Increased vascular penetration and nerve growth in the meniscus: a potential source of pain in osteoarthritis

Sadaf Ashraf,[1] Helen Wibberley,[1] Paul Ian Mapp,[1] Roger Hill,[2] Deborah Wilson,[2] David Andrew Walsh[1,2]

[1]Academic Rheumatology, Arthritis Research UK Pain Centre, University of Nottingham, Nottingham, UK
[2]Sherwood Forest Hospitals NHS Foundation Trust, Sutton in Ashfield, UK

Correspondence to
Ms Sadaf Ashraf, Academic Rheumatology, Arthritis Research UK Pain Centre, University of Nottingham, Clinical Sciences Building, City Hospital, Hucknall Road, Nottingham NG5 1PB, UK; mbxsa1@nottingham.ac.uk

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ABSTRACT

Objectives Meniscal damage is a recognised feature of knee osteoarthritis (OA), although its clinical relevance remains uncertain. This study describes vascular penetration and nerve growth in human menisci, providing a potential mechanism for the genesis of pain in knee OA.

Methods Menisci obtained post mortem were screened on the basis of high or low macroscopic tibiofemoral chondropathy as a measure of the presence and degree of OA. Forty cases (20 per group) were selected for the study of meniscal vascularity, and 16 (eight per group) for the study of meniscal innervation. Antibodies directed against α-actin and calcitonin gene-related peptide (CGRP) were used to localise blood vessels and nerves by histochemistry. Image analysis was used to compare vascular and nerve densities between groups. Data are presented as median (IQR).

Results Menisci from knees with high chondropathy displayed degeneration of collagen bundles in their outer regions, which were more vascular than the inner regions, with an abrupt decrease in vascularity at the fibrocartilage junction. Vascular densities were increased in menisci from the high compared with low chondropathy group both in the synovium (3.8% (IQR 2.6–5.2), 2.0% (IQR 1.4–2.9), p=0.002) and at the fibrocartilage junction (2.3% (IQR 1.7–3.1), 1.1% (IQR 0.8–1.9), p=0.003), with a greater density of perivascular sensory nerve profiles in the outer region (high chondropathy group, 144 nerve profiles/mm^2 (IQR 134–189); low chondropathy group, 119 nerve profiles/mm^2 (IQR 104–144), p=0.049).

Conclusion Tibiofemoral chondropathy is associated with altered matrix structure, increased vascular penetration, and increased sensory nerve densities in the medial meniscus. The authors suggest therefore that angiogenesis and associated sensory nerve growth in menisci may contribute to pain in knee OA.

INTRODUCTION

Osteoarthritis (OA), one of the most common joint diseases,[1] is a major source of pain and disability in the ageing population.[2–4] It is associated with loss of articular cartilage (chondropathy), sclerosis and osteophyte formation in the subchondral bone. Chondropathy alone does not equate to OA, but emerging research shows chondropathy scores are representative of the presence and severity of OA.[5]

Pain is the predominant symptom of OA, but little is certain about the mechanisms by which this pain arises. Pain has been suggested to originate from articular cartilage,[6] periosteum,[7] subchondral bone,[8] synovium,[9] ligaments, muscle and joint capsule.[6 10–12]

Angiogenesis may contribute to pain in OA by enabling growth of new unmyelinated sensory nerves.[13 14] Angiogenesis and sensory nerves are seen in the synovium and at the osteochondral junction in OA[15] penetrating into non-calcified articular cartilage and osteophytes.[6] These perivascular, unmyelinated nerve fibres containing substance P and calcitonin gene-related peptide (CGRP) are implicated in mediating sustained burning pain described by patients with OA.[16 17]

The menisci are cresenteric wedges of fibrocartilage, located between the femoral condyles and the tibial plateaux.[18] They increase joint congruity, dispersal of weight and proprioception and shock absorption, and reduce friction.[19 20]

Meniscal tissue consists of cells suspended in an extracellular matrix of collagen (mainly type 1), glycoproteins, proteoglycans and elastin.[21] Disruption of collagen bundles, proteoglycan loss, perimeniscal synovitis and calcification not limited to the outer, peripheral portion of the meniscus[22–26] are described as meniscal degeneration.[27 28] A strong association between meniscal degeneration, articular cartilage damage, and joint space narrowing has been reported.[29 30] As well as being a consequence of OA, meniscal changes may contribute to OA structural damage.[27 30 31]

Meniscectomy in OA can lead to a reduction in pain,[32 33] although mechanisms by which this occurs are yet to be elucidated. Myelinated and unmyelinated nerve fibres, and free nerve endings, have been localised in the meniscus,[34–37] with perivascular nerves containing substance P.[38] Blood vessels and nerves penetrate the outer portion of the normal human meniscus, reaching as far as its middle third, with the innermost portion remaining avascular and aneural.[34 35] Large variations have been reported in the degree of vascular penetration and innervation between individuals, but it is unclear whether disease contributes to this heterogeneity.[18] Two studies comparing human menisci with extensive degeneration with normal menisci found no change in vascularity and innervation,[39 40] while another indicated increased penetration of blood vessels into the inner portion of the osteoarthritic meniscus.[26] Most previous studies of human menisci have used small numbers of surgical samples, and the heterogeneity of human meniscal tissue and high prevalence of OA may have compromised their ability to associate changes with disease.

Elucidation of the mechanisms involved in the generation of pain is needed to enable the

development of more effective therapeutic and surgical strategies. This study examines the potential role of meniscus in the genesis of pain in OA through angiogenesis and nerve growth. Furthermore, the vasculature plays important roles in tissue repair, and a better understanding of the vascular and neural anatomy of both healthy and diseased meniscus may help in the development of new treatments for meniscal injuries and OA.

We hypothesised that vascular penetration into the meniscus is increased in OA and is associated with increased sensory nerve growth.

MATERIALS AND METHODS

Patients and sample selection

All procedures were approved by Nottingham Research Ethics Committee 1 (08/H0403/132). Knees were collected post mortem from patients after consent from the next of kin (person identified by the patient or those accompanying the patient if the patient was incapacitated).[41] The Academic Rheumatology joint tissue repository containing 288 post-mortem cases was screened for cases displaying either high or low macroscopic chondropathy scores in the tibiofemoral joint as a measure of the presence and degree of OA.[5] Identification of meniscal nerves required special fixation of tissues, available for a subgroup of 52 repository cases. Cases did not have rheumatoid arthritis or other inflammatory joint diseases as determined by case notes review and interview with bereaved relatives.

Chondropathy score

The extent and severity of loss of articular cartilage integrity for the medial and lateral femoral condyles and tibial plateaux were determined for each case by a single assessor (RH) immediately after tissue harvesting, as previously described.[5 42] Severity of chondropathy was graded 0–4, and the percentage of the area of each articular surface that displayed each grade was estimated. Briefly,

Grade 0 = normal, smooth, unbroken surface, homogeneous white to off-white colour.

Grade 1 = swelling and softening, a light brown homogeneous coloration.

Grade 2 = superficial fibrillation, lightly broken surface, white to off-white/light brown in colour.

Grade 3 = deep fibrillation, coarsely broken cartilage surface, dark brown, grey or red in colour.

Grade 4 = subchondral bone exposure, stippled white and dark brown/red in colour.

Chondropathy scores were calculated using the formula[5 42]:

Score = (Grade 1×0.14) + (Grade 2×0.34) + (Grade 3×0.65) + Grade 4

The scores (possible range 0–100) from each articular surface were summed to give a total score (possible range 0–400) for the joint, with 0 indicating no evidence of chondropathy. Forty cases (20 per group) were then selected for the study of meniscal vascularity and 16 cases (eight per group) for the study of meniscal innervation, based on the following criteria.

High chondropathy group

Knees were included on the basis of highest total joint chondropathy score plus, to maximise the likelihood of including cases with medial compartment OA, the presence of at least some grade 3 or 4 chondropathy in the medial compartment.

Low chondropathy group

Knees were included on the basis of lowest total joint chondropathy score plus, in order to minimise the risk of including cases with tibiofemoral OA, the absence of grade 3 or grade 4 chondropathy in either the medial or lateral compartment.

Only medial menisci were examined for these studies. Demographic details of patient groups are given in table 1.

Sample preparation

All samples were processed on site at King's Mill Hospital by an experienced laboratory technician (RH) following standardised procedures. Coronal slices (2 mm thick) from the midline of medial menisci were fixed in neutral buffered formalin (containing 4% formaldehyde) for 48 h at room temperature and wax embedded, or were fixed in Zamboni's solution (2% (w/v) paraformaldehyde, 15% (v/v) picric acid in phosphate buffer, pH 7.3)[43] overnight at 4°C. Samples fixed in Zamboni's solution were transferred to 15% (w/v) sucrose in phosphate-buffered saline (PBS/sucrose) at 4°C for 5 days and then through a 1:1 mixture of PBS/sucrose and optimum cutting temperature (OCT) embedding matrix into 100% OCT for a further 7 days at 4°C before being mounted on to cork blocks, and then snap-frozen in melting isopentane and stored at −80°C.

Histochemistry

Tissue sections (5 µm thick) from wax blocks were cut in a Reichert–Jung rotary microtome. H&E stains were used to score tissue architecture of collagen bundles and Safranin-O staining to enable evaluation of proteoglycan content. Vascular smooth muscle and pericytes, markers of mature blood vessels,[44] were localised using mouse monoclonal antibody to α-actin (clone 1A4; 1:2000 dilution), a biotinylated horse anti-mouse secondary antibody (1:100 dilution), and visualised with avidin–biotin–peroxidase complex, developed with diaminobenzidine (DAB). Tissues sections (15 µm thick) from OCT blocks were cut in a motorised cryostat and thaw mounted on to Superfrost slides. Sensory nerves were immunolocalised using rabbit polyclonal antibody to CGRP, a biotinylated goat anti-rabbit secondary antibody (1:100 dilution) with peroxidase-labelled avidin–biotin complex, visualised by the nickel-enhanced DAB method.[45] Sections were dehydrated and mounted in distyrene, plasticiser and xylene. All antibodies were diluted in 0.03% serum from the host species of the secondary antibody (horse or goat) in PBS plus 0.05% bovine serum albumin.

Table 1 Patient details and chondropathy scores (possible range 0–400) for samples used in the study of meniscal vascularity and nerve growth

Patient/sample characteristics	Vascularity			Nerve growth		
	High chondropathy (n=20)	Low chondropathy (n=20)	p Value	High chondropathy (n=8)	Low chondropathy (n=8)	p Value
Male (N (%))	14 (70)	11 (55)	0.32	5 (63)	3 (38)	0.32
Age (years)	69 (65–81)	41 (34–46)	<0.0001	66 (65–72)	46 (33–55)	0.005
Chondropathy score (possible range 0–400)	203 (198–245)	24 (15–27)	<0.0001	199 (181–240)	33 (19–46)	0.0002

Data for age and chondropathy scores are medians (IQR). p<0.05 indicates significant difference between high and low chondropathy groups.

Image analysis and quantification

Meniscal degeneration was defined according to the modified Copenhaver classification based on change in appearance of collagen bundles[46 47] using the following criteria:

Grade 0 = homogeneous eosinophilic staining collagen. Collagen bundles closely set with normal chondrocytes.

Grade 1 = mild cleft formation of collagen bundles with irregular eosinophilic staining and reduction in the chondrocytes.

Grade 2 = severe cleft and cyst formation of collagen bundles accompanied by hypocellular regions.

The appearance of collagen bundles was analysed separately within the outer and inner regions of the meniscus using a ×20 objective lens (figure 1).

Vascularity was measured as percentage of tissue section area that was occupied by actin-positive blood vessels (≤150 μm diameter). Vascular densities were measured, using a ×4 objective lens within each of eight consecutive fields along the entire transverse midline from the inner tip towards the periphery, in all fields along the sagittal fibrocartilage junction between the inner and outer meniscal regions and within the synovium (figure 1). Each field was 1.4 mm long and had an area of 1.68 mm^2. Vascularity was remeasured by the same observer blinded to initial measurements in order to evaluate the repeatability of the initial measurements.

Numbers of CGRP-positive nerve profiles that were associated with blood vessels in the outer region of the meniscus were counted with a ×40 objective lens in six fields of view that displayed the highest vascular densities with associated nerve profiles. Each field of view comprised an area of 16 800 μm^2. CGRP-positive nerves were counted as associated with blood vessels if they were within 25 μm of the nearest vessel profile.

Synovial ingrowth towards the tip of the meniscus was measured as the length (mm) of the synovium attached to the inner region of the meniscus.

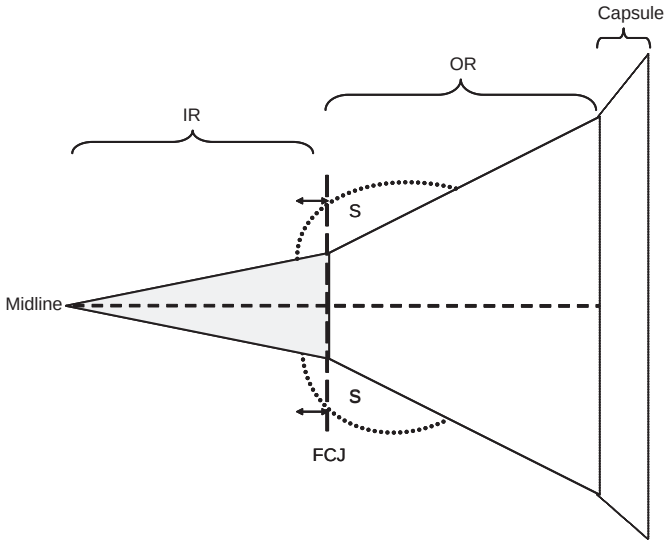

Figure 1 Diagram illustrating the method of quantifying meniscal vascularisation and nerve growth. Blood vessels were analysed (1) sequentially and within each consecutive field along the outer region (OR) of the entire fibrocartilage junction (FCJ) (vertical dashed line) separating the OR from the inner region (IR), (2) along the entire midline (horizontal dashed line) of the meniscus, and (3) in the synovium (S) adjacent to the FCJ. Nerve profiles associated with blood vessels were analysed in the OR of the menisci. The extent of synovial ingrowth (arrows) towards the meniscal tip was measured in the IR.

All image analysis was carried out by a single observer, who was blinded to the disease group, using a Zeiss Axioscop-50 microscope (Carl Zeiss, Welwyn Garden City, UK). Images were captured using a 3-CCD video camera module (model KY-F55B; JVC, Yokohama, Japan) and analysed using KS300 image analysis software (Imaging Associates, Thame, UK).

Statistical analysis

Data were analysed using SPSS V.14.0 with comparison between groups made using the Mann–Whitney U test and associations between variables expressed as Spearman's correlation coefficients. Measurement error between repeat vascularity measurements is expressed as a repeatability coefficient.[48] Repeatability of vascular density measurements was determined using synovia for six cases (three from high and three from low chondropathy groups). Data are presented as median (IQR) in the text, and, for clarity, graphically as mean±SEM, unless otherwise stated. $p<0.05$ was taken to indicate statistical significance. Graphs were created using Prism V.4.03 (GraphPad Software, San Diego, California, USA).

Reagents

Polyclonal anti-α CGRP (human) antibody (Ref: T-4239) was from Peninsula Labs (St Helens, UK). Biotinylated affinity-purified goat anti-rabbit and horse anti-mouse secondary antibodies and peroxidase-labelled streptavidin–biotin complex ABC Elite Kit were from Vector Laboratories (Peterborough, UK). DPX mounting medium and PBS were from VWR (Lutterworth, UK). OCT compound and Mayers H&E were from Raymond Lamb (Eastbourne, UK). Monoclonal mouse antibody to α-actin (clone 1A4) and all other reagents were from Sigma-Aldrich (Poole, UK).

RESULTS

Meniscal structure

Inner and outer regions of the meniscus were distinguished by differences in matrix structure and proteoglycan staining. The inner region of the meniscus displayed large collagen bundles with a regular arrangement, whereas the outer region fibres were arranged in smaller bundles with a more irregular orientation (figure 2). The junction separating these two regions we refer to as the fibrocartilage junction (figures 1 and 2). Synovium, localised to the surface of the outer region of the meniscus, extended to approximately the fibrocartilage junction in menisci from the low chondropathy group (figure 2A). In menisci from the high chondropathy group, synovium appeared thicker and extended over part of the inner region (figure 2D). Menisci from the high chondropathy group had a more fragmented appearance than menisci from the low chondropathy group, displaying more clefts in the matrix of the outer region (figure 2F), whereas menisci from the low chondropathy group displayed homogeneous eosinophilic matrix (figure 2C). Proteoglycan staining was present in the inner, but not in the outer, region of the menisci from both the low and high chondropathy groups. No difference was seen in the intensity of proteoglycan staining between groups. Blood vessels of various sizes were observed in the outer region of the meniscus, ranging from arteries with a continuous layer of smooth muscle to capillaries with walls of single cell thickness (figures 2 and 3).

The extent of synovial ingrowth towards the tip of the meniscus was greater in the high chondropathy group (0.12 mm (IQR 0.04–0.20)) than in the synovia from the low chondropathy group (0.00 mm (IQR 0.00–0.00), p=0.003).

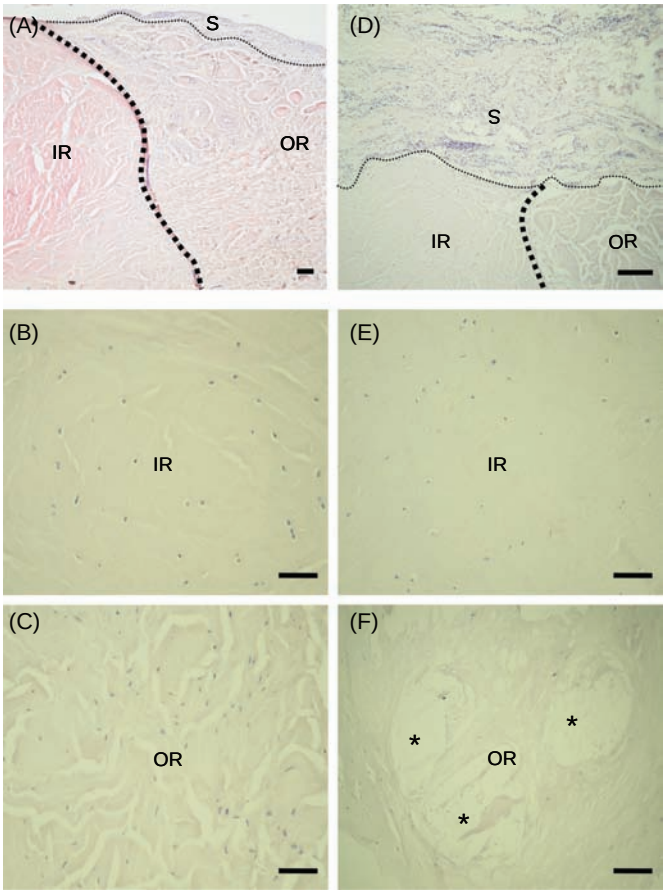

Figure 2 Structure of human menisci. H&E staining of menisci from the low (A–C) and high (D–F) chondropathy groups. In menisci from the low chondropathy group, a distinct fibrocartilage junction (large dotted line) (A) separated the inner region (IR), characterised by regular matrix fibre bundles (B), from the outer region (OR) which displayed irregular fibre bundles (C). A thin synovium (S) was adherent to the OR, but did not extend over the IR (A). In menisci from the high chondropathy group, a thick layer of synovium (S) extended over the intact IR (D, E), with the OR having progressive disorientation of fibre bundles bordering hypocellular regions, having clefts (asterisk) (F). Scale bar = 100 μm.

Grading of collagen bundle appearance indicated greater degeneration in the outer region of menisci from high chondropathy cases (grade 1 (IQR 0–1)) than from low chondropathy cases (grade 0 (IQR 0–0), p=0.048). Greater collagen bundle irregularity was associated with higher chondropathy (r=0.43, p=0.005). No significant difference was demonstrated in collagen bundle appearance in the inner region between high (grade 1 (IQR 0–1)) and low chondropathy groups (grade 0 (IQR 0–1), p=0.35) (figure 2B,E).

Vascularity

Repeatability for synovial vascularity density was 1.9 for 95% of pairs of observations (ie, the difference between two measurements for the same case is expected to be <1.9% for 95% of pairs). Vascular densities were greater in the high than in the low chondropathy group both in the synovium (3.8% (IQR 2.6–5.2), 2.0% (IQR 1.4–2.9), p=0.002) and at the fibrocartilage junction (2.3% (IQR 1.7–3.1), 1.1% (IQR 0.8–1.9), p=0.003). A degree of vessel penetration into the inner region along the midline of the menisci was observed in both groups (figure 3). The outer meniscal region was more vascular than the inner

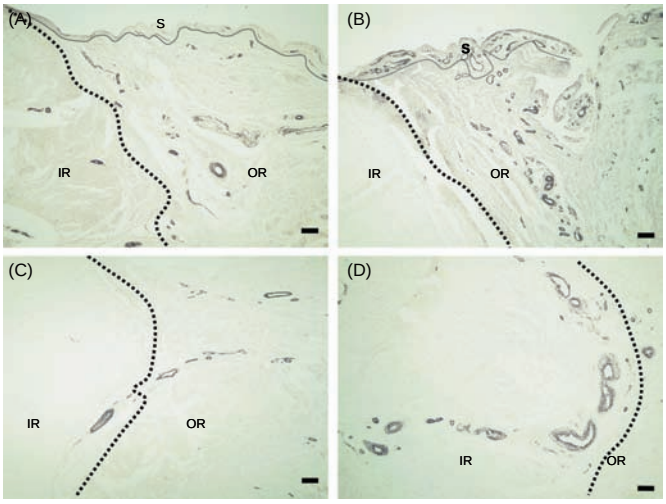

Figure 3 Vascularity of human menisci. α-actin-positive vasculature in menisci from the low (A and C) and high (B and D) chondropathy groups. In the low chondropathy group, low densities of vessels were found in the outer region (OR), both in the synovium (S) and along the fibrocartilage junction (large dotted line) (A), with vascular penetration into the inner region (IR) along the midline (C). In menisci from the high chondropathy group, high densities of vessels were observed in the OR, both in the synovium and along the fibrocartilage junction (B) with increased penetration into the IR along the midline (D). Scale bar = 100 μm.

region, with an abrupt decrease in vascularity in field 4 along the midline from the meniscal tip, which corresponded to the region of the fibrocartilage junction separating the inner from the outer region of the menisci (figures 3 and 4). Vascular densities in field 4 (the fourth consecutive microscopic field from the meniscal tip) were greater in the high than in the low chondropathy group (1.0% (IQR 0.3–1.5), 0.3% (IQR 0.0–0.8), p=0.015), indicating a greater degree of vascular penetration in the high chondropathy group. Meniscal vascularity at the fibrocartilage junction, in field 4, or in the synovium was not associated with collagen bundle appearance in the outer region (r=0.16, p=0.33, r=0.08, p=0.61 and r=0.21, p=0.20, respectively). Synovial vascular density was associated with meniscal vascularity at the fibrocartilage junction (r=0.46, p=0.003) and in field 4 (r=0.46, p=0.003).

Sensory innervation

CGRP-immunoreactive nerve profiles were identified alongside blood vessels (small arterioles) and sometimes at a distance from them (figure 5). These vessels and nerves were commonly observed in the outer region near the fibrocartilage junction of the meniscus. The inner region of the meniscus was mostly aneural. Menisci from the high chondropathy group had a greater number of perivascular sensory nerve profiles in the outer region (144 nerve profiles/mm^2 (IQR 134–189)) compared with menisci from the low chondropathy group (119 nerve profiles/mm^2 (IQR 104–144)) (p=0.049). Nerve profiles in the outer region of the menisci were associated with chondropathy (r=0.59, p=0.015), but not with collagen bundle appearance (r=0.10, p=0.97).

DISCUSSION

We found that menisci from knees with high tibiofemoral chondropathy had increased blood vessel densities near the fibrocartilage junction, associated with a greater number of

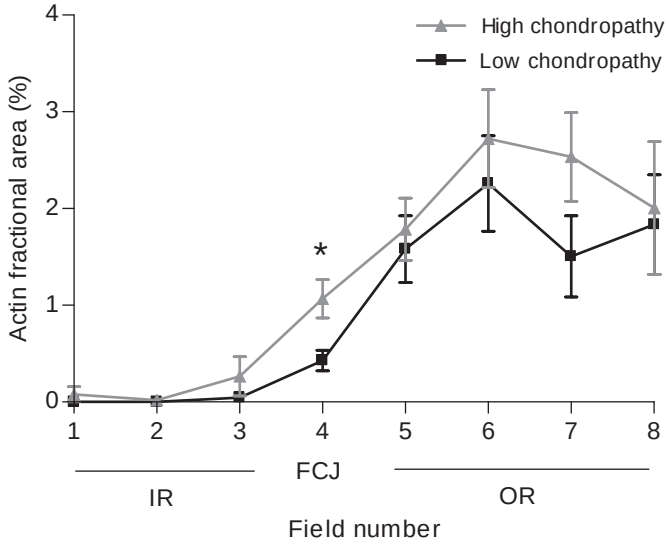

Figure 4 Depth of vascular penetration along the midline of menisci. The highest densities of α-actin-positive vessels were observed in the outer region (OR) of the menisci, where there was no significant difference in vascular density between the low and high chondropathy groups. Fewer vessels were observed in the inner region (IR) of both groups. Menisci from the high chondropathy group were characterised by increased vascular density in the fourth field of view, which corresponded to the region of the fibrocartilage junction, indicating increased penetration of vessels from the outer into the inner meniscal region. Each field was 1.4 mm in length. *$p<0.05$.

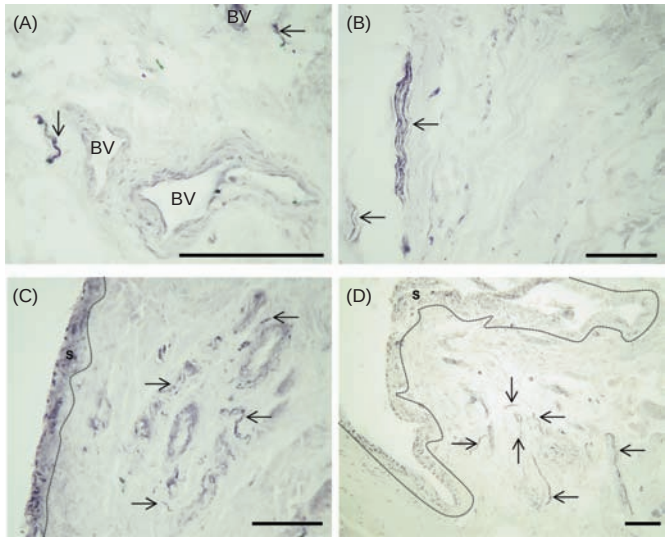

Figure 5 Calcitonin gene-related peptide-immunoreactive (sensory) nerve profiles in the outer region of human menisci. (A) Nerve profiles (arrows) associated with small arterioles (BV). (B) Apparently free nerve profiles not associated with small arterioles. (C) Meniscus from low chondropathy group having few nerve profiles associated with small arterioles. (D) Meniscus from high chondropathy group having several nerve profiles associated with small arterioles. Sample of menisci from high (A, B and D) or low (C) chondropathy cases. Dotted line indicates the boundary of the synovium (S). All scale bars = 100 μm.

perivascular sensory nerves. Angiogenesis and increased meniscal innervation are therefore possible mechanisms contributing to knee pain in tibiofemoral OA.

We have confirmed an association between disorganisation of collagenous matrix structure and chondropathy in human knees, and demonstrated increased penetration of blood vessels and nerves towards the meniscal tip with high chondropathy scores. Vascularisation of normally avascular tissues has also been demonstrated in articular cartilage in OA and inner regions of the annulus fibrosus of the intervertebral disc in back pain.[49] Meniscal angiogenesis may be a homoeostatic response for minimising meniscal damage in OA. Vascularisation changes tissue biomechanics and may predispose to further damage. Interventional studies would be required to determine whether meniscal angiogenesis protects against, or contributes to, joint damage in OA.

Our findings show that increased angiogenesis previously observed in OA synovium[13 14] extends to include synovium attached to menisci. Increased synovial vascularity was associated with vascularisation at the fibrocartilage junction of the meniscus, suggesting that angiogenesis in these different joint compartments may be regulated by common factors. Synovial angiogenesis is associated with synovitis in OA, and may be driven by increased expression of angiogenic factors such as vascular endothelial growth factor by cells within the inflamed synovium.[13 14] Furthermore, the meniscus is closely apposed to articular cartilage in vivo, and vascular endothelial growth factor is also upregulated by superficial chondrocytes in OA.[50] The stimuli to meniscal blood vessel growth deserve further study, but may originate, as well as from cells within the meniscus itself, from adjacent structures such as the synovium and articular cartilage.

We have demonstrated CGRP-positive sensory nerve profiles in the outer region of menisci, and most of these nerves were associated with blood vessels. CGRP is co-localised with substance P in unmyelinated sensory nerve fibres, and our work therefore extends previous findings that perivascular nerves containing substance P were located in the peripheral portion of torn menisci.[38] We show an increase in sensory innervation in the outer region of menisci from knees with high chondropathy scores. Nerve growth in menisci may contribute to pain in OA through both an increased density of nociceptive fibres and increased sensitivity that occurs during nerve growth.[51] Recent controlled trials of nerve growth factor blockade have demonstrated its ability to inhibit OA knee pain,[52] although the rapid onset of its analgesic effect may suggest an action on sensitisation rather than on nerve growth. CGRP-immunoreactive nerves were localised adjacent to blood vessels within the meniscus, and sensory nerve growth follows blood vessel growth in a variety of tissues.[53 54] Angiogenesis inhibition may therefore also have analgesic potential by reducing aberrant innervation in the osteoarthritic knee.[55]

Substance P and CGRP released from sensory nerve terminals can amplify the inflammatory response[56 57] as well as initiating angiogenesis, with the potential to contribute to both pain and structural damage.[58] It remains possible that the increased sensory innervation in menisci from knees with chondropathy contributes to, as well as being a consequence of, meniscal angiogenesis.

We used macroscopic appearance of chondropathy as an indicator of tibiofemoral OA. Chondropathy scores for our high chondropathy group were similar to those previously observed at total joint replacement surgery for knee OA (257 (IQR 228–283)),[5] all of whom fulfilled the American College of Rheumatology revised criteria for OA.[61] Synovial and meniscal angiogenesis do not appear to be restricted to knees undergoing arthroplasty. Our findings of meniscal pathology are consistent with MRI studies showing meniscal

abnormalities associated with symptomatic radiographic OA compared with asymptomatic and non-radiographic groups.[59 60] Chondropathy alone, however, is insufficient to diagnose knee OA,[61] and further studies would be required to determine whether the meniscal changes that we have observed are associated with other features of OA. During selection of cases for this study, three were excluded from the high chondropathy group because of non-availability of meniscal tissue. Menisci can be completely disrupted in severe OA and the current findings may only be relevant to cases where menisci remain intact. The prevalence and severity of OA increase with age, and it was not possible in this study to exclude the possibility that differences in vascularity and innervation between high and low chondropathy groups may have been age- rather than disease-related. It is not known whether patients from whom the menisci were obtained had knee pain, and our proposal that meniscal pathology may be associated with knee pain remains speculative. Despite these limitations, however, our use of post-mortem tissues permitted the analysis of two discrete patient groups, with menisci harvested and processed and chondropathy assessed by identical methodologies, avoiding differences between surgical and control specimens that can often limit interpretation of pathological studies.

In conclusion, we have shown increased vascularity and innervation of menisci from knees displaying high chondropathy scores. Neovascularisation and innervation may contribute to both structural change and pain in OA. Inhibition of angiogenesis and/or nerve growth may each offer the potential to prevent meniscal innervation, and interventional studies are needed to further elucidate the contribution of, and the links between, angiogenesis, nerve growth and pain.

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Competing interests None.

Ethics approval This study was conducted with the approval of the Nottingham Research Ethics Committee (08/H0403/132).

Provenance and peer review Not commissioned; externally peer reviewed.

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