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Mesenchymal progenitor cells combined with pentosan polysulfate mediating disc regeneration at the time of microdiscectomy: a preliminary study in an ovine model

Laboratory investigation

David Oehme, M.B.B.S. (Hons),[1] Peter Ghosh, D.Sc., Ph.D., F.R.S.C.,[1,2] Susan Shimmon, B.Sc.,[2] Jiehua Wu, Ph.D., M.Sc.,[2] Courtney McDonald, B.Sc. (Hons), Ph.D.,[1] John M. Troupis, M.B.B.S., F.R.A.N.Z.C.R.,[3] Tony Goldschlager, M.B.B.S., Ph.D., F.R.A.C.S.,[1] Jeffrey V. Rosenfeld, M.D., M.S., F.R.A.C.S.,[4,5] and Graham Jenkin, Ph.D.[1]

[1]The Ritchie Centre, Monash Institute of Medical Research, Monash University, Clayton, Victoria; [2]Proteobioactives Research Laboratories, Brookvale, New South Wales; [3]Diagnostic Imaging, Monash Health, Clayton; [4]Department of Surgery, Monash University, Clayton; and [5]Department of Neurosurgery, Alfred Hospital, Melbourne, Victoria, Australia

Object. Following microdiscectomy, discs generally fail to undergo spontaneous regeneration and patients may experience chronic low-back pain and recurrent disc prolapse. In published studies, formulations of mesenchymal progenitor cells combined with pentosan polysulfate (MPCs+PPS) have been shown to regenerate disc tissue in animal models, suggesting that this approach may provide a useful adjunct to microdiscectomy. The goal of this preclinical laboratory study was to determine if the transplantation of MPCs+PPS, embedded in a gelatin/fibrin scaffold (SCAF), and transplanted into a defect created by microdiscectomy, could promote disc regeneration.

Methods. A standardized microdiscectomy procedure was performed in 18 ovine lumbar discs. The subsequent disc defects were randomized to receive either no treatment (NIL), SCAF only, or the MPC+PPS formulation added to SCAF (MPCs+PPS+SCAF). Necropsies were undertaken 6 months postoperatively and the spines analyzed radiologically (radiography and MRI), biochemically, and histologically.

Results. No adverse events occurred throughout the duration of the study. The MPC+PPS+SCAF group had significantly less reduction in disc height compared with SCAF-only and NIL groups ($p < 0.05$ and $p < 0.01$, respectively). Magnetic resonance imaging Pfirrmann scores in the MPC+PPS+SCAF group were significantly lower than those in the SCAF group ($p = 0.0213$). The chaotropic solvent extractability of proteoglycans from the nucleus pulposus of MPC+PPS+SCAF-treated discs was significantly higher than that from the SCAF-only discs ($p = 0.0312$), and using gel exclusion chromatography, extracts from MPC+PPS+SCAF-treated discs also contained a higher percentage of proteoglycan aggregates than the extracts from both other groups. Analysis of the histological sections showed that 66% ($p > 0.05$) of the MPC+PPS+SCAF-treated discs exhibited less degeneration than the NIL or SCAF discs.

Conclusions. These findings demonstrate the capacity of MPCs in combination with PPS, when embedded in a gelatin sponge and sealed with fibrin glue in a microdiscectomy defect, to restore disc height, disc morphology, and nucleus pulposus proteoglycan content.
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Key Words • **lumbar disc degeneration** • **microdiscectomy** • **stem cell** • **mesenchymal progenitor cell** • **pentosan polysulfate** • **sheep**

Microdiscectomy is one the most common surgical procedures performed by spine surgeons, with more than 300,000 procedures performed each year in the United States.[58] While microdiscectomy has a high success rate at relieving sciatica, it fails to address the underlying problem of disc degeneration. Following microdiscectomy, discs fail to undergo spontaneous regeneration and patients may experience chronic low-back pain and recurrent disc prolapse.[12] The reported incidence of low-back pain following microdiscectomy ranges from 10% to 75%, with 10% of patients experiencing severe

Abbreviations used in this paper: AF = anulus fibrosus; AF1 = anular half containing the microdiscectomy; AF2 = anular half contralateral to the microdiscectomy; CEP = cartilaginous endplate; DHI = disc height index; GuHCl = guanidine hydrochloride; MPC = mesenchymal progenitor cell; MSC = mesenchymal stem cell; NIL = no treatment; NP = nucleus pulposus; PG = proteoglycan; PPS = pentosan polysulfate; SCAF = scaffold; VB = vertebral body.

This article contains some figures that are displayed in color online but in black-and-white in the print edition.

disabling back pain[13,14,45,88] and 20% of patients being dissatisfied with the outcome of surgery 2 years postoperatively.[84] Symptomatic recurrent disc prolapse occurs in as many as 7%–25% of patients.[5,12,14,15,82] Measures that could be implemented at the time of microdiscectomy to prevent or alleviate these unwanted side effects would improve surgical outcomes for many patients worldwide.

Advances in cell biology and tissue engineering have led to significant progress in the field of biological treatments to induce disc regeneration. Multiple preclinical animal studies have demonstrated the efficacy of intradiscally injected mesenchymal stem cells (MSCs) to induce disc regeneration.[1,2,16,18,20,21,25,31–34,38,39,49,53,56,60,64–66,69,73,83,87,90] Allogeneic mesenchymal progenitor cells (MPCs) are the earliest uncommitted clonogenic population of bone marrow stromal cells and have also successfully repaired the extracellular matrix following their injection into degenerative ovine discs.[25] Moreover, when ovine MPCs combined with the chondrogenic agent pentosan polysulfate (PPS) were embedded in a gelatin sponge and placed in a biodegradable cage that was implanted into the interbody space of ovine cervical spines, the cage became filled with a predominately cartilaginous matrix.[27] Allogeneic human MPCs are currently being investigated in clinical trials for the treatment of back pain in patients with degenerative disc disease (http://clinicaltrials.gov/show/NCT01290367).

Notwithstanding the availability of autologous or allogeneic MPCs as a potential modality for regenerating a damaged disc following microdiscectomy, a major hurdle is the maintenance of the cells within the disc space immediately following the procedure. The problem does not present for simple injection of cells into the nucleus pulposus (NP) of discs where the anulus is circumferentially intact and thus preventing MPC leakage from the site. Following microdiscectomy in cases in which the anulus is typically breached by the surgeon, the transplanted cells may leak out and localize in peridiscal sites. This efflux of cells out of the NP might not only reduce or eliminate the cells' ability to repair the target disc, but the potential arises for adverse side effects such as the inadvertent formation of osteophytes.[81] A possible solution would be to implant a formulation of the MPCs, with PPS, in a bioresorbable matrix into the defect, followed by sealing the anular defect with a biocompatible glue, such as fibrin glue.[86]

In the present study, we describe experiments undertaken in an ovine model to test the hypothesis that the surgical implantation of a gelatin sponge seeded with a formulation of MPCs and PPS into a disc defect induced by microdiscectomy, followed by sealing of the defect with a fibrin glue, would promote disc regeneration.

Methods

Study Design

Six adult East Friesian/Merino male sheep underwent a standardized lumbar microdiscectomy procedure at 3 lumbar levels—L2–3, L3–4, and L4–5. The L1–2 and L5–6 discs served as untreated controls.[55] Disc defects after microdiscectomy received either no treatment (NIL), a gelatin/fibrin glue scaffold only (SCAF), or MPCs and PPS in the same scaffold (MPC+PPS+SCAF). Necropsies were undertaken at 6 months and the spines isolated. All spines underwent radiological analysis using radiography and MRI. The discs of 3 spines subsequently underwent biochemical analysis and the remaining 3 were submitted to histological analysis.

Allogeneic MPCs

The ovine MPCs were prepared from iliac crest bone marrow aspirates of adult Border Leicester Merino crossbred sheep that had been screened for mycoplasmas and other common ovine pathogens according to published procedures.[28,89] The authenticity of the ovine MPCs was confirmed using multilineage differentiation assays and flow cytometry, demonstrating that the MPCs expressed the MSC markers CD29, CD44, CD146, CD166, and HSP-90 and lacked expression of the hemopoietic and vascular endothelial markers CD31, CD14, and CD45.[25,28] Following culture, total cell count and cell viability were determined and the ovine MPCs were resuspended in cryopreservation medium (42.5% ProFreeze/7.5% DMSO/50% Alpha-MEM) containing PPS (bene pharmaChem GmbH) to the required concentration (see below). The formulation was then cryopreserved and stored in the vapor phase of a long-term liquid nitrogen storage tank until transfer to the operating theater.

Vials containing 5×10^6 ovine MPCs plus 125 μg PPS in 500 μl were used for the present study. Prior to administration, recovery of the frozen preparations was achieved by rapid thawing at 37°C. Cell number and viability were determined using a Neubauer hemocytometer (Invitrogen). Where cell counts or viabilities were less than 80%, cells were disposed of and a new vial used. Discs treated with MPCs+PPS+SCAF received doses of 1×10^6 ovine MPCs plus 25 μg PPS in 100 μl of cryopreservation medium.

Surgical Technique and Administration of MPCs+PPS

All surgical and experimental procedures were approved by the Monash Medical Centre Animal Ethics Committee and conformed to the Australian code of practice for the care and use of animals for scientific purposes (7th edition, 2004).

Sheep were fasted for 24 hours prior to surgery and anesthetized using intravenous thiopentone (10–15 mg/kg) followed by intubation and isofluorane inhalation (2%–3% in oxygen) prior to being placed in the right lateral position. Following the subcutaneous administration of local anesthetic (bupivacaine 0.5%), the L2–3, L3–4, and L4–5 lumbar intervertebral discs were exposed using a minimally invasive left lateral retroperitoneal approach (Fig. 1A).[55] Intraoperative lateral radiographs were acquired to identify the correct levels and to allow for calculation of disc height index (DHI). The L2–3, L3–4, and L4–5 discs then underwent a standardized microdiscectomy. A 5 × 3–mm rectangular anular incision was made in the left anterolateral disc using an 11-blade scalpel (Fig. 1B). Using pituitary rongeurs, a subtotal mi-

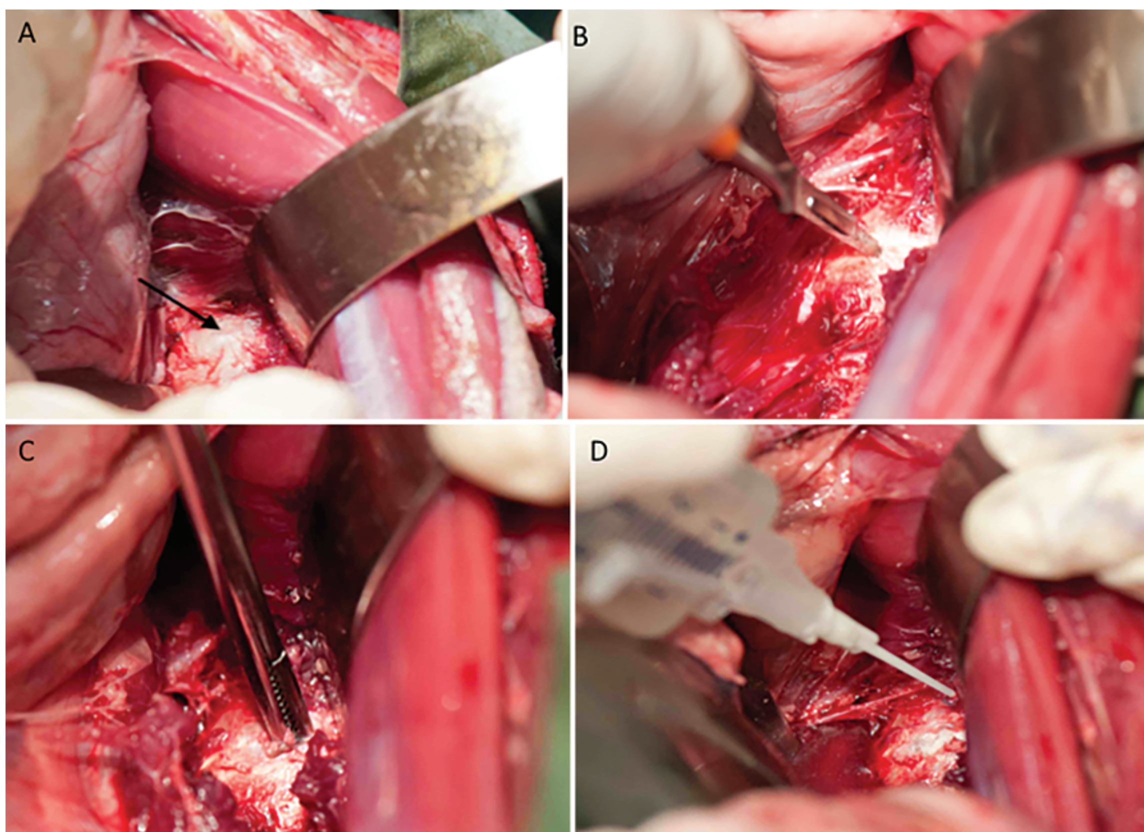

Fig. 1. Intraoperative digital photographs. **A:** Lateral retroperitoneal exposure of ovine lumbar disc *(arrow)*. **B:** A 5 × 3–mm rectangular anular incision being made in the left anterolateral disc. **C:** Removal of AF and NP tissue similar to what is performed in a human microdiscectomy procedure. **D:** Sealing of outer disc with fibrin glue following the application of MPCs+PPS to a Gelfoam sponge within the microdiscectomy defect.

crodiscectomy was performed, with removal of 0.2 g of intervertebral disc including nucleus pulposus and annulus fibrosus tissue (Fig. 1C).

Following the microdiscectomy procedure, discs levels were randomized to receive the following treatment allocations as shown in Table 1: 1) for the NIL group, only the microdiscectomy procedure was performed, with no further treatment administered; 2) for the SCAF group, a 5 × 5 × 3–mm piece of absorbent gelatin sponge (Gelfoam, Pfizer) was inserted into the microdiscectomy defect, and the disc was subsequently sealed with fibrin glue (Tisseel, Baxter); and 3) for the MPC+PPS+SCAF group, 100 µl of MPCs+PPS (from vial containing 5 × 10^6 MPCs + 125µg PPS) was injected onto 5 × 5 × 3–mm piece of gelatin sponge within disc defect, and immediately following application of MPCs+PPS, the disc space was sealed with fibrin glue to prevent efflux of the cell solution or expulsion of gelatin sponge (Fig. 1D).

TABLE 1: Treatment allocations for 6 animals to eliminate bias due to altered biomechanics or anatomical variations at different spinal levels

Spinal Level	Treatment		
	2 Sheep	2 Sheep	2 Sheep
L2–3	NIL	SCAF	MPCs+PPS+SCAF
L3–4	SCAF	MPCs+PPS+SCAF	NIL
L4–5	MPCs+PPS+SCAF	NIL	SCAF

Following administration of disc treatments, a routine layered closure was performed using absorbable sutures (Vicryl, Ethicon). Animals received a fentanyl patch (Durogesic 75 µg/hr) for postoperative analgesia and, once recovered, were immediately returned to deep litter holding pens with other sheep. All animals were intensely monitored for 5 days postoperatively by clinical veterinarians who assessed for pain, additional pain medication requirements, gross neurological dysfunction, and abnormal behavior.

After 1 week, following veterinarian approval, animals were transferred to open pasture, where they continued to be monitored on a weekly basis.

At 24 weeks following surgery, all animals were sacrificed by intravenous injection of 150 mg/kg of pentobarbital by a clinical veterinarian. The lumbar spines were removed, isolating a segment from the sacral midpoint to the thoracolumbar junction for subsequent radiological, biochemical, and histological analyses.

Radiological Analysis

Lateral and anteroposterior lumbar spinal digital radiographs (Radlink, Atomscope HF 200A) of all sheep were obtained preoperatively and prior to postmortem assessment with the lumbar spine in situ. Using standard-

ized methods, DHI measurements were calculated and recorded by an observer blinded to treated spinal levels, using digital image processing software (Osirix X v4.1.2).[47] In this method, the mean intervertebral disc height is calculated by averaging the measurements obtained from the anterior, middle, and posterior portions of the intervertebral disc and dividing that by the average height of the adjacent vertebral bodies.

Immediately following necropsy, the lumbar spines were harvested from the thoracolumbar junction to the midpoint of the sacrum, placed on ice, and then immediately transferred to a Monash Biomedical Imaging unit for MRI analysis (3-T Skyra Widebore MRI, Siemens). Sagittal and axial T2-weighted MRI sequences of the entire lumbar spine explant (L1–S1) were acquired for each animal. Using sagittal T2-weighted sequences, 3 blinded observers (a radiologist [J.T.], neurosurgeon [T.G.], and neurosurgery resident [D.O.]) determined the Pfirrmann MRI disc degeneration scores for all lumbar discs.[59]

Biochemical Analysis

Lumbar disc segments allocated for biochemical analysis were bisected by making a horizontal cut through the midline of the anulus fibrosus (AF), and images of the 2 sections from each spinal level were recorded digitally. The NP and AF regions were identified by their different morphological appearances and were separated from each other and their vertebral attachments by careful dissection. The AF tissues were subdivided into two halves, AF1 (the anular half containing the microdiscectomy) and AF2 (the anular half contralateral to the microdiscectomy) regions, as shown in Fig. 2. Tissues were then finely diced, weighed, lyophilized, and reweighed to determine water content. The dehydrated AF1 and AF2 tissues were subsequently solubilized using a papain digestion buffer (50 mM sodium acetate [pH = 6.0]) containing 2 mg/ml papain (Sigma-Aldrich Chemical) at 60°C for 16 hours.[11] The digested tissues were then centrifuged at 3000g for 15 minutes and supernatants diluted to a standard volume (the stock digest solution). Aliquots of the stock solution were analyzed for sulfated glycosaminoglycan content (an index of proteoglycan [PG] content),[19] hydroxyproline (to derive collagen content),[74] and DNA content[42] as an index of cell numbers. The lyophilized NP tissues were weighed and separated into 2 parts. One aliquot was subjected to digestion with the same papain buffer as for the AF tissues and the supernatants analyzed for sulfated glycosaminoglycans, hydroxyproline, and DNA levels as described above. The remaining NP tissues were extracted for 48 hours at 4°C with buffered 4-M guanidine hydrochloride (GuHCl) (Sigma-Aldrich Chemical) containing enzymes inhibitors as described previously.[50] The 4-M GuHCl–extracted PGs were then diluted to 2-M GuHCl with 0.5-M acetate buffer pH 6.5 containing enzyme inhibitors and aliquots (0.5 ml) subjected to gel permeation chromatography using a calibrated Sepharose CL-2B (1.0 × 29.5–cm) column (GE Life Science) eluting with the acetate buffer in the presence and absence of hyaluronan (SupaArt, SKK). Eluted fractions were assayed for the presence of glycosaminoglycans, as described above, and used to construct chromatographic profiles showing the PG concentration in the fractions from Vo to Vt. From these profiles, the areas under the curves corresponding to PG aggregates (V = Vo) and nonaggregatable PGs (V = ca 50 ml) were determined.

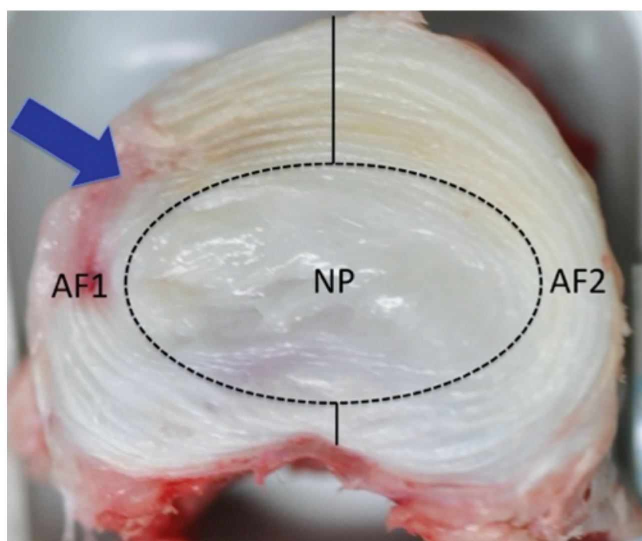

Fig. 2. Digital image of ovine intervertebral disc sectioned in the horizontal plane demonstrating the 3 regions undergoing separate biochemical analysis. The AF tissues were subdivided into 2 halves, AF1 being the anular half undergoing the microdiscectomy *(arrow)* and AF2 being the anular half contralateral to the microdiscectomy.

Histological Analysis

The individual disc segments, consisting of intervertebral disc and 1 cm of adjacent vertebral body, were isolated and fixed in multiple changes of 10% neutral buffered formalin for 8 days prior to transfer to 70% ethanol. Discs were then transported to Ratliff Histology Consultants for methylmethacrylate resin–based tissue embedding. Coronal sections of entire disc segments were cut using a motorized sliding microtome and stained using H & E, Goldner's trichrome and Safranin-O/Fast Green.

A semiquantitative ovine lumbar intervertebral disc histological grading system was used based on Bergknut and colleagues' canine histological grading system, which has been previously validated.[6] Each half of the disc was scored separately (for example, AF1 and AF2), incorporating all disc elements (AF and NP), cartilaginous endplates (CEPs), and adjacent vertebral bodies (VBs). In addition, the scoring of notochordal cell presence was eliminated due to the chondrodystrophoid phenotype of the sheep disc. The sum of all components (AF, NP, CEP, and VB) from both halves of the disc yielded a total disc histological score ranging from 0 (no degeneration) to 54 (extreme degeneration).

Statistical Analysis

All data analysis and storage was performed using a Prism 5.0d (GraphPad Software) and Excel (2011, Microsoft) software. A p value < 0.05 was considered statistically significant. Parametric data were analyzed using 1-way ANOVA, and the Tukey's multiple comparison

test was performed when significant differences in means were observed. Nonparametric data were analyzed using Kruskal-Wallis test of median values followed by Dunn's multiple comparison test. Treated groups were compared using the 2-tailed Student t-test followed by Mann-Whitney U-tests.

Results

Adverse Events

No surgical complications or cell-related adverse events were observed in any animal throughout the duration of the 6-month study. All animals remained well and were feeding throughout the duration of the experiment. No clinical manifestations of gross neurological injury, ongoing pain, or abnormal behavior were observed in any animal by study veterinarians.

Disc Height

Mean reductions in DHI for controls, NIL, SCAF, and MPC+PPS+SCAF discs were 0.0026, 0.0073, 0.0041, and 0.0032, corresponding to a mean decrease in disc height of 2.59%, 17.68%, 21.39%, and 9.60%, respectively (Fig. 3). The MPC+PPS+SCAF discs had significantly less loss of DHI compared with NIL and SCAF-only groups (9.6% vs 17.68%, $p < 0.05$ and 21.39%, $p < 0.01$). The increased loss of DHI observed between all microdiscectomy-treated levels (NIL, SCAF, and MPC+PPS+SCAF) and controls was significant (NIL & SCAF, $p < 0.0001$; MPC+PPS+SCAF, $p < 0.05$).

Magnetic Resonance Imaging

Median Pfirrmann degeneration scores for the discs that underwent microdiscectomy and that received NIL, SCAF, or MPC+PPS+SCAF were 3.0, 3.0 and 2.0, respectively. The scores for the MPC+PPS+SCAF group were significantly lower than those for the SCAF group ($p = 0.0213$) (Fig. 3). The scores for the MPC+PPS+SCAF group were also lower than those for the NIL group, but this difference was not significant.

The median Pfirrmann score for nonoperated control levels (L1–2 and L5–6 discs) was 1.5. The scores for the MPC+PPS+SCAF-treated spines, although higher, were not statistically different from those for the uninjured controls (2.0 vs 1.5, $p < 0.05$) (Fig. 3). Conversely, Pfirrmann scores for the NIL and SCAF groups were significantly higher than those for the uninjured controls ($p < 0.05$ for NIL, $p < 0.001$ for SCAF).

Biochemistry

The PG and collagen content in the 3 regions (NP, AF1, and AF2) of the control and discs undergoing microdiscectomy and subjected to each treatment regimen are shown in Figs. 4 and 5. Although the mean PG level of the MPC+PPS+SCAF-treated discs was higher than the corresponding Nil- or SCAF-treated discs, the differences were not statistically significant but were significantly lower than that of control discs ($p < 0.05$) (Fig. 4). In contrast, the PG content of the AF1 and AF2 regions of the MPC+PPS+SCAF-treated discs was equivalent to that of controls and higher than of the SCAF-treated discs ($p = 0.053$) (Fig. 5A and B). The percentage of collagen in the NP (Fig. 4) or AF (Fig. 5C and D) regions at all spinal levels, irrespective of their treatments, was statistically equivalent, notwithstanding some variations in individual mean values.

While the mean level of 4-M GuHCl–extracted PGs from the NP of all treated discs was less than that from the noninjured untreated controls, discs treated with PPS+MPCs+SCAF had significantly higher levels than

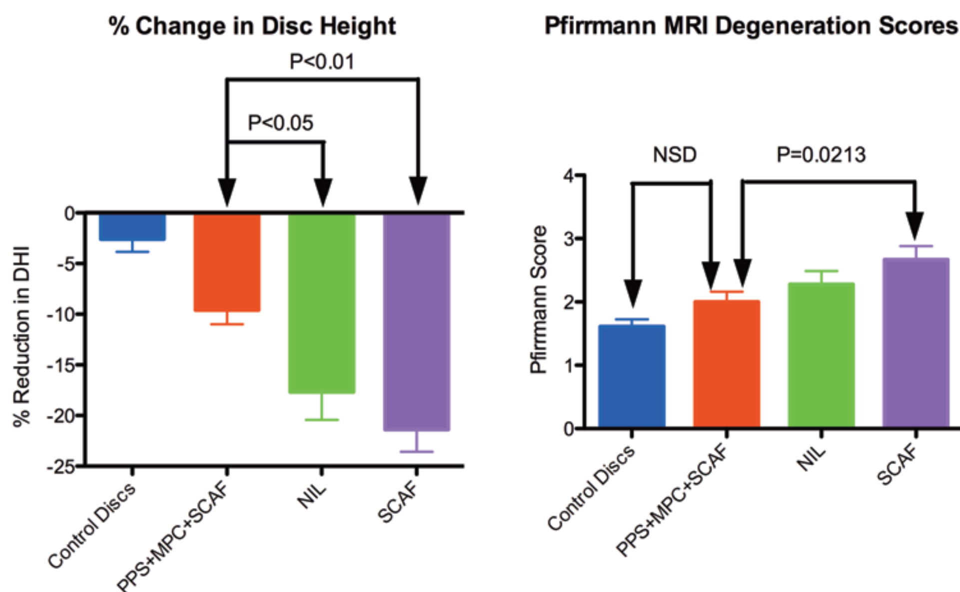

Fig. 3. Left: The MPC+PPS+SCAF-treated discs had significantly less loss of DHI when compared with NIL- and SCAF-treated discs (9.6% vs 17.68 [$p < 0.05$] and 21.39% [$p < 0.01$]) over the 24-week period following microdiscectomy. **Right:** Pfirrmann degeneration scores for the MPC+PPS+SCAF group were significantly lower than those for the SCAF group ($p = 0.0213$) and were not statistically different from those for the noninjured controls.

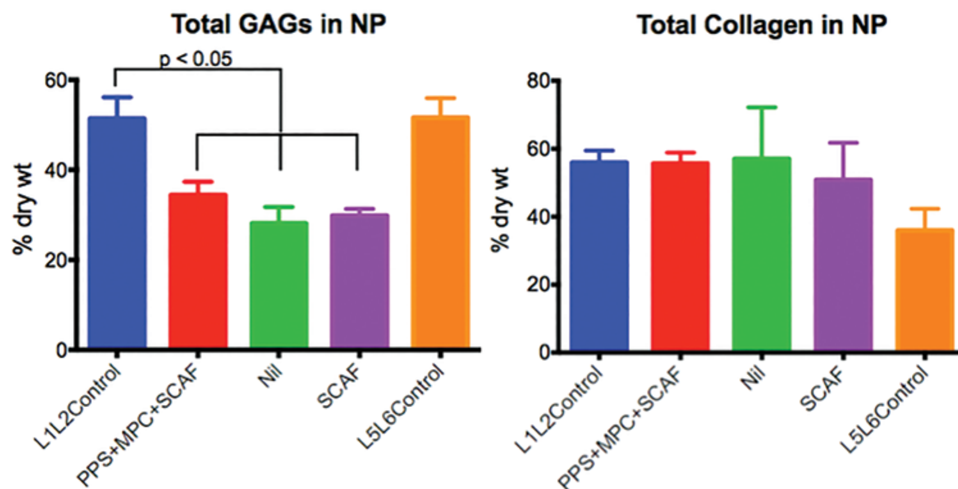

Fig. 4. The mean PG level of the MPC+PPS+SCAF-treated discs was higher than the corresponding levels of the NIL- or SCAF-treated discs, although the differences were not statistically significant. **Left:** The mean PG content of all discs undergoing microdiscectomy was significantly lower than it was for the control discs ($p < 0.05$). **Right:** The percentage of collagen in the NP of all treatment levels was statistically equivalent. GAGs = glycosaminoglycans.

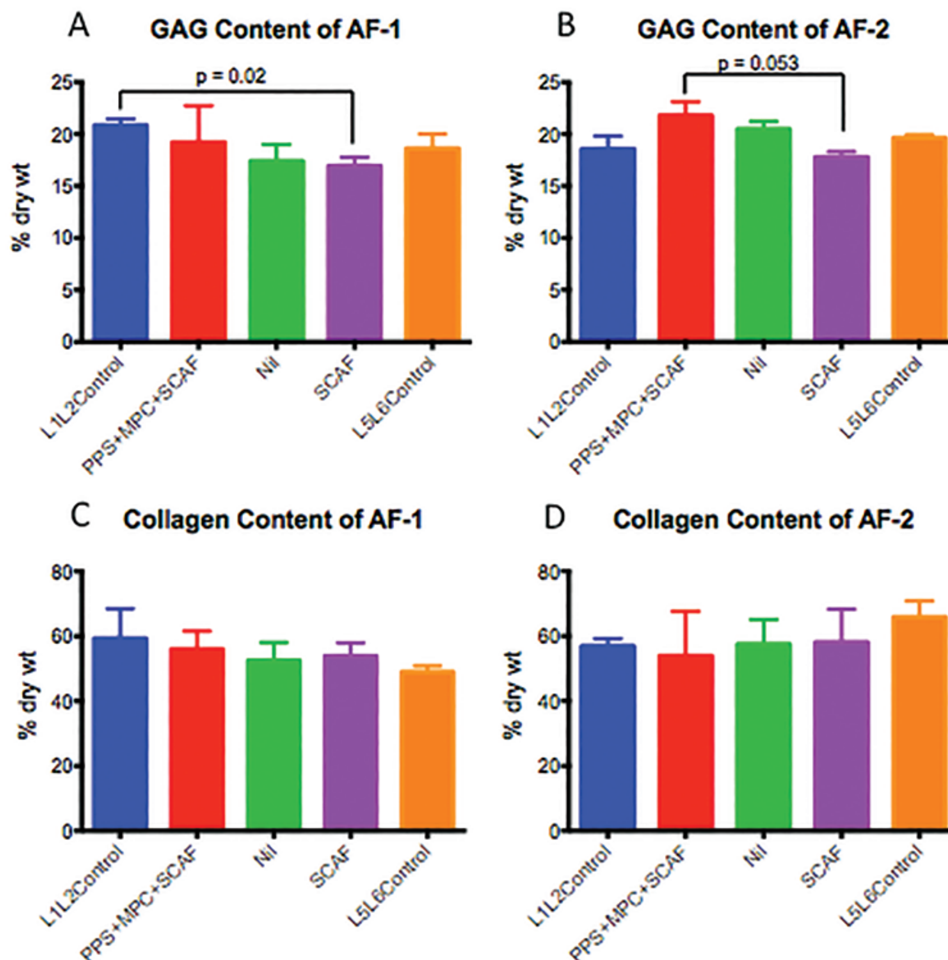

Fig. 5. **A and B:** The PG content of the AF1 and AF2 regions of the MPC+PPS+SCAF-treated discs was equivalent to that of controls and higher than that of the SCAF-treated discs ($p = 0.053$). **C and D:** The percentage of collagen in the AF1 and AF2 regions at all spinal levels, irrespective of their treatments, was statistically equivalent.

SCAF alone ($p = 0.0312$) and equivalent levels to the extractability of PGs from the L5–6 discs (Fig. 6A). When the 4-M GuHCl extracts were applied to the gel permeation chromatography column to separate the PG populations on the basis of their molecular size, we observed that the PGs extracted from the PPS+MPC+SCAF-treated discs were capable of forming a higher proportion of PG aggregates than those extracted from the L1–2 control discs ($p < 0.005$) (Fig. 6C). The percentage of PG aggregates present in the extracts of the NIL- or SCAF-treated NPs was equivalent to that of the L1–2 control NPs (Fig. 6C).

No significant differences were observed between DNA contents of the NP or AF regions of control, NIL-, SCAF-, or the MPC+PPS+SCAF-treated groups (data not shown).

Histology

Despite differences in mean values that were observed, the total disc histological scores obtained using the modified Bergknut canine histological grading system[6] failed to demonstrate statistically significant differences between the various treatment regimens ($p < 0.05$). Visual inspection of sections from 2 of 3 MPC+PPS+SCAF-treated discs showed less degenerative changes compared with the corresponding NIL- or SCAF-treated discs, as exemplified in Fig. 7. The 2 MPC+PPS+SCAF-treated discs that scored favorably also demonstrated increased healing of the injured AF regions (Fig. 7).

Discussion

The results of this study have demonstrated that it is possible to deliver allogeneic MPCs into open, surgically created disc defects, thereby promoting healing and the regeneration of an extracellular matrix within the disc. Discs undergoing microdiscectomy that received MPCs+PPS+SCAF showed significantly improved outcomes in terms of restoration of disc height, Pfirrmann MRI score, and integrity of the PG extracted from the NP relative the corresponding defect controls. Moreover, these preclinical findings highlight the feasibility of surgeons being able to administer regenerative therapies at

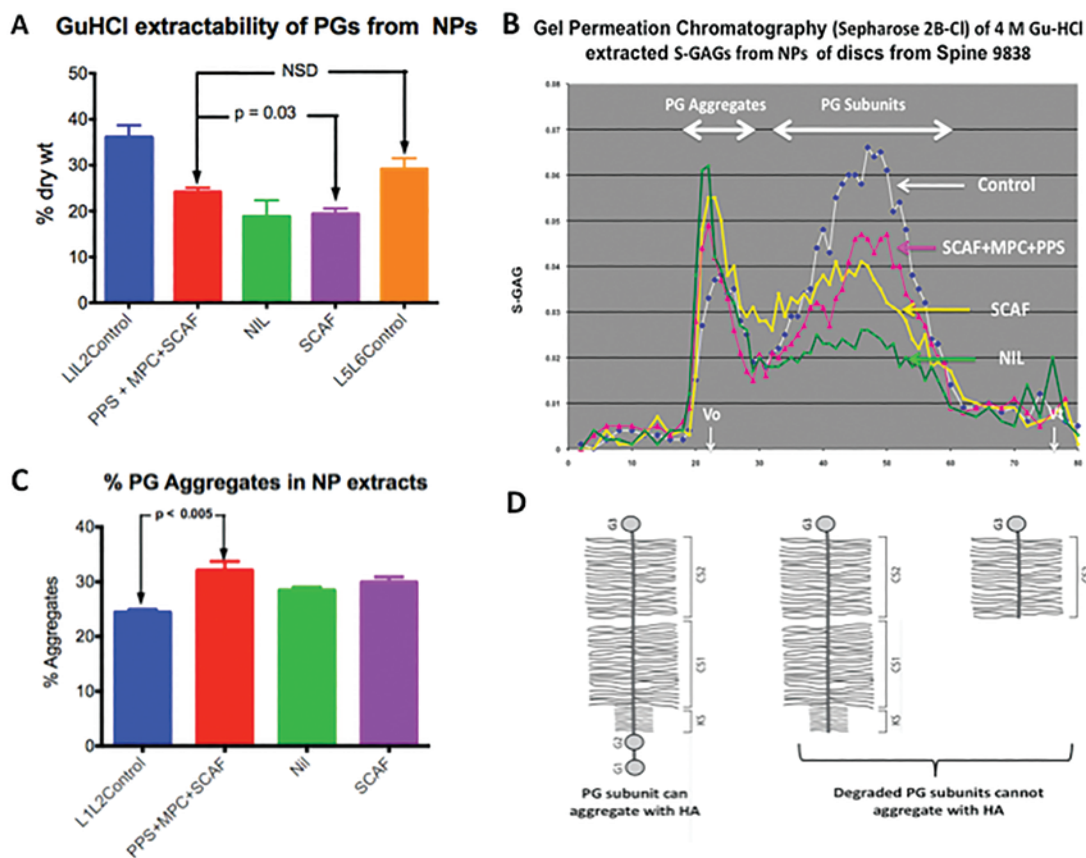

Fig. 6. A: Graph showing mean extractability values of PGs from the NP of all treated discs were less than those of the noninjured untreated controls; however, values of discs treated with PPSs+MPC+SCAF were significantly higher than those of SCAF alone ($p = 0.0312$) and equivalent to those of PGs from the L5–6 control discs. **B:** Chromatographic profiles showing the PG concentration in the fractions from Vo to Vt. From these profiles, the areas under the curves corresponding to PG aggregates (V = Vo) and nonaggregatable PGs (V = ca 50 ml) were determined. **C:** Graph showing that PGs extracted from the PPS+MPC+SCAF-treated discs were capable of forming a higher proportion of PG aggregates than those extracted from the L1–2 control discs ($p < 0.005$), whereas the extracts of the NIL- or SCAF-treated NPs were equivalent to levels of the L1–2 controls. **D:** Schema demonstrating the globular regions (G1–G2) of the PG subunit interacting with hyaluronic acid (HA) to form the PG macromolecular aggregates *(far left)*. The MPC+PPS+SCAF group had a higher proportion of these newly formed aggregates. NSD = not significantly different.

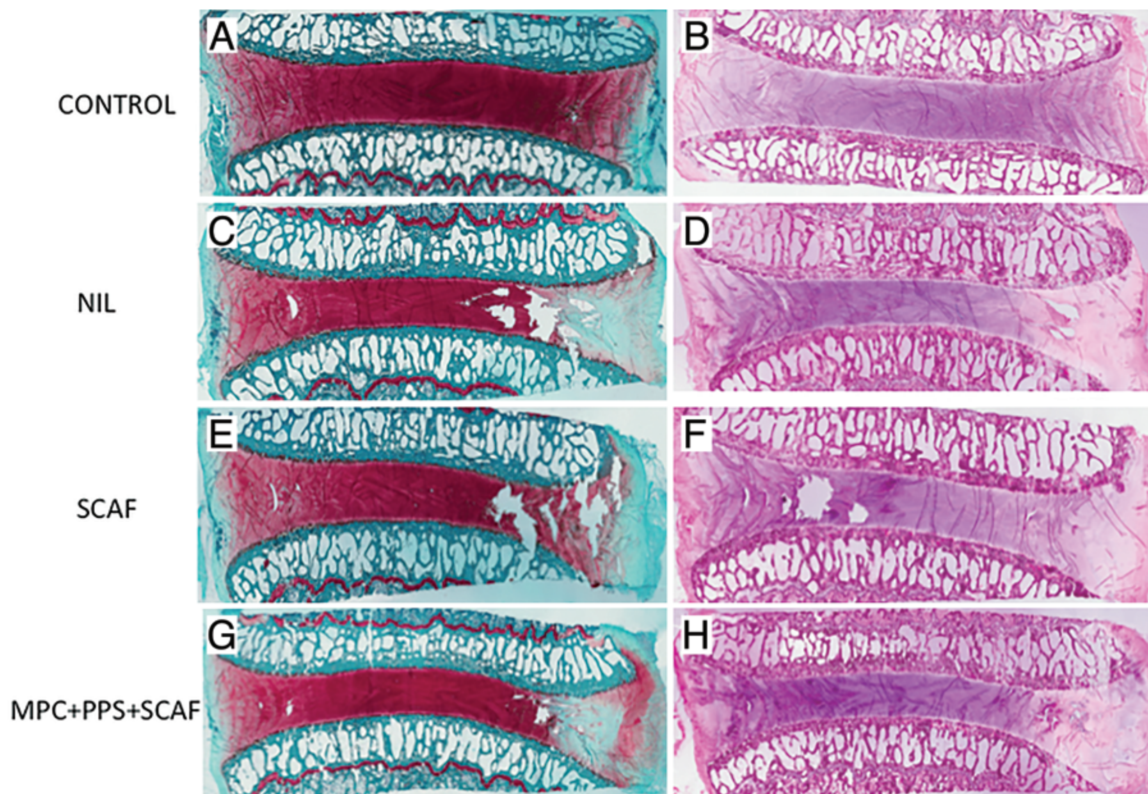

Fig. 7. Sections stained with Safranin-O/Fast Green **(A, C, E, and G)** and H & E **(B, D, F, and H)** from one animal, demonstrating L5–6 control disc and NIL-, SCAF-, and MPC+PPS+SCAF-treated discs. The right aspect of NIL, SCAF and MPC+PPS+SCAF sections underwent the microdiscectomy procedure. The MPC+PPS+SCAF disc sections (G and H) demonstrate focal collagenous healing within the disc defect with less structural degeneration of the NP and AF when compared with NIL (C and D) and SCAF (E and F) specimens. Original magnification ×2.

the time of common surgical procedures, such as microdiscectomy, where, usually, no attempt is made to instigate repair of the damaged disc.

In both preclinical and human clinical trials, chondrocyte-like cells of the NP and bone marrow–derived MSCs have been transplanted into degenerate discs in an attempt to regenerate the extracellular matrix.[1,2,10,16,20,22,25,30–32,34–38,46,48,49,53,54,56,57,60,62,64–67,69,73,83,90] The EuroDISC study, by Meisel and colleagues,[49] investigated the percutaneous transplantation of autologous disc chondrocytes. Following microdiscectomy, disc chondrocytes were harvested and expanded in vitro and were subsequently injected into the NP 3 months postoperatively. Treated patients had significantly less back pain, and an increased NP T2 signal was documented on MRI, in both the treated and adjacent discs. There are, however, several limitations and impracticalities associated with the use of disc cells. Treatment is limited to patients requiring disc surgery; otherwise patients not undergoing disc surgery would require harvesting of cells from an adjacent disc, which would inappropriately accelerate degeneration at that level. In addition, the expense, expertise, and process of isolating, expanding, and storing disc cells under GMP (good manufacturing practice) conditions will exclude such therapy from being available to most spinal surgery centers.

In contrast, MSCs can be readily isolated from a variety of tissues including bone marrow, adipose tissue, and synovium;[17,63,91] they are reported to be nonimmunogenic[44,61] and to lack the potential to undergo malignant transformation following transplantation in the recipient.[7] Mesenchymal stem cells possess the capacity for self-renewal, thus maintaining their undifferentiated phenotype in multiple subcultures, but when exposed to the appropriate stimuli, they can undergo differentiation into cells of the mesenchymal lineage such as chondrocytes, osteocytes, tenocytes, and adipose cells. They can be isolated from bone marrow aspirates by their propensity to adhere to plastic culture plates, a technique that allows them to be separated from most of the other cellular components of the marrow that do not adhere. However, the MSCs isolated by this means consist of a mixed population of MSC clones at various stages of differentiation together with contaminant mononuclear cells and fibroblasts.

The earliest uncommitted clonogenic populations of bone marrow stromal cells, designated MPCs, can be distinguished by their expression of specific cell surface antigens including STRO-1, VCAM-1 (CD106), STRO-3 (tissue nonspecific alkaline phosphatase), STRO-4 (HSP-90b), and CD146.[26,29,70] By using magnetic beads coupled to antibodies to these specific antigens, it is possible to immuno-select particular clones from the mixed cell population. Mesenchymal progenitor cells isolated in this manner when expanded in culture can generate cell banks of purified cells from a single donor, which retain extensive proliferative capacity and differentiation

potential.[28,29,89] Combined with hyaluronic acid, MPCs have been demonstrated to reconstitute the disc extracellular matrix when injected into the degenerate ovine NP.[25] Human MPCs, isolated using the same methods, are currently under investigation in clinical trials for the treatment of back pain due to degenerative disc disease (http://clinicaltrials.gov/show/NCT01290367).

The microdiscectomy procedure is the most common procedure performed by spine surgeons. Up to 90% of patients will have significant improvements in sciatica and radiculopathy following the procedure. Nevertheless, the disc fails to undergo spontaneous regeneration and patients may experience chronic low-back pain, persistent sciatica and radiculopathy, and recurrent disc prolapse.[12] Currently no therapy is available to surgeons to administer to patients, at the time of the operation, to prevent these negative outcomes.

In the development of the present study, we hypothesized that MPCs could also be administered to the disc during the same microdiscectomy procedure, when the surgeon observes the disc, rather than during a second procedure at a later date. To seal the cells within the defect a commercially available fibrin glue (Tisseel) was used. Given their biocompatibility, biodegradability, and low immunogenicity, fibrin glue matrices have been used as a cell-delivery vehicles in numerous tissue-engineering applications to facilitate cell attachment, growth, and differentiation into several tissue types.[86]

In an earlier study undertaken by our group, we successfully used a gelatin sponge as a support scaffold for implantation of MPCs, alone or in combination with PPS, into a biodegradable interbody cage fixed between the C3–4 and C4–5 intervertebral spaces of sheep cervical spines.[27] The formulation of MPCs with PPS promoted the deposition of cartilage within the cage, whereas MPCs alone generated some cartilage, but mostly bone.[27] This finding was consistent with earlier laboratory investigations that showed that human MPCs cultured in the presence of PPS proliferated, were more viable, and underwent chondrogenic differentiation but not osteogenic differentiation.[26] Pentosan polysulfate is approved for the treatment of interstitial cystitis in the US,[3] but it is also an anti-osteoarthritic drug.[23,24,43] Apart from its antiinflammatory properties,[8,9,40,41,71,72,85] PPS is a potent leukocyte elastase[4] and aggrecanase (ADAMTS-4 and ADAMTS-5) inhibitor.[77–80] Pentosan polysulfate has been shown to mediate part of its antiinflammatory effects by downregulating TNF-α activity via the suppression of NF-Kβ nuclear translocation and inhibition of the MAPKs pathways.[75,76,85] Since the levels of TNF-α[68] and ADAMTS-4/ADAMTS-5[51] are elevated in degenerative discs and have been implicated in the degradation of disc PGs, the combination of PPS with MPCs in the present study offered several potential advantages over the use of MPCs alone.

The fate of the cells once implanted into the disc space and their mechanism of action has yet to be resolved. The concept that the stem cells, once administered, differentiate into chondrocyte-like disc cells and begin to reconstitute the extracellular matrix of the disc is one possible mechanism of action. However, it is also likely that the transplanted cells act to rescue or modulate resident disc cells, by the release of soluble factors, to increase matrix production and prevent cell death and apoptosis. The PGs extracted by 4-M GuHCl from the discs implanted with MPC+PPS+SCAF were shown by size exclusion chromatography to consist of populations more capable of aggregating with hyaluronic acid (Figs. 6B and 6C). This finding indicates that the globular regions (G1–G2) of the PG subunit core protein, which interacts with HA to form the macromolecular aggrecan complex, were largely intact (Fig. 6D). This circumstance can only occur if the PGs are newly synthesized or, following synthesis, if their rate of degradation is diminished. While MPCs are known to release trophic factors, such as the bone morphogenetic proteins, that would promote new PG synthesis by the resident disc cells,[52] the anti-TNFα transduction and antiproteolytic activities of PPS could also reduce their rate of degradation.

The view that preservation of disc PGs was a significant mode of action in the MPC+PPS+SCAF-implanted discs was supported by the observation that this combination was superior to NIL- or SCAF-treated discs in terms of preserving disc height (loss of disc height 9.6% vs 17.68, $p < 0.05$; and 21.39%, $p < 0.01$). In addition, MRI degeneration scores were lower in the MPC+PPS+SCAF-treated discs than NIL- and SCAF-treated discs, with the difference between MPC+PPS+SCAF and SCAF groups reaching significance. Similarities in MRI degeneration scores between the MPC+PPS+SCAF group and normal controls may indicate that the addition of MPCs+PPS+SCAF at the time of microdiscectomy restores disc morphology toward its prediscectomy state.

The statistical analysis of the histological sections did not show any significant differences ($p > 0.05$). The low number of specimens in the histological analysis likely contributed to this unfavorable result. Nonetheless, on visual inspection, in 2 of 3 animals treated with MPCs+PPS+SCAF, we observed less structural degeneration than we did in those treated with NIL and SCAF on routine staining. This finding applied not only to the NP, but also to the AF, which exhibited less disruption of its lamella structure (Fig. 7). In the histological group, one animal that scored poorly was the result of tissue-section processing artifacts, which significantly confounded our ability to achieve statistical significance in the scoring of these sections. If the MPCs+PPS+SCAF, in fact, did not only restore the NP matrix but also assisted recovery of the anular fibrocartilage, this would have important and valuable repercussions for the prevention of recurrent disc prolapse.

There are several important limitations to the present study. One limitation is the small sample size that limits the study's power. Despite this, statistical significance was still reached in 3 outcomes measures (DHI, MRI, NP PG extractability, and aggregation). A second limitation of the study was that the microdiscectomy model does not exactly parallel the human clinical situation. The traditional microdiscectomy performed via a posterior approach in the humans is unable to be performed in the sheep. The extension of the ovine spinal cord into the sacral region and the ossification, or at least calcification,

of the posterior longitudinal ligament necessitate lateral retroperitoneal approaches in the sheep. In addition, the microdiscectomy procedure was performed on otherwise healthy adult ovine discs, which contained largely normal resident cells. In the humans, the microdiscectomy procedure is performed on degenerative prolapsed discs, which would contain a high proportion of nonviable cells and an inferior disc matrix. These points are important when considering translation of the results from animal models to the human setting. Although currently there is no ovine model of disc prolapse, we have initiated a research program to address this deficiency.

Despite these limitations, the present study does demonstrate the feasibility of administering stem cell therapies at the time of microdiscectomy to initiate disc repair. Spine surgeons are well positioned to administer regenerative disc therapies at the time of common spinal operations. Similar to the administration of commonly used hemostatic, antiadhesion, or dural sealing products, spine surgeons could similarly administer cell-based therapies to the disc when it is on view at the time of surgery. It is particularly relevant to the microdiscectomy procedure, in which, typically, no attempt is made to repair the disc and in which the procedure itself can contribute to further degeneration within the disc.

Conclusions

Preliminary results demonstrate the potential regenerative capabilities of MPCs combined with PPS, when administered in adhesive fibrin scaffold at the time of microdiscectomy, to restore the extracellular matrix of the intervertebral disc. This novel approach provides an alternative method for administering cell-based therapies to the damaged intervertebral disc. Although further preclinical studies are required prior to translation into clinical practice, it is conceivable that a similar approach to disc repair in humans could be instituted by spine surgeons in the foreseeable future.

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Disclosure

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Address correspondence to: David Oehme, M.B.B.S.(Hons), P.O. Box 6178, South Yarra, Victoria 3141, Australia. email: drdavidoehme@mac.com.