst rats may show only minor changes in static weight-bearing[19,39]. As has been shown previously[23], significant alterations (principally in CT and LS) were seen in the unoperated left leg, confirming the importance of comparison to preoperative baseline data rather than an unoperated 'control' joint for gait studies in animals.

Results from gross and histopathology scores suggested a similar degree of degeneration after all three meniscal destabilisation procedures, confirming the lack of any substantial protective effect from residual meniscal tissue. There was a modest reduction in tibial cartilage modified Mankin-type score and osteophytosis following mid-body transection versus complete meniscectomy, although this could not be resolved at $P<0.05$ in this relatively low-powered pilot study. However, the immunohistology results were consistent with less advanced cartilage damage in MBT compared with MX or CPR. In the latter two models the increased cleavage of aggrecan by ADAMTS (NITEGE staining) and chondrocyte MMP-13, support a role for these enzymes in the pathogenesis of OA cartilage degradation in the sheep as in other species[40-43]. The presence of the MMP-cleaved aggrecan neoepitope DIPEN in sham-operated joints, is consistent with previous data on MMP-driven aggrecanolysis in normal cartilage growth and remodelling[44]. Loss of DIPEN in early OA 3 months after MX and CPR, may be associated with further C-terminal proteolysis and loss of the neoepitope, and/or loss of the entire HA-binding fragment from the tissue[45]. Increased DIPEN is only seen in late stage cartilage breakdown[46-47], confirming the early nature of the OA in the current models. That similar pathology and molecular mechanisms of cartilage degradation were seen in MX and CPR suggests that despite the potential for contribution of the meniscus itself to the enzyme burden in the joint[48], it

is the loss of meniscal mechanical functions that are predominantly responsible for early cartilage pathology in these models.

The reason for the somewhat reduced cartilage damage following MBT is unclear. It is difficult to reconcile with any protective effect of the residual meniscal tissue for two reasons. Firstly, the osteochondral section sampled corresponded with the site of meniscal incision (which obviously distracted post-surgery, given the substantial span of regrowth tissue typically present at necropsy); secondly, a similar protective effect was not seen with cranial pole meniscal release. Subjective evaluation of lesion distribution *(Figure 3)* suggested the potential for differences in pathology score to be artefactual of the single mid-condylar coronal section sampled, relative to the specific site of cartilage lesions, which seemed to vary between models. This focal shift may be attributable to a biomechanical effect, for example relative rotation of the tibia as has been shown in human meniscectomy patients[37], rather than any protection from remnant meniscal tissue. However, since the lesion distribution suggested conversely that mid-body transection should result in a *more* centralised tibial lesion compared to other techniques, this effect should not account for any reduction in pathology following this technique.

The application of both a modified Mankin-type[27] and the Pritzker[28] histopathological scoring methods allowed useful comparison of these methods in this animal model of early OA. Results showed that while the two methods were significantly correlated, the Pritzker system potentially yielded relatively lower scores for moderate cartilage lesions, due to its greater reliance on structural defects (depth of fissures or erosion) over concomitant matrix and cellular changes (Figure 5). For the same reason, the Pritzker system potentially derived higher scores for sham-operated controls, since some degree of cartilage fissuring and superficial erosion in otherwise fairly normal cartilage is a common finding in the MFC and uncovered MTP of ‘normal’ mature

sheep. Due to these higher scores for SHAM animals, Pritzker scores did not differ significantly between treatment groups for the MFC, with only stage but not grade increasing significantly after meniscal surgery. However, a statistically significant increase in Pritzker score could only be resolved in the MTC by separately scoring those areas originally covered by the meniscus. It was noted that, when taken alone, the structure sub-component (0-10) of the modified Mankin-type score also failed to resolve a statistically significant difference between treatment groups. Taken together, this suggests that for early OA when structural cartilage damage may be modest, as in the current study, Mankin-type histopathological scoring systems will provide more discriminative results.

Given the lack of substantial differences between techniques, this sheep study adds to the growing consensus that any intervention destroying the ability of a meniscus to resist tensile hoop stresses, will result in a similar degree of cartilage degeneration. This supports similar conclusions from related studies of hemimeniscectomy in sheep[16] and hemimeniscectomy[15] and meniscal release[12,17] in dogs. Further, work in rabbits has shown, despite the reknowned capacity for meniscal regeneration in this species, similar degenerative results from either cranial or caudal meniscotibial desmotomy, although differences were noted in histologic repair[49]. Together, these results argue against the conclusion of earlier authors[10,11], of a chondroprotective effect from preservation of destabilised meniscal tissue, although a protective effect versus focal subchondral sclerosis was suggested in the CPR model. It should be noted that this conclusion may not apply equally to lateral meniscal surgery techniques, given the added presence of the popliteus tendon (which may potentially provide some mechanical constraint on meniscal extrusion), and the greater meniscal coverage of lateral tibial condyle, which may be responsible for the greater cartilage disturbance seen following lateral

meniscectomy, despite lower contact stresses[6,50]. It should also be noted that cartilage degeneration would be expected to be progressively more severe had a six month endpoint been used[18,25], thus we cannot exclude that differences between the three surgeries would emerge in a longer term study.

We conclude from this study that although minor differences were suggested between meniscal surgery techniques for the induction of OA models, the primary pathological outcomes are similar at 3 months post-surgery. However, a few subtle differences in outcomes were revealed (location of tibial erosions; reduced aggrecanase-mediated neoepitope in MBT tibial cartilage; reduced subchondral bone sclerosis in CPR femur) that may become important in longer-term. The type of surgery performed may be more significant where clinical outcomes such as force-plate GRF analysis are important, since these differences are more likely related to non-cartilage joint components, thus may be more likely to be influenced by the nature of the meniscal intervention. Where gait outcomes are not required, the lesser gait disturbance in the cranial pole release model, in the absence of a concurrent reduction in OA progression (with the possible exception of focal bone sclerosis), may recommend this as the preferred surgical model for both practical and ethical reasons.

Conflicts of interest

This study was independently funded by the authors, who have no conflicts of interest to disclose.

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Contributions

M.A.C. was principal author involved in conception and design, animal surgery and force plate studies, analysis and interpretation of data, drafting and critical revision of the manuscript. R.A.R. was involved in obtaining funding, conception and design, animal surgery and force plate studies, pathology scoring, and critical revision of the manuscript. G.C. was involved in study design, animal surgery, data analysis and critical revision of the manuscript. A.D. was involved in animal surgery and force plate studies, data analysis, and critical revision of the manuscript. D.B. was involved in pathology scoring, data analysis, and critical revision of the manuscript. M.M.S. was involved in conception and design, pathology scoring, statistical analysis and interpretation of data, and drafting and critical revision of the manuscript. C.B.L. was involved in obtaining funding, conception and design, pathology scoring, immunohistology scoring, analysis and interpretation of data, and critical revision of the manuscript.

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Figure Legends

Fig.1. Composite plots of mean, normalised vertical ground reaction forces as a proportion of body weight (BW) at 2.5, 8 and 12 weeks post-surgery, by treatment group. Dashed lines indicate baseline values; n=4 sheep per group.

Fig.2. Plots of *(a)* peak vertical force (PVF), and *(b)* vertical impulse versus weeks post-surgery, in the operated (R) leg after complete meniscectomy (○), cranial pole meniscal release (□), or mid-body meniscal transection (△). Data are percentage ± 95% C.I. of paired baseline values.

Fig.3. Representative images of *in situ* meniscal remnants 12 weeks after meniscectomy (MX), mid-body meniscal transection (MBT), or cranial pole meniscal release (CPR). Asterisks (*) indicate gelatinous 'meniscoid' regrowth after meniscectomy, and spanning the distracted defect after MBT. Medial movement of the cranial pole after CPR appeared to be minimal (arrow).

Fig.4. Composite overlays combining the areas of visible cartilage erosion on the medial tibial condyle after meniscectomy (MX), mid-body meniscal transection (MBT), or cranial pole meniscal release (CPR). Lesions after CPR appeared to be more likely to be restricted to the cranial half of the condyle, while lesions after MX appeared more widespread.

Fig. 5. X-Y plot of modified Mankin-type versus Pritzker histopathology scores, for MFC and MTP cartilage. The lines of best fit (solid line) and theoretical intersection of maxima and minima (dotted line) are indicated.

Fig. 6. Examples of medial tibial condyle histopathology from each treatment group. Note the degree of variation in cellularity and matrix proteoglycan loss, concomitant with only minor structural damage. Mankin-type (0-29) and Pritzker (0-24) scores are A: 4 and 4.5; B: 10 and 6; C: 17 and 15.75; D: 17.5 and 16 respectively.

Fig. 7. Immunostaining of cartilage from each treatment group for: the ADAMTS-generated aggrecan neoepitope NITEGE (A-D); the MMP-generated aggrecan neoepitope DIPEN (F-I); and MMP-13 (K-N). The images show staining in the full depth of cartilage from the region of the medial tibial plateau protected by the meniscus where the cartilage lesion develops (Fig 6; approximately 1/3rd of the distance from the abaxial joint margin), and are representative of 3 replicates from each group (Sham = arthrotomy alone, MX = meniscectomy, CPR = cranial pole meniscal release, MBT = mid-body meniscal transection). Negative controls (E, J, O) were stained with a species matched IgG. The chevron shows the location of the tidemark between calcified (below) and non-calcified cartilage in each section. All images are at the same magnification, the scale bar in E = 100µ.

Table 1

Vertical ground reaction forces in operated and contralateral hindlegs 2.5, 8 and 12 weeks after meniscal surgery, by surgery group

		2.5wks			8wks			12wks	
	MX	**CPR**	**MBT**	**MX**	**CPR**	**MBT**	**MX**	**CPR**	**MBT**
LEFT (CONTRALATERAL) LEG									
CT (s)	0.54 (0.52-0.57)	0.61 (0.59-0.63)	0.62 (0.56-0.69)	0.59 (0.57-0.61)	0.65 (0.62-0.68)	0.63 (0.58-0.67)	0.57 (0.52-0.62)	0.64 (0.58-0.69)	0.66 (0.60-0.73)
%	110 (95-125)	102 (96-109)	116 (110-121) †	119 (106-133)+	108 (101-115)	116 (111-122) †	115 (95-135)	106 (96-116)	124 (110-137) †
AVF (BW)	0.27 (0.24-0.30)	0.25 (0.23-0.28)	0.25 (0.24-0.26)	0.24 (0.23-0.25)	0.24 0.22-0.26)	0.24 (0.23-0.26)	0.25 (0.24-0.27)	0.27 (0.25-0.29)	0.26 (0.25-0.27)
%	105 (96-114)	95 (88-103)	99 (87-111)	92 (91-94) †	90 (81-99)	96 (89-102)	99 (92-106)	100 (94-106)	102 (94-110)
PVF (BW)	0.40 (0.34-0.45)	0.39 (0.37-0.41)	0.38 (0.35-0.41)	0.35 (0.33-0.36)	0.36 (0.33-0.39)	0.37 (0.33-0.40)	0.37 (0.36-0.39)	0.40 (0.37-0.43)	0.39 (0.37-0.41)
%	106 (93-119)	95 (90-100)	101 (88-114)	93 (90-96) †	89 (78-100)	97 (85-109)	99 (93-106)	98 (91-106)	104 (91-116)
IMP (BW.s)	0.15 (0.13-0.16)	0.15 (0.14-0.17)	0.16 (0.14-0.18)	0.14 (0.14-0.14)	0.15 (0.14-0.17)	0.15 (0.14-0.16)	0.14 (0.13-0.16)	0.17 (0.16-0.18)	0.17 (0.15-0.19)
%		97 (85-118)	115 (102-129)	110 (99-120)	97 (88-106)	111 (105-117) †	114 (92-	107 (92-121)	126 (112-

	114 (92-138)						137)		140) †
LS ($BW.s^{-1}$)	3.48 (2.95-4.00)	3.08 (2.81-3.34)	2.84 (2.58-3.09)	2.61 (2.35-2.86)	2.72 (2.41-3.04)	2.58 (2.20-2.96)	2.99 (2.73-3.26)	3.04 (2.55-3.53)	2.56 (2.39-2.73)
%	99 (84-114)	92 (86-99)	92 (79-104)	74 (63-86) †	82 (67-98)	83 (71-96) +	85 (75-96) +	91 (82-99)	84 (67-100)
RIGHT (OPERATED) LEG									
CT (s)	0.5 (0.46-0.53)	0.57 (0.56-0.59)	0.57 (0.52-0.62)	0.56 (0.53-0.59)	0.60 (0.53-0.67)	0.61 (0.59-0.62)	0.55 (0.54-0.56)	0.63 (0.60-0.66)	0.61 (0.59-0.64)
%	102 (87-116) *	98 (94-103)	105 (99-111) *	114 (100-128) *	102 (89-116)	112 (104-120) +	112 (102-123)	107 (101-114)	114 (102-127)
AVF (BW)	0.22 (0.19-0.24)	0.22 (0.19-0.25)	0.22 (0.19-0.25)	0.22 (0.19-0.26)	0.23 (0.22-0.24)	0.22 (0.20-0.25)	0.55 (0.54-0.56)	0.25 (0.22-0.28)	0.25 (0.23-0.26)
%	84 (73-96) *+	85 (77-94) *†	83 (72-94) +	87 (73-102)	91 (82-100)	84 (75-93) †	112 (102-123)	98 (89-107)	92 (88-96) †
PVF (BW)	0.33 (0.29-0.37)	0.33 (0.28-0.38)	0.34 (0.30-0.37)	0.32 (0.28-0.37)	0.34 (0.30-0.38)	0.33 (0.31-0.36	0.35 (0.32-0.38)	0.36 (0.31-0.42)	0.37 (0.36-0.37)
%	91 (77-106) *	86 (78-95) *+	86 (76-96) +	90 (76-103)	90 (84-96) †	85 (79-92) †	96 (84-107)	97 (87-106)	94 (92-96) †
IMP (BW.s)	0.11 (0.10-0.11)	0.13 (0.11-0.14)	0.13 (0.10-0.15)	0.12 (0.10-0.15)	0.14 (0.12-0.16)	0.14 (0.12-0.15)	0.13 (0.12-0.14)	0.16 (0.14-0.18)	0.15 (0.14-0.17)

%	85 (76-94)	84 (74-95) +	88 (74-103)	99 (81-117)	93 (78-107)	95 (86-104) *	103 (97-109)	106 (92-120)	106 (91-120)
LS ($BW.s^{-1}$)	2.43 (2.12-2.75)	2.62 (2.11-3.13)	2.36 (1.86-2.85)	2.42 (2.15-2.69)	2.64 (2.44-2.84)	2.28 (1.92-2.64)	2.77 (2.52-3.01)	2.70 (2.24-3.16)	2.46 (2.16-2.75)
%	74 (55-92)	83 (70-97)	74 (55-88) †	73 (54-92) +	85 (74-95) +	69 (59-79) †	83 (66-100)	86 (74-98)	75 (61-90) +

MX, meniscectomy; CPR, cranial pole release; MBT, mid-body transection; CT, contact time; AVF, PVF, average and peak vertical force; IMP, vertical impulse (AVF x CT); LS, loading slope. Values are above: means (95% confidence interval), n=4/gp; below: percentage (95% confidence interval) variation from baseline. *, R (operated) differs from L (unoperated) leg, paired t-test $P<0.05$; †,+, differs significantly from baseline (Week 0) values, paired t-test † $P<0.05$, + $P<0.10$.

Table 2

Gross joint pathology, modified Mankin-type and Pritzker *cartilage histopathology scores, by treatment group*

	SHAM	**MX**	***P***	**CPR**	***P***	**MBT**	***P***
Gross pathology score							

Cartilage erosion	1.3 (0.2-2.4)	5.5 (4.7-6.3)	0.004	5.3 (4.7-6.0)	0.004	6.0 (5.3-6.7)	0.004
Osteophytes	2.5 (1.7-3.3)	5.7 (4.7-6.6)	0.005	5.7 (4.2-7.2)	0.006	7.2 (6.8-8.0)	0.004 [a]
Sum	3.8 (2.4-5.3)	11.2 (9.5-12.9)	0.004	11.0 (9.2-12.8)	0.004	13.2 (12.0-14.3)	0.004
Modified Mankin-type histopathology score							
Aggregate score (0-29)							
MFC	4.7 (0.4-9.0)	12.4 (9.1-15.8)	0.037	13.3 (9.4-17.3)	0.045	12.5 (8.3-16.7)	0.025
MTP	7.1 (3.0-11.1)	17.6 (7.4-17.3)	0.006	15.2 (14.9-20.3)	0.031	12.3 (10.0-20.5)	0.15 [b]
Structure score (0-10)							
MFC	1.5 (0-3.7)	2.7 (0.3-5.1)	0.17	3.0 (2.0-4.0)	0.078	3.2 (0.7-5.6)	0.13
MTP (MTP-C)	1.9 (0-4.1)	6.0 (4.0-8.0)	0.11	5.5 (3.5-7.5)	0.11	4.2 (1.7-6.7)	0.20
Pritzker histopathology score							

Grade (0-6)							
MFC	2.6 (1.8-3.4)	2.8 (2.1-3.4)	0.76	2.9 (2.8-3.1)	0.45	3.2 (2.4-3.9)	0.32
MTP-C	0.5 (0-1.2)	3.1 (2.4-3.7)	0.003	2.8 (1.6-4.0)	0.006	3.1 (2.4-4.1)	0.003
MTP-U	3.8 (3.1-4.4)	3.3 (2.5-4.2)	0.47	3.5 (2.9-4.1)	0.60	3.0 (2.7-3.3)	0.06
Stage (0-4)							
MFC	1.3 (0.7-2.0)	3.3 (2.7-4.0)	0.001	2.7 (2.0-3.3)	0.021	2.7 (2.0-3.3)	0.021
MTP-C	0.7 (0-1.6)	3.3 (2.7-4.0)	0.004	2.8 (1.9-3.8)	0.016	3.2 (2.2-4.1)	0.003
MTP-U	3.0 (2.5-4.2)	3.5 (2.8-4.2)	0.027	3.7 (3.3-4.1)	0.073	3.7 (3.3-4.1)	0.073
Score (Grade x Stage; 0-24)							
MFC	3.9 (0.7-7.1)	9.1 (6.1-12.1)	0.044	7.8 (5.7-9.9)	0.074	8.8 (5.0-12.5)	0.086
MTP-C	1.2 (0-3.1)	10.7 (6.8-14.5)	0.003	9.3 (3.6-14.9)	0.009	10.0 (5.2-14.8)	0.006
MTP-U	11.5 (8.0-15.0)	12.3 (7.8-16.8)	0.78	13.0 (9.7-16.3)	0.55	10.9 (9.9-11.9)	0.76

MFC, MTP: medial femoral condyle, medial tibial plateau; MTP-C, MTP-U, covered and uncovered (ie. normally by meniscus) areas of MTP. Data are means (confidence interval), n=6/gp. P values indicate significant difference from SHAM; a, b, MBT differs from MX at $P<0.10$ level, a: $P=0.054$, b: P=0.055

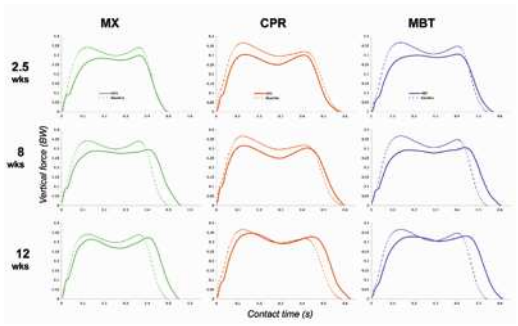
Table 3

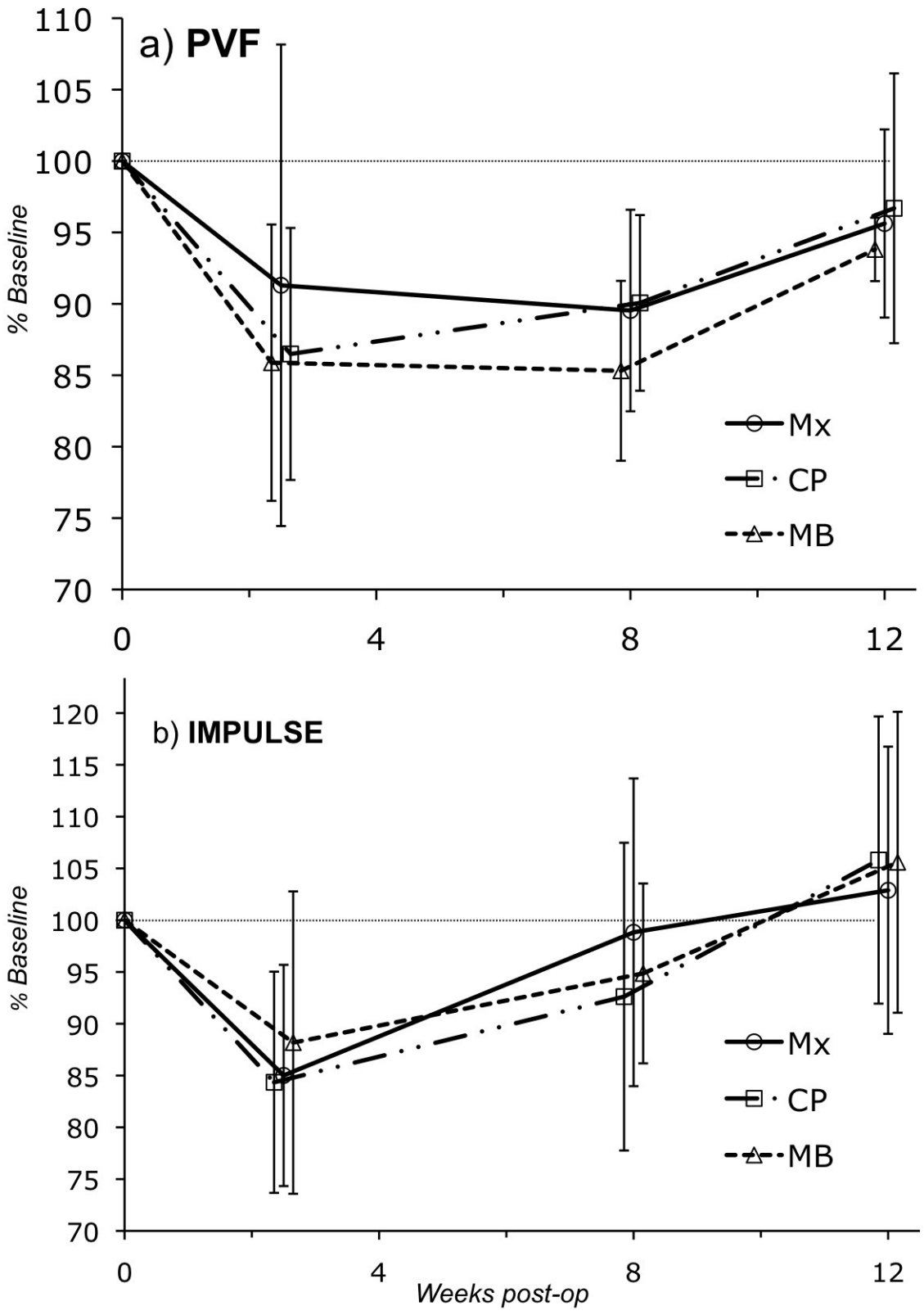
Histological cartilage and subchondral bone thickness, by region and treatment group

	SHAM	**MX**	***P***	**CPR**	***P***	**MBT**	***P***
Cartilage thickness (mm)							
Medial Femoral Condyle (MFC)							
Inner	1.51 (1.27-1.74)	1.38 (1.26-1.51)	0.36	1.54 (1.29-1.79)	0.87	1.50 (1.34-1.66)	0.97
Middle	1.74 (1.45-2.04)	1.36 (1.15-1.56)	0.06	1.37 (1.10-1.63	0.09	1.52 (1.35-1.69)	0.30
Outer	0.85 (0.70-1.00)	0.88 (0.67-1.10)	0.79	1.00 (0.90-1.10)	0.14	1.06 (0.94-1.18)	0.06
Medial Tibial Plateau (MTP)							
Inner	1.66 (1.48-1.83)	1.63 (1.48-1.79)	0.84	1.42 (1.31-1.52)	0.044	1.61 (1.42-1.81)	0.72

					a		
Middle	1.32(1.15-1.50)	1.40 (1.21-1.60)	0.58	1.19 (1.00-1.39)	0.34	1.22 (0.93-1.51)	0.55
Outer	0.85 (0.77-0.93)	0.73 (0.63-0.84)	0.11	0.80 (0.69-0.91)	0.49	0.83 (0.74-0.93)	0.82
Subchondral plate (mm)							
Medial Femoral Condyle (MFC)							
Inner	0.71 (0.65-0.78)	0.71 (0.58-0.84)	0.96	0.68 (0.60-0.76)	0.52	0.76 (0.69-0.83)	0.38
Middle	0.85 (0.69-1.02)	1.35 (1.08-1.63)	0.012	0.97 (0.82-1.12)	0.30 b	1.20 (1.01-1.40)	0.022
Outer	0.49 (0.37-0.60)	0.59 (0.44-0.73)	0.31	0.64 (0.35-0.94)	0.35	0.60 (0.54-0.66)	0.11
Medial Tibial Plateau (MTP)							
Inner	1.02 (0.85-1.19)	0.98 (0.84-1.13)	0.76	0.95 (0.86-1.03)	0.48	0.92 (0.80-1.05)	0.40
Middle	1.29 (1.05-1.52)	1.26 (1.11-1.42)	0.87	1.29 (1.12-1.47)	0.96	1.22 (0.92-1.52)	0.74
Outer	0.89 (0.75-1.03)	0.95 (0.68-1.21)	0.72	0.77 (0.62-0.92)	0.29	0.98 (0.79-1.18)	0.45

Data are means (confidence interval), n=6/gp. *P* values indicate significant difference from SHAM; a, CPR < MX *P*=0.045; b, CPR < MX *P*=0.038




a) PVF
% Baseline
110
105
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95
90
85
80
75
70
0
4
8
12
Mx
CP
MB
b) IMPULSE
% Baseline
120
115
110
105
100
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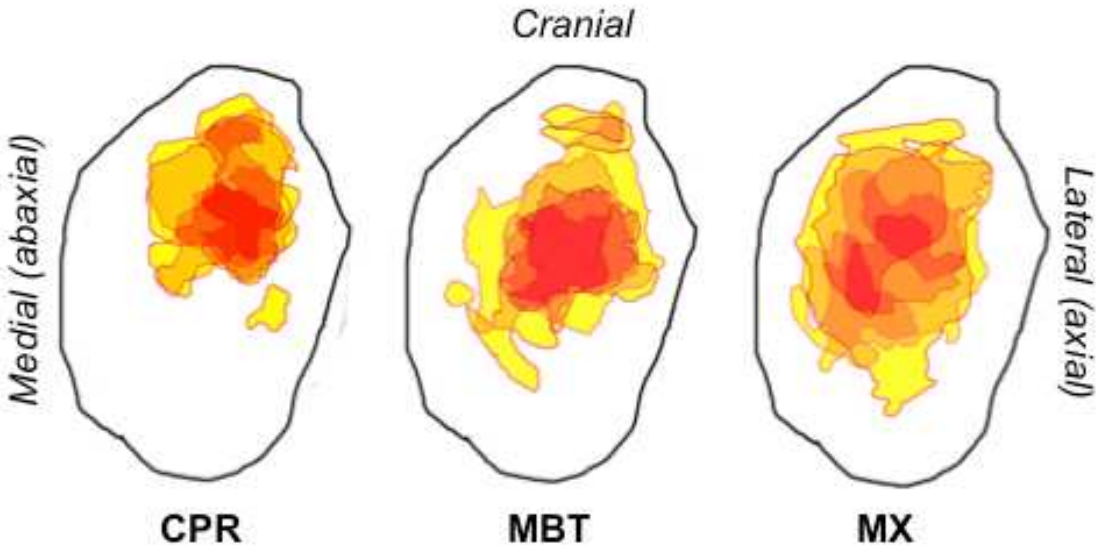
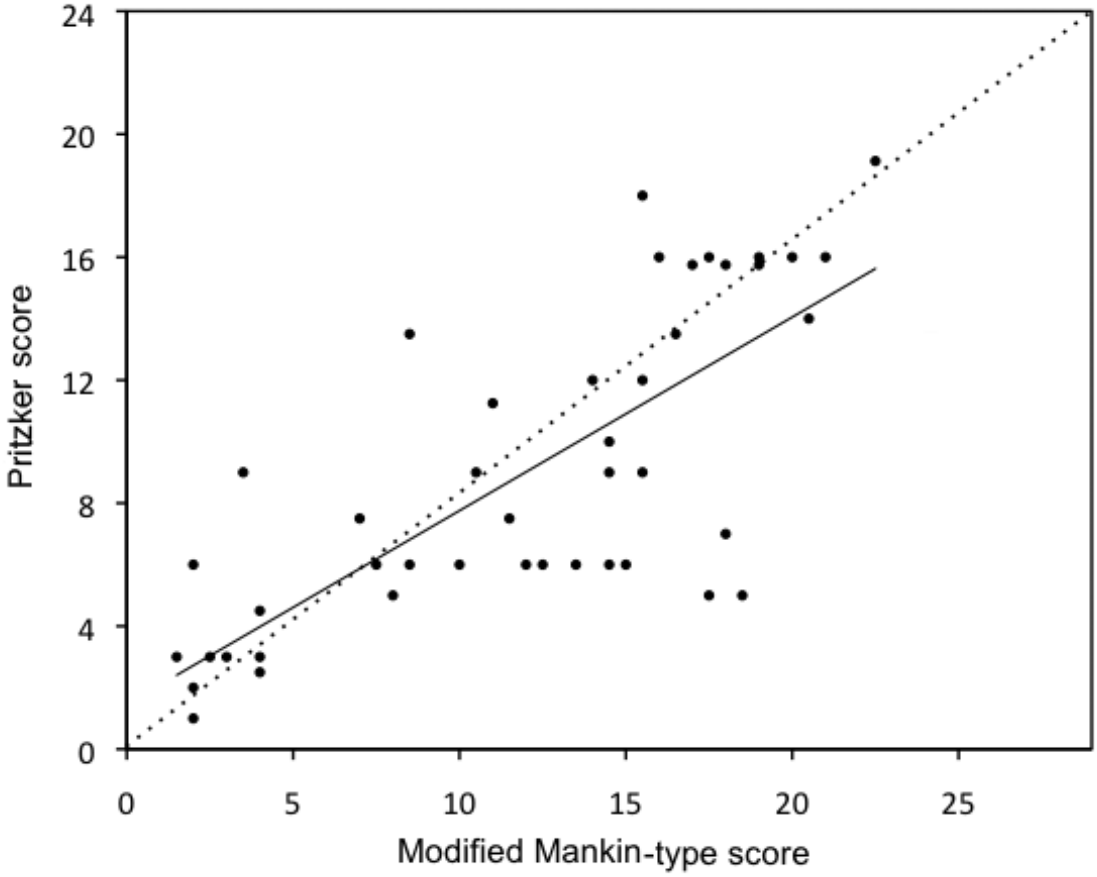
12
Weeks post-op
Mx
CP
MB


MX


MBT


CPR

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Modified Mankin-type score
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8
12
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24
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10
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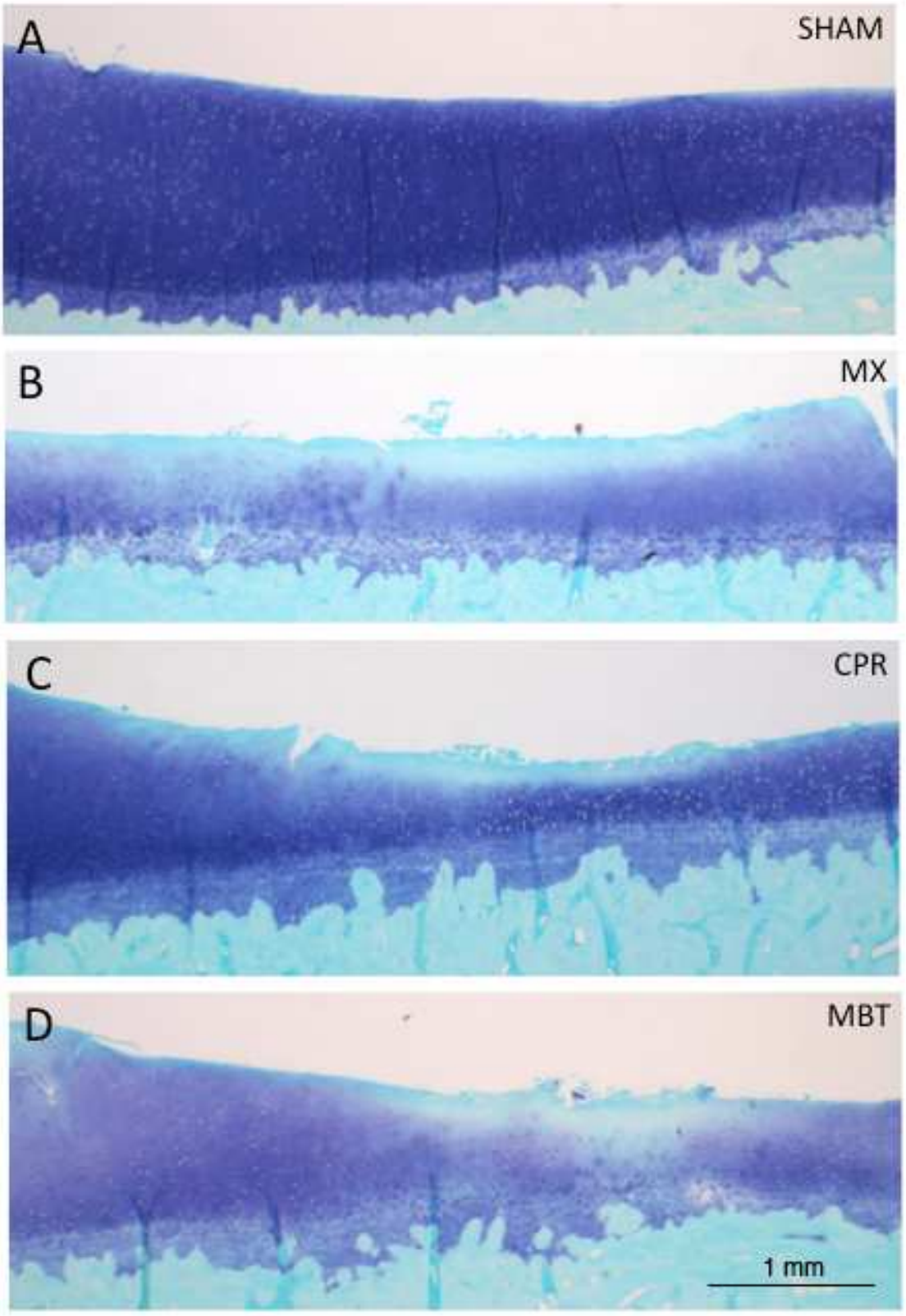
20
25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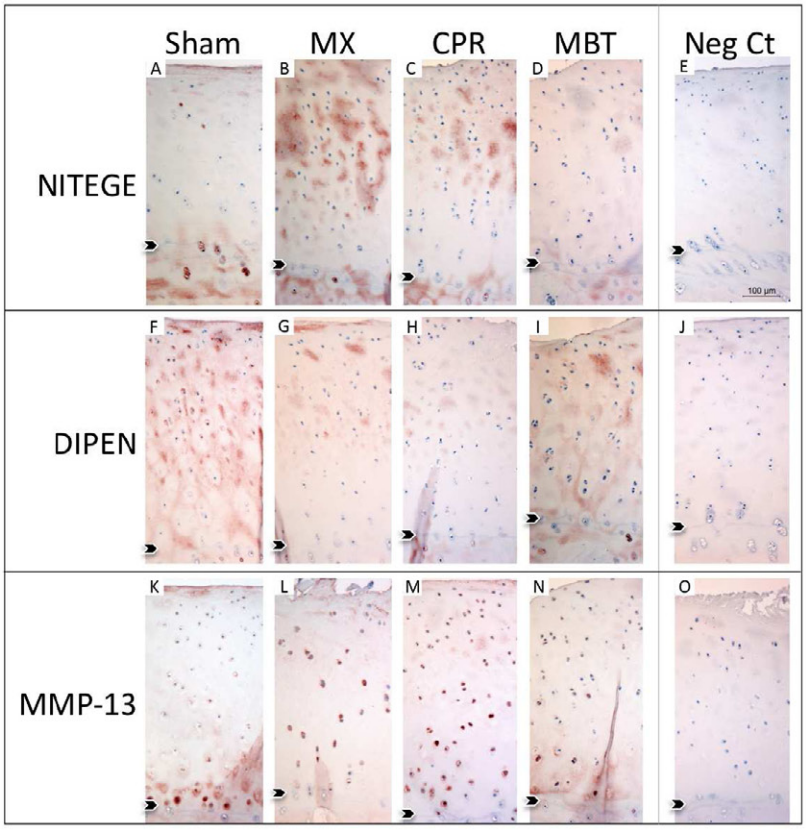
Sham
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