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Intra-articular implantation of collagen scaffold carriers is safe in both native and arthrofibrotic rabbit knee joints

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Objectives

Sustained intra-articular delivery of pharmacological agents is an attractive modality but requires use of a safe carrier that would not induce cartilage damage or fibrosis. Collagen scaffolds are widely available and could be used intra-articularly, but no investigation has looked at the safety of collagen scaffolds within synovial joints. The aim of this study was to determine the safety of collagen scaffold implantation in a validated *in vivo* animal model of knee arthrofibrosis.

Materials and Methods

A total of 96 rabbits were randomly and equally assigned to four different groups: arthrotomy alone; arthrotomy and collagen scaffold placement; contracture surgery; and contracture surgery and collagen scaffold placement. Animals were killed in equal numbers at 72 hours, two weeks, eight weeks, and 24 weeks. Joint contracture was measured, and cartilage and synovial samples underwent histological analysis.

Results

Animals that underwent arthrotomy had equivalent joint contractures regardless of scaffold implantation (-13.9° *versus* -10.9°, equivalence limit 15°). Animals that underwent surgery to induce contracture did not demonstrate equivalent joint contractures with (41.8°) or without (53.9°) collagen scaffold implantation. Chondral damage occurred in similar rates with (11 of 48) and without (nine of 48) scaffold implantation. No significant difference in synovitis was noted between groups. Absorption of the collagen scaffold occurred within eight weeks in all animals

Conclusion

Our data suggest that intra-articular implantation of a collagen sponge does not induce synovitis or cartilage damage. Implantation in a native joint does not seem to induce contracture. Implantation of the collagen sponge in a rabbit knee model of contracture may decrease the severity of the contracture.

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Article focus

- A total of 96 rabbits were randomly allocated to four experimental groups: arthrotomy alone; arthrotomy and collagen scaffold placement; contracture surgery; and contracture surgery and collagen scaffold placement.
- Animals were killed at 72 hours and two, eight and 24 weeks.
- Joint contracture, chondral damage and synovitis were assessed.

Key messages

- Intra-articular implantation of a collagen sponge does not induce synovitis, cartilage damage or fibrosis with contracture in the rabbit joint.
- All collagen sponges were resorbed in this model by eight weeks.
- Collagen sponges may be considered a safe carrier for intra-articular administration of pharmacological agents.

Strength and limitations

- Strengths: large sample size, four study groups, multiple time points, validated scales for grading.
- Limitations: assessment of chondral damage was difficult due to artefacts at the time of sectioning.

Introduction

Arthrofibrosis, or fibrosis of the tissues around synovial joints, remains a difficult clinical problem in orthopaedics, despite numerous treatment modalities.[1,2,3] In order to study the pathophysiology behind arthrofibrosis, animal models have been developed using the New Zealand White rabbit knee.[4,5] These models involve an intracapsular knee injury followed by Kirschner-wire (K-wire) immobilisation for eight weeks, and result in substantial contracture that resembles the human biomechanical, cellular, and molecular response to injury, with increased myofibroblasts and transforming growth factor beta (TGF-β) activity.[6-12]

Previous studies have demonstrated significant upregulation of myofibroblasts early in the process of joint contracture (within two weeks of injury),[12] as well as notable changes in messenger ribonucleic acid (mRNA) expression within the first 72 hours of injury. Furthermore, microarray analysis of mRNA has demonstrated that the most notable changes in mRNA expression in capsular tissues occurred within the first 24 to 72 hours after injury.[13,14] Other studies have suggested the possible influence of mast cells on the development of arthrofibrosis in the four weeks after injury.[11] The early onset of changes, both on the cellular and molecular level, seems to indicate the need for early intervention to prevent or abort the development of arthrofibrosis. Using these animal models, attempts to find an effective anti-fibrosis medication are underway. Ketotifen, a mast cell stabiliser, has already shown some promise in reducing contracture severity in a rabbit model.[15] Decorin, while demonstrating change in the local mRNA expression, did not affect the overall degree of contracture.[13]

If administration of a pharmacological agent is proven to be effective in the prevention or treatment of joint contracture, the route of administration becomes critical. Systemic administration may lead to unpredictable pharmacological levels at the joint, and could be associated with side effects. Local intra-articular administration is very appealing, but would require the use of a carrier for sustained administration and release. However, intra-articular implantation of a carrier could potentially have adverse effects, such as cartilage damage, an inflammatory synovial response, or fibrosis.

Collagen, a naturally occurring polymer, presents an attractive option for intra-articular drug delivery. Currently, collagen scaffolds are clinically employed for (or under investigation for use in) ophthalmologic applications,[16] local antibiotic delivery,[17-24] local wound care,[25-27] and healing of bone defects or generation of bone fusion.[28-32] Despite the ongoing research into the clinical applications of collagen scaffold implantation, the safety of intra-articular implantation of a collagen scaffold is largely unknown.

We hypothesised that a collagen scaffold could be placed in an intra-articular location in the rabbit knee, without causing alteration of joint range of movement (ROM), significant cartilage damage, or synovial inflammatory reaction, as assessed by mechanical and histological analysis. Secondly, we hypothesised that intra-articular placement of a collagen scaffold in a rabbit model of arthrofibrosis would not alter the expected joint contracture, nor cause damage to the articular cartilage, nor create a synovial inflammatory reaction, as assessed by mechanical and histological analysis. Thirdly, we hypothesised that collagen scaffold absorption would occur within eight weeks.

Materials and Methods

After obtaining approval from our departmental Review Board and Institutional Animal Care and Use Committee, 102 skeletally mature female New Zealand White rabbits (using the same gender rabbits increased likelihood of homogeneity), were randomly and equally divided into four study groups: sham arthrotomy (SA); collagen scaffold arthrotomy (CSA); sham Mayo contracture model (MCM); and collagen scaffold Mayo contracture model (CMCM), with six animals assigned as non-operative histological controls (Fig. 1). National Institutes of Health guidelines for the care and use of laboratory animals were observed at all times. Rabbits in the arthrotomy groups underwent an arthrotomy, as described below. Rabbits assigned to the arthrofibrosis model underwent the same initial procedure to develop a contracture as described in previous studies and briefly outlined below.[5,12-14,33] Collagen scaffolds used in this study were commercially available collagen sponges, derived from purified bovine tendon, currently used in dental applications. These scaffolds are pre-sterilised, eliminating the need for sterilisation. The sterile scaffolds were fashioned into 1 cm × 1 cm implants prior to implantation.

Initial procedure – arthrotomy (groups SA and CSA). Under appropriate inhalational anaesthesia, a lateral parapatellar arthrotomy was completed on the right knee. Of the 48 rabbits assigned to the arthrotomy cohort, 24 underwent placement of a 1 cm × 1 cm collagen scaffold (CSA), whereas 24 underwent arthrotomy alone (SA). The scaffold was placed in the medial recess of the suprapatellar pouch, taking care not to trap the sponge within the patellofemoral articulation. The arthrotomy and wound were then closed with absorbable sutures. All animals in the arthrotomy cohort were allowed free cage activity (0.19 m^3) following the operation.

Initial procedure – arthrofibrosis (groups MCM and CMCM). Under appropriate inhalational anaesthesia, a lateral parapatellar arthrotomy was performed on the right knee. The patella was subluxed medially. Cortical drill holes, 3 mm in diameter, were made in the non-cartilaginous portions of the medial and lateral femoral condyles,

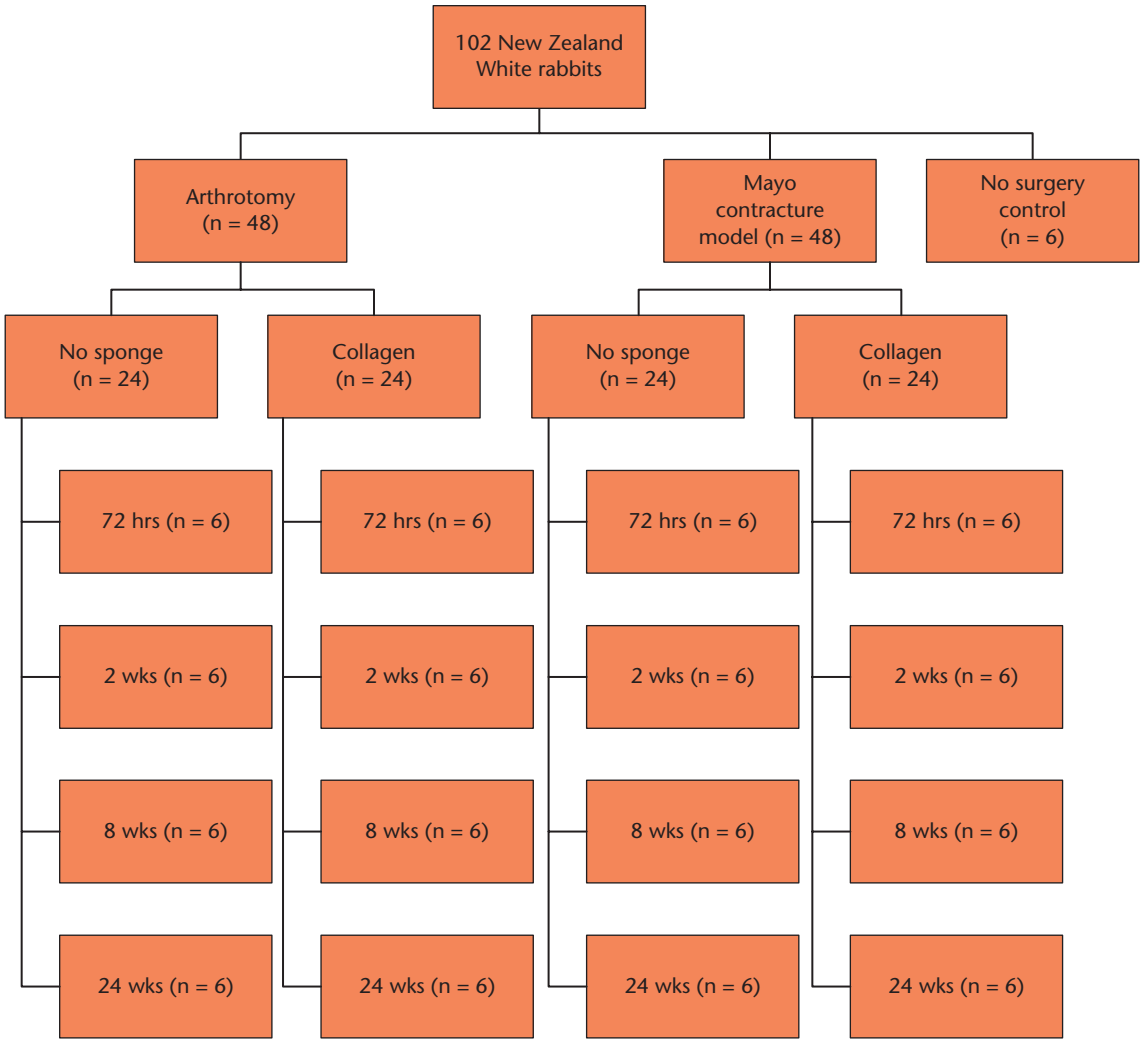

Fig. 1

Organisational chart demonstrating study design.

taking care to avoid the insertion of the collateral ligaments. The anterior and posterior cruciate ligaments were transected sharply, leaving the collateral ligaments intact. The knee was hyperextended 45°, disrupting the posterior capsule. Of the 48 animals assigned to undergo Mayo contracture model surgery, 24 underwent placement of a 1 cm × 1 cm collagen scaffold (CMCM) in the medial recess of the suprapatellar pouch, taking care not to entrap the scaffold in the patellofemoral articulation. In the remaining 24 animals that underwent contracture model surgery, no scaffold was placed (MCM). The knee was then hyperflexed, and separate incisions were made along the anterior tibia and the lateral thigh. A 1.6 mm K-wire was advanced through a drill hole in the tibia ~5 cm distal to the tubercle and secured. The wire was retrieved through the lateral thigh incision, and bent to capture and immobilise the femur in flexion. All wounds were irrigated and closed with absorbable sutures. The animals were then allowed free cage activity (0.19 m^3) following the operation.

Second procedure (eight weeks, groups MCM and CMCM). Animals in the arthrofibrosis groups (MCM and CMCM) assigned to be killed at 24 weeks underwent a second surgical procedure at week eight to remove the wire used for immobilisation. Under inhalational anaesthesia, the previous incision along the anterior tibia and lateral thigh were utilised to remove the K-wire. Any bridging heterotopic ossification along the K-wire path was disrupted without forced ROM of the knee joint. The incisions were then closed with absorbable suture. All animals were then allowed free cage mobilisation (0.19 m^3) for 16 weeks.[5,13,14]

Biomechanical testing. All animals were assigned to be killed at designated time points (72 hours, two weeks, eight weeks, 24 weeks), in equal number (six) per time point (Fig. 1). Animals were killed using an overdose of sodium pentobarbital. The operative limb and contralateral (internal control) limb were then disarticulated, and excess skin and soft tissues dissected away, but tissues immediately around the knee were left undisturbed. The

femur and tibia were transected approximately 7 cm from the joint line. In animals from groups MCM and CMCM killed at 72 hours, two weeks, and eight weeks, the K-wire was removed to permit testing. Both limbs then underwent mechanical testing in a custom device, which is calibrated using known weights, and has been previously validated.[5] The centre of rotation of the knee is placed over the torque cell, and its position confirmed fluoroscopically. Both long bones are secured by intramedullary rods attached to the torque cell. An extension torque was then applied at 1° per second to a maximum torque of 20 newton centimetres (NCm), as has been previously described. Loss of extension (joint contracture) was defined as the difference between the angle at which the non-operative limb reached 10 NCm and the angle at which the operative limb reached 10 NCm.

Gross macroscopic and histological analysis. The specimens were further dissected for histological samples. A thorough inspection of the joint was conducted, documenting the presence or absence of the scaffold. Gross histological changes, when present, were observed. A small window of synovium was taken from the medial synovium adjacent to the quadriceps tendon and patella. Both femoral condyles, as well as the medial tibial plateau, were harvested. All samples were then fixed in formalin for histological examination.

For all synovial specimens from all cohorts, the fixed samples were embedded in paraffin blocks, sectioned, and underwent haematoxylin and eosin staining. All samples were examined by an experienced veterinary pathologist (RM) blinded to treatment allocation. Synovial changes were scored according to a previously described system[34] to document synovitis. In nine of 90 operative limbs and 24 of 90 non-operative limbs, the synovial cell layer could not be definitively identified. In these samples, the peri-articular tissue sections were still reviewed for synovitic changes and inflammation, and assigned a score to the best ability of the pathologist.

Three bony specimens per limb (medial and lateral femoral condyles, and medial tibial plateau) underwent decalcification in a solution of 20% formic acid, and were subsequently sectioned, and stained with Safranin O/Fast Green. These samples were then examined by an experienced and blinded veterinary pathologist (RM) to determine the rate of degenerative articular (hyaline cartilage) changes by comparing contralateral limb controls and by examination of control limbs from non-operative animals. A binary grading system was employed, assigning samples with no change, or no change greater than those due to artefact a 0 value, and those samples with change greater than those due to artefact, a 1. The scores for each sample on each animal were then added together, yielding a composite value for each limb ranging from 0 (no change in any sample) to 3 (change greater than artefact in both femoral condyles and medial tibial plateau). For analysis, the number of animals in each experimental group demonstrating each sum total (0 to 3) was reported. Changes in both operative and non-operative limbs were recorded and reported.

Statistical analysis. Joint contracture was defined as the ROM of the non-operative limb minus the ROM of the operative limb. Data from all time points at death were pooled for ROM analysis. Joint contracture values were compared for equivalence using the two one-sided test (TOST) procedure. Sample size was determined based on an equivalence limit of 15° (+ or -7.5°), which was decided *a priori* to represent a clinically meaningful limit. Significance for the TOST procedure was set at < 0.05, generating 95% confidence intervals (CI) for the difference in means.

Cartilage and synovial histology were not compared for statistical equivalence, as an *a priori* definition of equivalence could not be made. Cartilage histology was reported in a descriptive manner, with mean rate of cartilage damage, mean relative risk of cartilage damage, risk differences and respective CIs reported for all groups. Synovial histology was reported in the same manner; in addition, a chi-squared analysis between groups SA and CSA, and between groups MCM and CMCM was also performed. Significance level was set at $p < 0.05$.

Scaffold dissolution data were largely descriptive, however, chi-squared analysis between time points was performed, with significance set at $p < 0.05$. Pseudotrochlea formation rates were also descriptive, although chi-squared analysis was performed to determine whether pseudotrochlea formation affected synovial inflammation. Again, significance was set at $p < 0.05$.

Results

Animals that underwent joint arthrotomy and placement of a collagen scaffold (CSA) demonstrated equivalent ROM when compared with animals that underwent arthrotomy alone (SA), with equivalence limits set at 15° (Fig. 2). No contracture was noted in either group of animals: in fact, the mean ROM for the operative knee was found to have increased by 13.9° and 10.9° (SA and CSA groups, respectively) compared with the non-operative extremity. The difference in the mean contractures between the two arthrotomy groups (SA and CSA) was -3.1°, with a 95% CI of -6.9° to 0.8°, well within the equivalence limits of -7.5° to 7.5°.

Animals that underwent Mayo contracture model surgery and placement of a collagen scaffold (CMCM) did not have an equivalent joint contracture formation compared with MCM animals, with equivalence limits set at 15°. When comparing the two groups at all time points, CMCM animals demonstrated a mean joint contracture of 41.8° (95% CI 22.8° to 60.7°), compared with 53.9° (95% CI 31.8° to 76°) in MCM animals. The mean contracture difference was calculated to be 12.2°, with the

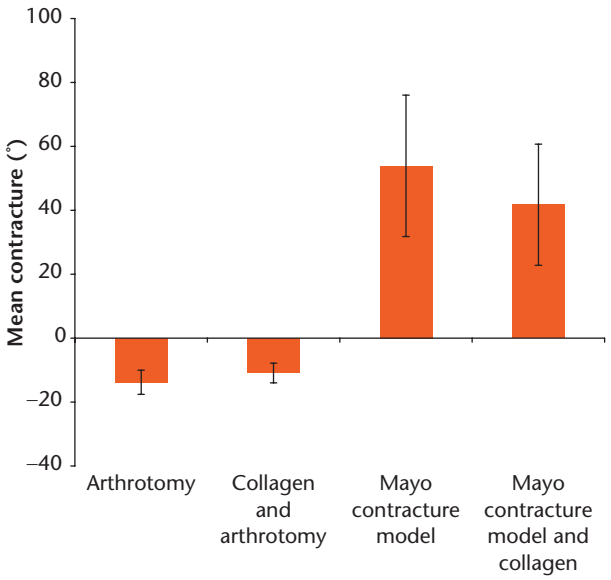

Fig. 2

Final joint contracture for all time points at death (mean ± 95% confidence intervals). The mean contractures of the arthrotomy and collagen + arthrotomy groups were statistically equivalent, with an equivalence limit of 15°.

Table I. Collagen scaffold absorption rate by surgical procedure and time of death

Group	Time of death	Scaffolds present at death, n (%)
Arthrotomy group		
	72 hrs	6 (*100*)
	2 wks	5 (*83.3*)
	8 wks	0 (*0*)
	24 wks	0 (*0*)
Mayo contracture model group		
	72 hrs	5 (*83.3*)
	2 wks	2 (*33.3*)
	8 wks	0 (*0*)
	24 wks	0 (*0*)
Overall		
	72 hrs	11/12 (*92*)
	2 wks	7/12 (*58*)
	8 wks	0 (*0*)
	24 wks	0 (*0*)

95% CI of -11.5° to 35.8° falling outside the equivalent limits of -7.5° to 7.5°.

Absorption of the collagen scaffold occurred within eight weeks in all animals (Table I). In CSA animals, the collagen scaffold was found at the time of death in all animals (six of six) killed 72 hours following the index procedure, in five of six animals killed two weeks following index procedure, and in no animals killed eight or 24 weeks following the index procedure. In CMCM animals, the collagen scaffold was found at the time of death in five of six animals at 72 hours following the index procedure, in two of six (33%) animals killed two weeks after the index procedure, and in no animals killed eight or 24 weeks after the index procedure. The difference between the 72-hour (five of six) and two-week (two of six) time

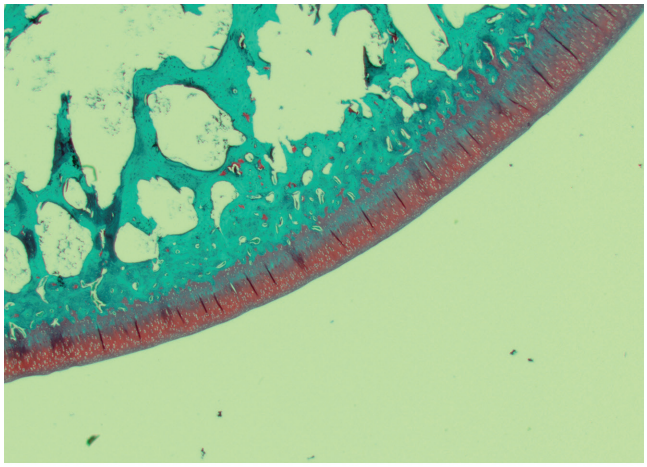

Fig. 3a

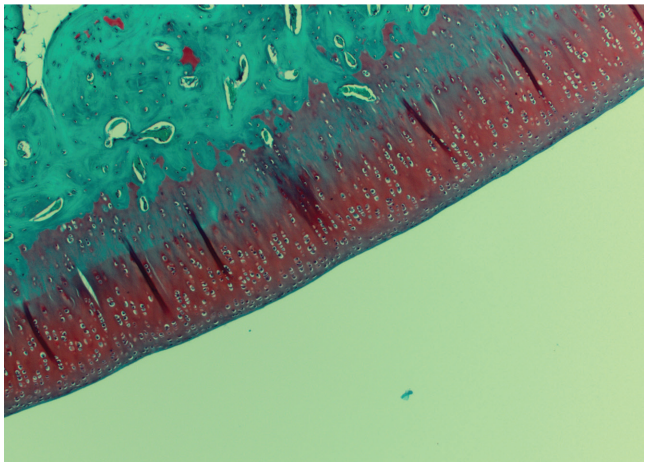

Fig. 3b

Cartilage sample of normal appearance from an animal that underwent arthrotomy alone, 1.25× magnification shown above (a), 4× magnification below (b).

points was not found to be significant (p = 0.24). When comparing the scaffold absorption rate in CSA animals and CMCM animals, the scaffold was found at two weeks in five of six CSA animals compared with only two of six CMCM animals. This difference was also not found to be significant (p = 0.24).

The vast majority of animals suffered no chondral damage greater than that due to sectioning artefact (Figs 3 and 4, Table II). Only three animals (two of which had collagen scaffolds implanted) suffered chondral changes in the operative knee greater than those due to artefact in all three samples (medial and lateral femoral condyles and medial tibial plateau). Collagen scaffold implantation resulted in an 8% increase (95% CI -12% to 28%) in the risk of any cartilage damage greater than artefact in animals that underwent arthrotomy, and a 4% decrease (95% CI -28% to 20%) in animals that underwent Mayo contracture model surgery (Fig. 5). Additionally, ten of 96 (10%) of the non-operative knees demonstrated chondral changes greater than those due to artefact. The overall risk of chondral damage in the operative limbs of CSA

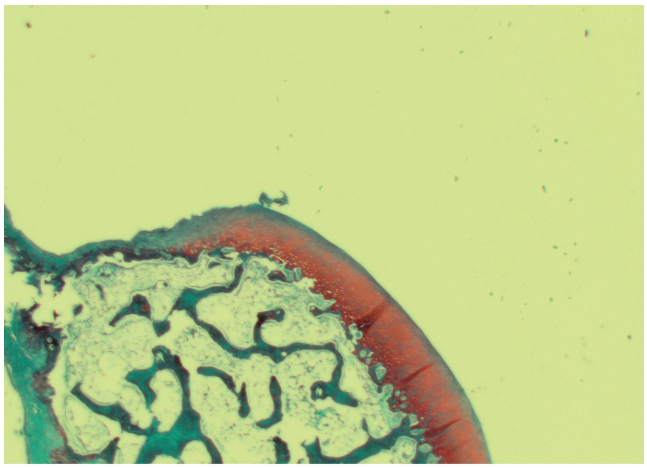

Fig. 4a

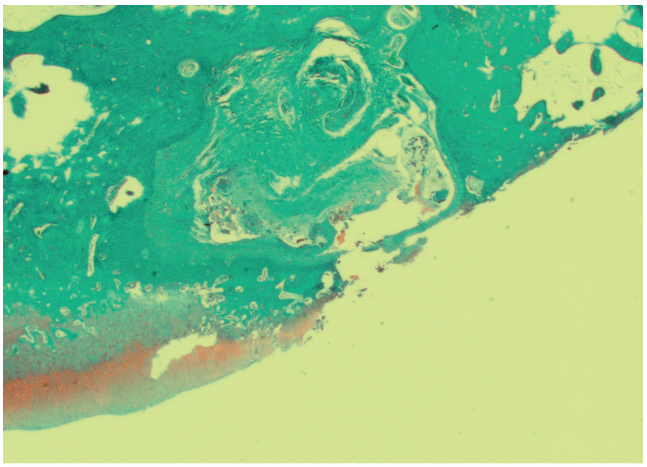

Fig. 5a

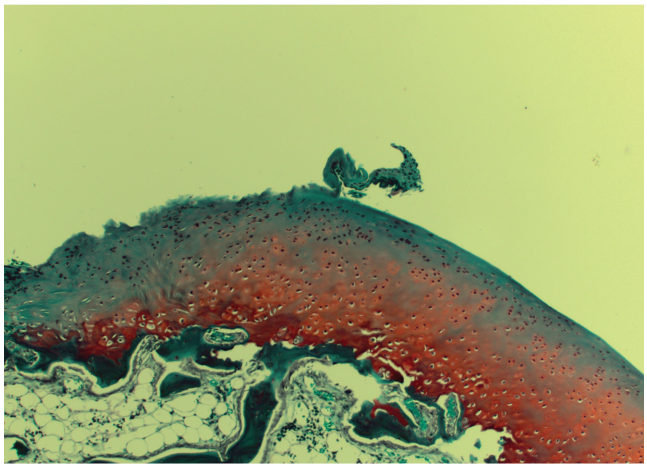

Fig. 4b

Cartilage sample from the operative knee of an animal undergoing Mayo contracture model surgery. These illustrations demonstrate changes due to artefact.

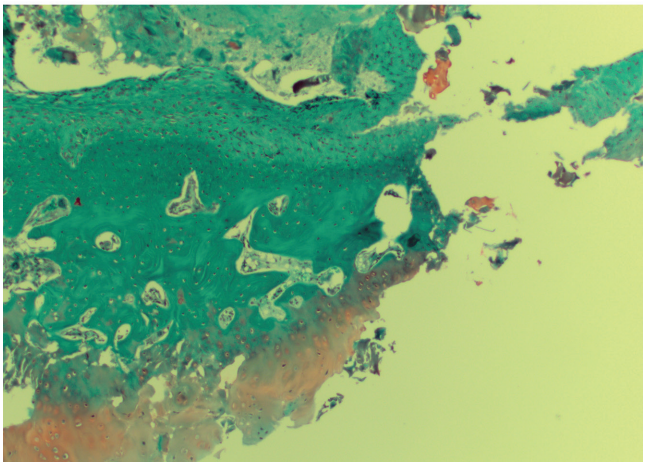

Fig. 5b

Cartilage samples (1.25× above, 4× below) from an animal undergoing Mayo contracture model surgery and collagen scaffold placement, demonstrating changes greater than those due to artefact.

Table II. Cartilage histology results of both operative and non-operative limbs, with relative risk of any cartilage damage, and the 95% confidence interval (CI) associated with risk difference

Study group (n = 24 each)	0	1	2	3	Risk (%)	Risk difference	95% CI
Operative limb cartilage histology: all time points, n (%)							
Arthrotomy	22 (*92*)	1 (*4*)	0 (*0*)	1 (*4*)	*8.3*	–	(-) 12% to 28%
Arthrotomy and collagen	20 (*83*)	3 (*13*)	1 (*4*)	1 (*4*)	*16.7*	8%	
Mayo contracture model	17 (*71*)	5 (*21*)	2 (*8*)	0 (*0*)	*29.2*	–	(-) 28% to 20%
Mayo contracture and collagen model	18 (*75*)	4 (*17*)	1 (*4*)	1 (*4*)	*25.0*	–4%	
Non-operative limb cartilage histology: all time points, n (%)							
Arthrotomy	21 (*88*)	2 (*8*)	1 (*4*)	0 (*0*)	*12.5*		(-) 17% to 25%
Arthrotomy and collagen	20 (*83*)	2 (*8*)	2 (*8*)	0 (*0*)	*16.7*	4%	
Mayo contracture model	*22* (*92*)	2 (*8*)	0 (*0*)	0 (*0*)	*8.3*		(-) 22% to 13%
Mayo contracture and collagen model	23 (*96*)	1 (*4*)	0 (*0*)	0 (*0*)	*4.2*	–4%	

and SA animals was not significantly different (p = 1.0) to the risk of chondral damage in the non-operative limbs. Conversely, the risk of chondral damage in the operative limbs of MCM and CMCM animals was significantly greater (p = 0.02) than the risk of chondral damage in non-operative limbs.

When the synovial samples were graded, no sample received a score higher than three of nine (indicative of mild synovitis). No significant difference was noted between the mean synovitis scores for the animals that underwent arthrotomy and those that underwent arthrotomy + collagen scaffold implantation (0.5 *versus*

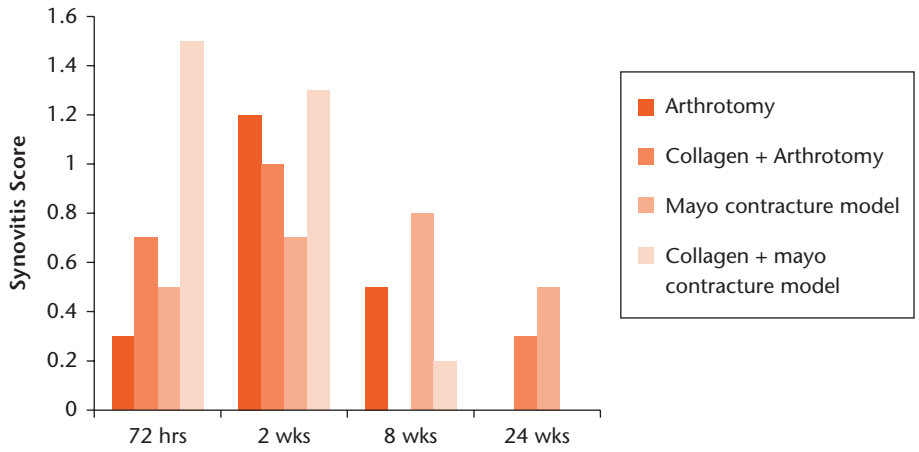

Fig. 6

Mean synovitis score by time and group (range 0 to 9). No significant difference was noted between any groups.

Table III. Synovitis scores

Study group (n = 24 each)	0	1	2	3	Mean	Mean difference	95% CI	p-value*
Operative limb synovitis score (grades 0 to 9): all time periods								
Arthrotomy	17 (*70.8*)	3 (*12.5*)	3 (*12.5*)	1 (*4.2*)	0.5	0	–0.5 to 0.5	1
Arthrotomy and collagen	16 (*66.7*)	6 (*25*)	0 (*0*)	2 (*8.3*)	0.5			
Mayo contracture model	16 (*66.7*)	4 (*16.7*)	1 (*4.2*)	3 (*12.5*)	0.63	–0.13	–0.7 to 0.5	0.69
Mayo contracture and collagen model	14 (*58.3*)	5 (*20.8*)	2 (*8.3*)	3 (*12.5*)	0.75			
Non-operative limb synovitis score (grades 0 to 9): all time periods								
Arthrotomy	24 (*100*)	0 (*0*)	0 (*0*)	0 (*0*)	0	–0.04	–0.1 to 0.04	0.32
Arthrotomy and collagen	23 (*95.8*)	1 (*4.2*)	0 (*0*)	0 (*0*)	0.04			
Mayo contracture model	23 (*95.8*)	0 (*0*)	1 (*4.2*)	0 (*0*)	0.08	0.04	–0.1 to 0.2	0.67
Mayo contracture and collagen model	23 (*95.8*)	1 (*4.2*)	0 (*0*)	0 (*0*)	0.04			

*chi-squared analysis.
The number of animals in each group per synovitis core is demonstrated, as is the mean synovitis score for each experimental group. The mean difference represents the difference between the collagen-implanted animals and the sham operation animals. No significant differences were noted between groups in operative or non-operative limbs

Table IV. Pseudotrochlea after arthrotomy

	Time of death	Pseudotrochlea present, n (%)
Collagen scaffold	72 hrs	0 (*0*)
	2 wks	1/6 (*16.6*)*
	8 wks	3/6 (*50*)
	24 wks	3/6 (*50*)
Overall (n = 24)		**6/24 (*25*)†**
No scaffold	72 hrs	0 (*0*)
	2 wks	3/6 (*50*)
	8 wks	1/6 (*16.6*)
	24 wks	0 (*0*)
Overall (n = 24)		**4/24 (*16.7*)**
All animals	72 hrs	0 (*0*)
	2 wks	3/12 (*25*)†
	8 wks	4/12 (*33.3*)
	24 wks	3/12 (*25*)
Total (n = 48)		**10/48 (*20.8*)**

Pseudotrochlea formation rate in animals undergoing arthrotomy ± scaffold placement
In one animal, indicated by *, the patella of the non-operative limb was found subluxed, and a pseudotrochlea had developed
†the sum totals do not include the animal in which a pseudotrochlea developed in the non-operative limb

0.5, p = 1.0) (Fig. 6, Table III). Similarly, no significant difference was noted between the mean synovitis scores for animals undergoing Mayo contracture model surgery and those undergoing Mayo contracture model surgery + collagen scaffold implantation (0.63 *versus* 0.75, p = 0.69). The mean synovitis score for the operative knee of animals that underwent Mayo contracture model surgery + collagen scaffold implantation was 0.75. The mean synovitis scores for animals that underwent arthrotomy + collagen scaffold placement (0.5), and those that underwent Mayo contracture model surgery + collagen scaffold placement (0.75), are indicative of "no synovitis" (values 0 to 1, not significant).

Interestingly, some animals undergoing arthrotomy ± placement of a collagen scaffold demonstrated signs at time of death of chronic medial patellar instability, with formation of a "pseudotrochlea" (Table IV). Such changes were not observed in any of the animals undergoing Mayo contracture model surgery. In CSA animals, these changes were noted in three of six animals at eight weeks and three of six animals at 24 weeks (six of 24 animals). In SA animals, such changes were found in three of six animals killed two weeks following the index procedure, and one of six animals at eight weeks after the index

procedure (four of 24 animals). Overall, ten (20.1%) of the 48 animals that underwent arthrotomy ± scaffold implantation displayed signs of chronic medial patellar instability and pseudotrochlea formation. The rate and grade of synovitis for animals in which a pseudotrochlea was found (ten of 48 animals) were compared against those of animals in which no pseudotrochlea was found (38 of 48), and no significant differences were found by chi-squared analysis ($p = 0.46$).

Discussion

In this study, we intended to investigate the safety of an intra-articular collagen scaffold drug delivery device on native rabbit knee joints, and on the knee joints of rabbit knees in a contracture model by assessing: stiffness secondary to joint fibrosis by comparing joint contracture development in study animals (CSA, CMCM) and sham animals (SA, MCM); the absorption kinetics of the intra-articular collagen scaffold by *ex vivo* observation at 72 hours and two, eight, and 24 weeks; and the histological effects of intra-articular collagen scaffold placement by comparing cartilage and synovial tissue samples of study animals and control animals.

Biomechanical testing of movement revealed statistical equivalence between CSA and SA animals, but did not demonstrate equivalence between CMCM and MCM animals. These data indicate that scaffold placement into a native knee by lateral arthrotomy poses equivalent risk of joint contracture as surgery alone, suggesting that implantation of a collagen scaffold in a non-contracted joint does not generate arthrofibrosis. Interestingly, when implanted in joints developing contracture, collagen scaffold implantation seemed to be protective of joint contracture. From a scientific perspective, this protective effect could make it difficult to determine whether using a collagen sponge for administration of a pharmacological agent would confound the assessment of reduction in contracture (due to the collagen sponge *versus* the pharmacological agent itself). On the other hand, from a practical perspective it is very attractive to consider the potential additive effects of collagen and antifibrotic drugs.

Absorption of the scaffold consistently occurred prior to the eight-week time point. Interestingly, our data may suggest a trend towards slower absorption in SA animals compared with CMCM. This is not unexpected given the increased surgical trauma suffered by animals in the Mayo contracture model, which likely leads to a greater inflammatory response, and consequently faster breakdown of the scaffold in the injury model animals. The suggestion of increased synovitis in all animals at the two-week mark would support this theory. In both groups of animals, however, the scaffold demonstrated slower breakdown than expected based on product literature (Integra Miltex Collagen Products, York, Philadelphia). This may be due to the relatively decreased vascularity of the joint space compared with other tissues in which these scaffolds are typically placed.

A limitation of this study is the sensitivity of the histological analysis, particularly of the histological analysis of the bone and cartilage samples. The generation of sectioning artefact in many samples limited our ability to detect subtle histological changes in cartilage. This limited the depth of our histological analysis of cartilage samples. Additionally, changes thought to be greater than those explained by artefact were noted in 10% of the non-operative limbs, for which artefact or routine age-related wear and damage were the only plausible explanations. This suggests that some of the changes in the operative limbs thought to be pathologic may indeed be related to artefact or routine wear rather than to the device or to iatrogenic (surgical) trauma. An additional limitation of the histological analysis was the difficulty in capturing synovial tissue on slides for examination. In samples from nine of 90 operative knees, synovial cells could not be isolated. This does limit our analysis of the synovium somewhat, as the synovial architecture could not be evaluated in every animal. Despite this, all samples were reviewed for inflammatory changes, and were scored accordingly, to the best of the veterinary pathologist's (RM) ability.

Despite the limitations in our histological analysis, both collagen (CSA, CMCM) and sham (SA, MCM) animals were found to have similar rates of chondral injury. These data suggest that the chondral damage noted was likely sequelae of injury or surgery, or iatrogenic at time of death, rather than secondary to mechanical wear or inflammatory changes due to collagen implantation. Additionally, the relative rarity of changes in all anatomic locations would suggest that collagen implantation did not cause wear throughout the joint, as would be expected in inflammatory arthritis. This is consistent with data from other experimental models, which do not suggest an adverse local reaction in collagen membranes or scaffold used for cartilage repair.[35-37] Additionally, collagen scaffolds have been noted to demonstrate faster release of pro-inflammatory cytokines than other scaffold materials such as polylactic acid, which may create a more chondrocyte-friendly environment.[38]

Animals in all groups (SA, CSA, MCM, and CMCM) experienced some degree of peri-articular synovial inflammation, particularly at the 72-hour and two-week time points. By the 24-week time point, this response had largely abated. No significant difference was noted in the rate of synovial inflammation in the study animals (CSA, CMCM) when compared with the sham surgery animals (SA, MCM). This is encouraging given that some studies investigating the intra-articular injection of drug delivery scaffolds have noted synovial inflammation as a result[39] since some inflammatory infiltrate is expected for breakdown of the scaffold.[16] These data suggest that implantation of the collagen scaffold itself does not lead to an increase in synovitis.

At the point of death of the animals undergoing arthrotomy ± scaffold placement, an interesting phenomenon was noted; several animals suffered chronic medial dislocation of the patella following the lateral arthrotomy. At the time of death, these animals were noted to have dislocated patellae, along with the formation of a fibrous pseudotrochlea medial to the actual trochlea. Interestingly, this did not occur in any of the Mayo contracture model animals, regardless of time of immobilisation, remobilisation status, or placement of a collagen scaffold. Given that this occurred in both sham arthrotomy animals as well as animals undergoing arthrotomy and collagen scaffold placement, it is likely to be related to the mechanical alignment of the rabbit knee and surgical disruption of the lateral structures which clearly play a role in stabilisation of the rabbit patella. Such changes have been noted in a rabbit model in the past, although by a different mechanism.[40] While interesting and a potential confounding factor, these findings do not limit the findings of this study, as they occurred with similar frequency in both collagen and sham arthrotomy animals, and did not demonstrate a significant effect on the rate or severity of synovitis.

In conclusion, intra-articular implantation of a collagen scaffold does not seem to lead to contracture, synovial inflammation, or chondral damage. Using equivalence analysis, collagen scaffolds implanted in joints undergoing contracture seem to be protective, which could be interpreted as a confounding factor if a collagen sponge is considered for testing of antifibrotic drugs in the future, but could also provide additive beneficial effects in the prevention and treatment of arthrofibrosis. Further studies are warranted to assess the potential of collagen scaffolds as an intra-articular carrier of antifibrotic pharmacological agents.

References

1. **Bong MR, Di Cesare PE.** Stiffness after total knee arthroplasty. *J Am Acad Orthop Surg* 2004;12:164-171.
2. **Mellema JJ, Lindenhovius AL, Jupiter JB.** The posttraumatic stiff elbow: an update. *Curr Rev Musculoskelet Med* 2016;9:190-198.
3. **Haller JM, Holt DC, McFadden ML, Higgins TF, Kubiak EN.** Arthrofibrosis of the knee following a fracture of the tibial plateau. *Bone Joint J* 2015;97-B:109-114.
4. **Hildebrand KA, Sutherland C, Zhang M.** Rabbit knee model of post-traumatic joint contractures: the long-term natural history of motion loss and myofibroblasts. *J Orthop Res* 2004;22:313-320.
5. **Nesterenko S, Morrey ME, Abdel MP, et al.** New rabbit knee model of posttraumatic joint contracture: indirect capsular damage induces a severe contracture. *J Orthop Res* 2009;27:1028-1032.
6. **Hildebrand KA, Zhang M, van Snellenberg W, King GJ, Hart DA.** Myofibroblast numbers are elevated in human elbow capsules after trauma. *Clin Orthop Relat Res* 2004;419:189-197.
7. **Hildebrand KA, Zhang M, Hart DA.** High rate of joint capsule matrix turnover in chronic human elbow contractures. *Clin Orthop Relat Res* 2005;439:228-234.
8. **Hildebrand KA, Zhang M, Hart DA.** Joint capsule matrix turnover in a rabbit model of chronic joint contractures: correlation with human contractures. *J Orthop Res* 2006;24:1036-1043.
9. **Hildebrand KA, Zhang M, Hart DA.** Myofibroblast upregulators are elevated in joint capsules in posttraumatic contractures. *Clin Orthop Relat Res* 2007;456:85-91.
10. **Hildebrand KA, Zhang M, Germscheid NM, Wang C, Hart DA.** Cellular, matrix, and growth factor components of the joint capsule are modified early in the process of posttraumatic contracture formation in a rabbit model. *Acta Orthop* 2008;79:116-125.
11. **Hildebrand KA, Zhang M, Salo PT, Hart DA.** Joint capsule mast cells and neuropeptides are increased within four weeks of injury and remain elevated in chronic stages of posttraumatic contractures. *J Orthop Res* 2008;26:1313-1319.
12. **Abdel MP, Morrey ME, Barlow JD, et al.** Myofibroblast cells are preferentially expressed early in a rabbit model of joint contracture. *J Orthop Res* 2012;30:713-719.
13. **Abdel MP, Morrey ME, Barlow JD, et al.** Intra-articular decorin influences the fibrosis genetic expression profile in a rabbit model of joint contracture. *Bone Joint Res* 2014;3:82-88.
14. **Abdel MP, Morrey ME, Grill DE, et al.** Effects of joint contracture on the contralateral unoperated limb in a rabbit knee contracture model: a biomechanical and genetic study. *J Orthop Res* 2012;30:1581-1585.
15. **Monument MJ, Hart DA, Befus AD, et al.** The mast cell stabilizer ketotifen fumarate lessens contracture severity and myofibroblast hyperplasia: a study of a rabbit model of posttraumatic joint contractures. *J Bone Joint Surg [Am]* 2010;92-A:1468-1477.
16. **Friess W.** Collagen–biomaterial for drug delivery. *Eur J Pharm Biopharm* 1998;45:113-136.
17. **Bennett-Guerrero E, Ferguson TB, Jr Lin M, et al.** Effect of an implantable gentamicin-collagen sponge on sternal wound infections following cardiac surgery: a randomized trial. *JAMA* 2010;304:755-762.
18. **Bennett-Guerrero E, Pappas TN, Koltun WA, et al.** Gentamicin-collagen sponge for infection prophylaxis in colorectal surgery. *N Engl J Med* 2010;363:1038-1049.
19. **Ipsen T, Jørgensen PS, Damholt V, Tørholm C.** Gentamicin-collagen sponge for local applications. 10 cases of chronic osteomyelitis followed for 1 year. *Acta Orthop Scand* 1991;62:592-594.
20. **Rutten HJ, Nijhuis PH.** Prevention of wound infection in elective colorectal surgery by local application of a gentamicin-containing collagen sponge. *Eur J Surg Suppl* 1997;578:31-35.
21. **Swieringa AJ, Goosen JH, Jansman FG, Tulp NJ.** In vivo pharmacokinetics of a gentamicin-loaded collagen sponge in acute periprosthetic infection: serum values in 19 patients. *Acta Orthop* 2008;79:637-642.
22. **Becker PL, Smith RA, Williams RS, Dutkowsky JP.** Comparison of antibiotic release from polymethylmethacrylate beads and sponge collagen. *J Orthop Res* 1994;12:737-741.
23. **Diefenbeck M, Mückley T, Hofmann GO.** Prophylaxis and treatment of implant-related infections by local application of antibiotics. *Injury* 2006;37:S95-S104.
24. **Zalavras CG, Patzakis MJ, Holtom P.** Local antibiotic therapy in the treatment of open fractures and osteomyelitis. *Clin Orthop Relat Res* 2004;427:86-93.
25. **Grzybowski J, Kołodziej W, Trafny EA, Struzyna J.** A new anti-infective collagen dressing containing antibiotics. *J Biomed Mater Res* 1997;36:163-166.
26. **Still J, Glat P, Silverstein P, Griswold J, Mozingo D.** The use of a collagen sponge/living cell composite material to treat donor sites in burn patients. *Burns* 2003;29:837-841.
27. **Singh O, Gupta SS, Soni M, et al.** Collagen dressing versus conventional dressings in burn and chronic wounds: a retrospective study. *J Cutan Aesthet Surg* 2011;4:12-16.
28. **Blumenthal NM, Koh-Kunst G, Alves ME, et al.** Effect of surgical implantation of recombinant human bone morphogenetic protein-2 in a bioabsorbable collagen sponge or calcium phosphate putty carrier in intrabony periodontal defects in the baboon. *J Periodontol* 2002;73:1494-1506.
29. **Choi SH, Kim CK, Cho KS, et al.** Effect of recombinant human bone morphogenetic protein-2/absorbable collagen sponge (rhBMP-2/ACS) on healing in 3-wall intrabony defects in dogs. *J Periodontol* 2002;73:63-72.
30. **Hou J, Wang J, Cao L, et al.** Segmental bone regeneration using rhBMP-2-loaded collagen/chitosan microspheres composite scaffold in a rabbit model. *Biomed Mater* 2012;7:035002.
31. **Shigeno K, Nakamura T, Inoue M, et al.** Regenerative repair of the mandible using a collagen sponge containing TGF-beta1. *Int J Artif Organs* 2002;25:1095-1102.
32. **Ueda H, Hong L, Yamamoto M, et al.** Use of collagen sponge incorporating transforming growth factor-beta1 to promote bone repair in skull defects in rabbits. *Biomaterials* 2002;23:1003-1010.
33. **Barlow JD, Hartzler RU, Abdel MP, et al.** Surgical capsular release reduces flexion contracture in a rabbit model of arthrofibrosis. *J Orthop Res* 2013;31:1529-1532.
34. **Krenn V, Morawietz L, Burmester GR, et al.** Synovitis score: discrimination between chronic low-grade and high-grade synovitis. *Histopathology* 2006;49:358-364.
35. **Dorotka R, Windberger U, Macfelda K, et al.** Repair of articular cartilage defects treated by microfracture and a three-dimensional collagen matrix. *Biomaterials* 2005;26:3617-3629.
36. **Frenkel SR, Toolan B, Menche D, Pitman MI, Pachence JM.** Chondrocyte transplantation using a collagen bilayer matrix for cartilage repair. *J Bone Joint Surg [Br]* 1997;79-B:831-836.

37. Toolan BC, Frenkel SR, Pachence JM, Yalowitz L, Alexander H. Effects of growth-factor-enhanced culture on a chondrocyte-collagen implant for cartilage repair. *J Biomed Mater Res* 1996;31:273-280.

38. Kwon H, Sun L, Cairns DM, et al. The influence of scaffold material on chondrocytes under inflammatory conditions. *Acta Biomater* 2013;9:6563-6575.

39. Ratcliffe JH, Hunneyball IM, Smith A, Wilson CG, Davis SS. Preparation and evaluation of biodegradable polymeric systems for the intra-articular delivery of drugs. *J Pharm Pharmacol* 1984;36:431-436.

40. Finsterbush A. Rotational knee strain resulting in patellar dislocation. An experimental study in rabbits. *Clin Orthop Relat Res* 1982;169:259-263.

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ICMJE Conflicts of Interest
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