

Reconstitution of degenerated ovine lumbar discs by STRO-3–positive allogeneic mesenchymal precursor cells combined with pentosan polysulfate

David Oehme, MBBS, PhD,¹ Peter Ghosh, DSc, PhD, FRSC,^{1,2,3}

Tony Goldschlager, MBBS, PhD, FRACS,^{1,3} Silviu Itescu, MD, PhD,³ Susan Shimon, BSc,²

Jiehua Wu, PhD, MSc,² Courtney McDonald, BSc, PhD,¹ John M. Troupis, MBBS, FRANZCR,⁴

Jeffrey V. Rosenfeld, MD, MS, FRACS,^{5,6} and Graham Jenkin, PhD¹

¹The Ritchie Centre, MIMR-PHI Institute, Monash University, Clayton, Victoria; ²Proteobioactives, Pty Ltd, Brookvale, New South Wales; ³Mesoblast Ltd, Melbourne; ⁴Diagnostic Imaging, Monash Health; ⁵Department of Surgery, Monash University, Clayton; and ⁶Department of Neurosurgery, Alfred Hospital, Melbourne, Victoria, Australia

OBJECTIVE Disc degeneration and associated low-back pain are major causes of suffering and disability. The authors examined the potential of mesenchymal precursor cells (MPCs), when formulated with pentosan polysulfate (PPS), to ameliorate disc degeneration in an ovine model.

METHODS Twenty-four sheep had annular incisions made at L2–3, L3–4, and L4–5 to induce degeneration. Twelve weeks after injury, the nucleus pulposus of a degenerated disc in each animal was injected with ProFreeze and PPS formulated with either a low dose (0.1 million MPCs) or a high dose (0.5 million MPCs) of cells. The 2 adjacent injured discs in each spine were either injected with PPS and ProFreeze (PPS control) or not injected (nil-injected control). The adjacent noninjured L1–2 and L5–6 discs served as noninjured control discs. Disc height indices (DHIs) were obtained at baseline, before injection, and at planned death. After necropsy, 24 weeks after injection, the spines were subjected to MRI and morphological, histological, and biochemical analyses.

RESULTS Twelve weeks after the annular injury, all the injured discs exhibited a significant reduction in mean DHI (low-dose group 17.19%; high-dose group 18.01% [$p < 0.01$]). Twenty-four weeks after injections, the discs injected with the low-dose MPC+PPS formulation recovered disc height, and their mean DHI was significantly greater than the DHI of PPS- and nil-injected discs ($p < 0.001$). Although the mean Pfirrmann MRI disc degeneration score for the low-dose MPC+PPS–injected discs was lower than that for the nil- and PPS-injected discs, the differences were not significant. The disc morphology scores for the nil- and PPS-injected discs were significantly higher than the normal control disc scores ($p < 0.005$), whereas the low-dose MPC+PPS–injected disc scores were not significantly different from those of the normal controls. The mean glycosaminoglycan content of the nuclei pulposus of the low-dose MPC+PPS–injected discs was significantly higher than that of the PPS-injected controls ($p < 0.05$) but was not significantly different from the normal control disc glycosaminoglycan levels. Histopathology degeneration frequency scores for the low-dose MPC+PPS–injected discs were lower than those for the PPS- and Nil-injected discs. The corresponding high-dose MPC+PPS–injected discs failed to show significant improvements in any outcome measure relative to the controls.

CONCLUSIONS Intradiscal injections of a formulation composed of 0.1 million MPCs combined with PPS resulted in positive effects in reducing the progression of disc degeneration in an ovine model, as assessed by improvements in DHI and morphological, biochemical, and histopathological scores.

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KEY WORDS degenerative; disc regeneration; mesenchymal precursor cell; pentosan polysulfate; sheep model

ABBREVIATIONS AF = annulus fibrosus; cABC = chondroitinase ABC; DHI = disc height index; GAG = glycosaminoglycan; HA = hyaluronic acid; MPC = mesenchymal precursor cell; MSC = mesenchymal stem cell; NP = nucleus pulposus; PG = proteoglycan; PPS = pentosan polysulfate.

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LOW-BACK PAIN is the leading cause of disability in the developed world.⁷³ Recent advances in cell biology and tissue engineering have led to increased research into the use of cell-based biological therapies for the treatment of disc degeneration. Preclinical and clinical studies have used mesenchymal stem cells (MSCs) and the chondrocyte-like cells derived from the nucleus pulposus (NP) of the disc or juvenile articular cartilage to repair the intervertebral disc.^{1,2,7,13–15,22–30,38,39,57,59,74,75} Unlike disc chondrocytes, MSCs can be readily isolated from a variety of tissues including bone marrow, adipose tissue, and synovium.^{9,76} Moreover, MSCs possess the capacity for self-renewal and thus maintain their undifferentiated phenotype in multiple subcultures, but when they are exposed to the appropriate stimuli, they can undergo differentiation into cells of the mesenchymal lineage, such as chondrocytes, osteocytes, and adipose cells.^{9,76} The earliest uncommitted clonogenic populations of bone marrow stromal cells, designated mesenchymal precursor cells (MPCs), can be distinguished and isolated by their expression of certain cell surface antigens including STRO-1, VCAM-1 (CD106), STRO-3 (tissue nonspecific alkaline phosphatase [TNAP]), STRO-4 (HSP-90b), and CD146.^{21,62}

In a previous study that used an ovine model of disc degeneration induced by the injection of the enzyme chondroitinase ABC (cABC) into lumbar disc NPs, STRO-3-positive and immunoselected allogeneic ovine MPCs formulated with hyaluronic acid (HA) were reported to restore disc extracellular matrix 6 months after intradiscal injection.¹⁸ A criticism of the cABC model is that the viability of the native disc cells is largely preserved after cABC injection, and these cells still possess the capacity to regenerate an extracellular matrix irrespective of the administration of MPCs. The ovine annular injury model of disc degeneration has been studied extensively^{12,36,41–43,45,47–49} and exhibits pathological events comparable to those observed for degenerated human discs, including degradation of the extracellular matrix, a decline in endogenous cell viability, and neovascularization.^{16,22,55,70,71} We therefore considered the ovine annulus fibrosus (AF) injury model to represent a more suitable large-animal model for the evaluation of novel biological therapies.

Although HA has been shown to exhibit profound effects on the morphogenesis and chondrogenic differentiation of embryonic MSCs,^{34,35} similar studies undertaken with adult bone marrow-derived MSCs have generated conflicting results.^{10,32,65} An *in vitro* study that investigated the ability of HA and the polysulfated polysaccharide pentosan polysulfate (PPS) to promote chondrogenic differentiation of MPCs in culture found that HA over a concentration range of 2.5–20 μ /ml inhibited MPC chondrogenesis rather than promoted it.¹⁹ In contrast, PPS markedly stimulated MPC chondrogenic differentiation and also promoted cell proliferation, reduced MPC apoptosis, and inhibited osteogenic differentiation.¹⁹ These *in vitro* findings suggest that the combination of MPCs with PPS could provide a useful means of stimulating chondrogenic differentiation of MPCs *in vivo*.

In this study, we report the results of investigations into the relative efficacy and safety of formulations of low and

high doses of MPCs and PPS on the reconstitution of degenerated discs by using an ovine model of disc degeneration induced by limited surgical injury to the periphery of the AF.

Methods

Study Design

Twenty-four adult East Friesian/Merino male sheep were subjected to a standardized 6 × 20-mm annular incision⁴⁴ at L2–3, L3–4, and L4–5. The L1–2 and L5–6 discs served as uninjured and untreated controls. Twelve weeks after injury, the NPs of degenerated discs were injected with the test formulations. One of the 3 degenerated lumbar discs from each animal in each group was injected with the MPC+PPS formulation at the concentrations shown in Table 1. An adjacent degenerated disc in each spine was injected with the noncellular components of the formulation (ProFreeze+PPS; Table 1, component controls), whereas the remaining degenerated disc received no treatment (nil-injected injured control). The 3 disc levels of each animal were randomly assigned for the treatments to eliminate any potential effects of spinal level on outcomes.

Necropsies were performed 6 months after the injections. All spines underwent radiological analysis (radiography and MRI). The lumbar discs of 6 animals from each treatment group were used for morphological and biochemical analysis. The lumbar discs from the remaining 6 animals of each experimental group were processed for histological assessments.

Preparation of Allogeneic Ovine STRO-3-Positive MPCs

The ovine MPCs were prepared from iliac crest bone marrow aspirates of adult Border Leicester Merino cross-bred sheep that had been screened for mycoplasmas and other common ovine pathogens using procedures described previously.^{21,62} The immunoselected ovine STRO-3-positive MPCs and their formulations with PPS (Bene Pharmachem GmbH & Co) were prepared under Good Manufacturing Practice by Biotest Laboratories Pty Ltd under contract to Mesoblast Ltd.

Surgical Technique and Administration of MPC Formulations

All the surgical and experimental procedures used for this study 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).

The sheep were fasted for 24 hours and then anesthetized using intravenous thiopentone (10–15 mg/kg) and inhaled isoflurane (2%–3% in oxygen) before being placed in the lateral position. The left L2–3, L3–4, and L4–5 intervertebral discs were exposed by using a minimally invasive left-lateral retroperitoneal approach.⁵¹

Using a custom scalpel with a 6-mm depth collar attached, a horizontal 6 × 20-mm annular incision was made in the left anterolateral aspect of the L2–3, L3–4, and L4–5 intervertebral discs. A routine layered closure was then performed by using absorbable sutures (Vicryl,

TABLE 1. Summary of the cellular and noncellular components of the formulations used in the study, their respective concentrations, and volumes injected into the discs

Experimental Group	MPC Formulation	Formulation Component Controls	Degenerated Disc Controls	Disc Analyses
Low-dose MPC+PPS (n = 12)	0.1 million MPCs + 100 µg PPS in 200 µL ProFreeze	100 µg PPS in 200 µL ProFreeze	Nil injected	Biochemistry (n = 6), histology (n = 6), radiology (n = 12)
High-dose MPC+PPS (n = 12)	0.5 million MPCs + 500 µg PPS in 200 µL ProFreeze	500 µg PPS in 200 µL ProFreeze	Nil injected	Biochemistry (n = 6), histology (n = 6), radiology (n = 12)

Ethicon). The animals received a fentanyl patch (Durogesic 75 µg/hr) for postoperative analgesia.

Twelve weeks later, the animals were subjected to an identical surgical procedure from the side contralateral to that of the annular injury. Using a 22-gauge needle connected to a preloaded syringe, the anterolateral midline of the AFs of the L2–3, L3–4, or L4–5 discs were penetrated to the depth of the center of the NP. Two hundred microliters of the test article was injected, and after the needle was carefully withdrawn, a layered closure was performed.

Twenty-four weeks after test article administration, each animal was killed humanely by an intravenous injection of 150 mg/kg of pentobarbital administered by a clinical veterinarian. Each animal's lumbar spine was removed en bloc for subsequent radiological, morphological, biochemical, and histological analysis.

Radiographic Analysis

Lateral lumbar spinal digital radiographs (Radlink, Atomscope HF 200A) of each sheep were obtained before study entry (before annular injury [Week 0]), before cell injection (Week 12), and before postmortem assessment with the lumbar spine in situ (Week 36). Using standardized methods, disc height index (DHI) measurements were calculated and recorded by an observer blinded to spinal treatment levels using digital image-processing software (Osirix X v4.1.2). In this method, the 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 of the adjacent vertebral bodies.³⁷

Immediately after necropsy, each lumbar spine was harvested from the thoracolumbar junction to the midpoint of the sacrum for MRI analysis (Siemens 3-T Skyra Widebore MRI). 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, 2 blinded observers determined the Pfirrmann MRI disc degeneration score for each of the lumbar discs.⁵³

Disc Morphology Assessment

The lumbar discs of the 6 animals from each treatment group dedicated for biochemical analysis were bisected horizontally, and high-resolution digital photographs of both halves of the disc were taken, incorporating the entire NP and AF. The digital images of these discs were scored blinded using a morphological grading system.

The morphological assessment of disc degeneration using the digital photographs was determined as an aggregate of the scores of 6 regions of each disc. The AF was divided into 4 quadrants (Fig. 1), and each quadrant was scored separately from 0 to 4 according to the criteria outlined in Table 2. The NP was divided into left (NP1) and right (NP2) halves and scored separately from 0 to 2 according to the criteria shown in Table 2. The sum of all component scores yielded a total disc degeneration score from 0 (normal) to 20 (severely degenerated).

Biochemical Analysis

The NP and AF regions were identified by their distinct morphological appearances and were separated from each other and their vertebral attachments by careful dissection. The AF tissues were subdivided into 2 halves: AF1 (the annular half containing the annular lesion) and AF2 (the annular half contralateral to the annular lesion). The NP was designated the nonlamellae gelatinous tissue remaining after the AF1 and AF2 regions were removed.

The tissues were finely diced, weighed, lyophilized, and reweighed to determine their dry weight. The anhydrous NP, AF1, and AF2 tissues were subsequently solubilized using a Papain digestion buffer (Papain 2 mg/ml [Sigma-Aldrich Chemical] dissolved in 50 mM sodium acetate [pH = 6.0]) at 60°C for 16 hours.⁸ The digested tissues were then centrifuged at 3000 G for 15 minutes, and the supernatants were diluted to a standard volume with the sodium acetate buffer (the stock digest solution). Aliquots of the stock solution were analyzed by using the methods

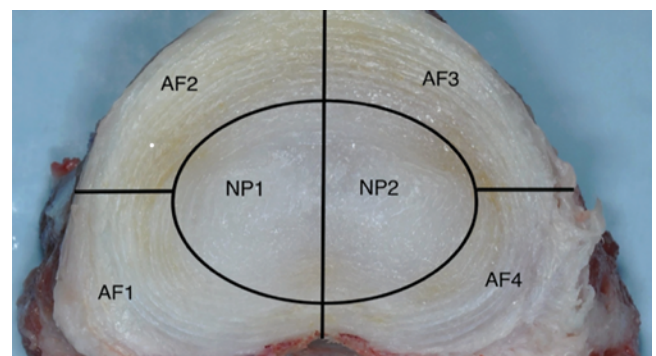


FIG. 1. Image of an ovine disc sectioned in the horizontal plane, showing the regions to be scored as part of the gross morphological grading system (see Table 2 for the criteria used for assessing the gross morphology scores). Figure is available in color online only.

TABLE 2. Criteria used for assessing the gross morphology scores for the 4 AF and 2 NP disc quadrants and for calculating the overall degeneration scores for each disc*

Score	Morphological Scoring System	
	Applied to Each AF Quadrant†	Applied to Each NP Half ‡
0	Normal disc: concentric lamellated AF structure maintained w/o disruption or discoloration; no distortion btwn AF–NP transition zone	Normal NP: no discoloration or hemorrhage; gelatinous hydrated appearance
1	Minor disruption: minor disruption/dyscoloration of lamella in outer half of AF zone; preservation of inner half of AF zone & AF–NP transition zone	Minor disruption: discoloration &/or hemorrhage <50% NP region; no fissuring; some evidence of nuclear dehydration
2	Moderate disruption: moderate disruption/dyscoloration of outer half of AF extending into inner half; preservation of AF–NP transition zone	Major disruption: discoloration & hemorrhage >50% NP region &/or presence of fissuring; dehydrated appearance
3	Major disruption: disruption/dyscoloration of entire AF w/ loss of AF–NP transition zone	
4	Complete disruption: complete loss of lamellated structure & AF–NP transition zone w/ extension into NP; bulging or prolapse of degenerated disc tissue	

* The sum of all regional scores (AF1a, AF1b, AF2a, AF2b, NP1, and NP2) yielded a total disc degeneration score between 0 (normal) and 20 (severely degenerated) for each disc.

† Quadrants are AF1a, AF1b, AF2a, and AF2b.

‡ Halves are NP1 and NP2.

described in the cited references for sulfated glycosaminoglycans (GAGs) (an index of proteoglycan [PG] content),¹¹ hydroxyproline (to obtain the collagen content),⁶⁴ and the DNA content as an index of cell numbers.³³

Histological Analysis

The individual disc segments, which encompassed approximately 1 cm of the adjacent vertebral bodies, were separated from the spines with a bandsaw. Each segment was fixed in multiple changes of 10% neutral buffered formalin for 8 days before transfer to 70% ethanol. Disc segments were then transported to Ratliff Histology Consultants for methylmethacrylate resin-based tissue embedding. Coronal sections of the entire disc segments were cut with a motorized sliding microtome and stained with H & E, Goldner's trichrome, and Safranin-O/Fast Green using standardized procedures.

A semiquantitative ovine lumbar intervertebral disc histological grading system was used based on a validated histological grading system.³ Each half of the disc was scored separately incorporating all disc elements (AF and NP), cartilaginous endplates, and adjacent vertebral bodies. However, the scoring of notochordal cell presence was eliminated because of the absence of these cells in chondrodystrophoid phenotype sheep discs.⁴⁰ The sum of all components (AF, NP, cartilaginous endplate, and vertebral body) from both halves of the disc yielded a total disc histological score from 0 (no degeneration) to 54 (extreme degeneration). Two blinded observers undertook the scoring of all histological sections and recorded the data for each group. The mean scores for the sections from each group were presented graphically by one of the investigators (P.G.) after breaking the assignment code.

Statistical Analysis

All experimental data were stored using Excel software (version 2011, Microsoft Corporation) and then transferred to Prism version 6.0e (GraphPad Software, Inc.) for sta-

tistical analysis and graphical representation. Parametric data were evaluated by using 2-way ANOVA and Tukey's multiple comparison test when significant differences in means were observed. Nonparametric data were evaluated by using the Kruskal-Wallis test of median values followed by Dunn's multiple comparison test. Treated groups were also compared by using the 2-tailed Student t-test followed by Mann-Whitney U-test or Wilcoxon matched-pairs rank test. A p value of < 0.05 was accepted as statistically significant.

Results

DHI

Twelve weeks after the annular injury, the low- and high-dose groups showed significant decreases in mean DHI of 17.19% and 18.01%, respectively (Fig. 2A and B). There were no significant differences in DHI changes observed between any of the injured discs or between the 2 control levels (Fig. 2A and B), which confirmed the validity of the model.

After administration of the test substances (12–36 weeks), discs injected with the low-dose MPC+PPS formulation exhibited a significant improvement in DHI compared with that in the nil- or PPS-injected groups ($p < 0.001$) (Fig. 2C). The recovery in DHI throughout the duration of the experiment (0–36 weeks) for this group was also significantly increased compared with that of the nil and PPS groups ($p < 0.001$) (Fig. 2E). No significant improvement in DHI was observed for the high-dose MPC+PPS-injected discs over 12–36 or 0–36 weeks (Fig. 2D and F).

Pfirschmann MRI Scores

Although the mean Pfirschmann MRI disc degeneration scores for the low-dose MPC+PPS-injected discs were lower than the corresponding nil- and PPS-injected disc scores, the differences were not significantly different (Fig. 3A). Similar findings were observed for the high-dose groups (Fig. 3B).

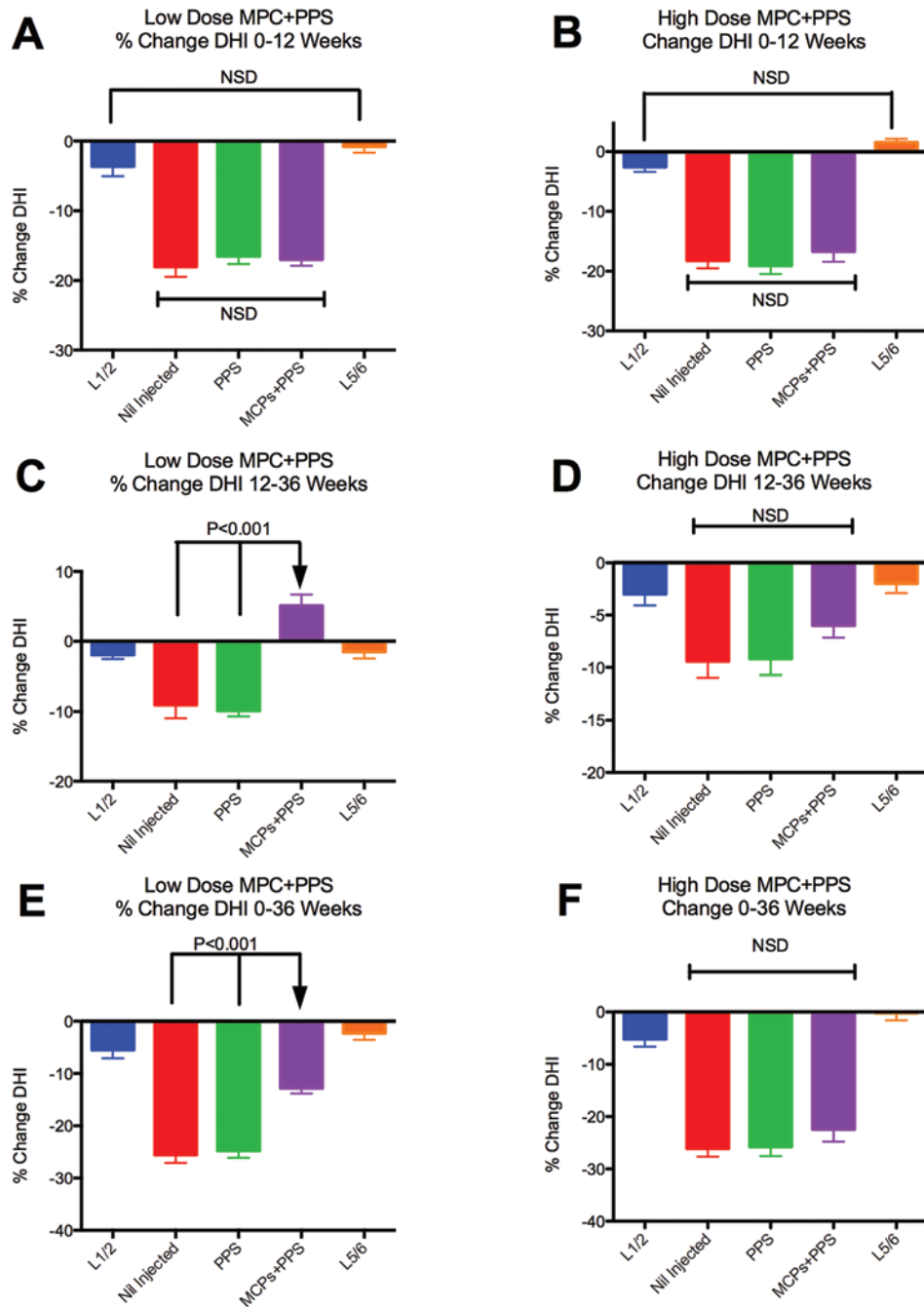


FIG. 2. A and B: Percentage changes in DHI for the low- and high-dose MPC groups, respectively, from baseline to 12 weeks after AF injury. **C and D:** Percentage changes in DHI for each of the low- and high-dose treatments, respectively, after their administration on Week 12 until necropsy on Week 36. Over the duration of the experiment (0–36 weeks), the low-dose MPC+PPS group significantly reduced the loss of disc height relative to the nil and PPS-only groups ($p < 0.001$). **E:** Disc heights did not return to noninjured control values. **F:** In the high-dose group, each injured disc showed significant losses in DHI relative to those of controls without any effect of treatment. NSD = no significant difference. Figure is available in color online only.

Disc Morphology Scores

The total disc morphology scores for the low-dose MPC+PPS-injected group were not significantly different from those for the noninjured controls, whereas the scores for the nil- and PPS-only-injected discs were significantly higher than those of the control discs ($p < 0.05$) (Fig. 4A).

In the high-dose group, all treatment discs had morphology degeneration scores significantly higher than those of the controls ($p < 0.05$) (Fig. 4B).

Biochemical Analysis

The mean GAG content of NPs from the low- and high-

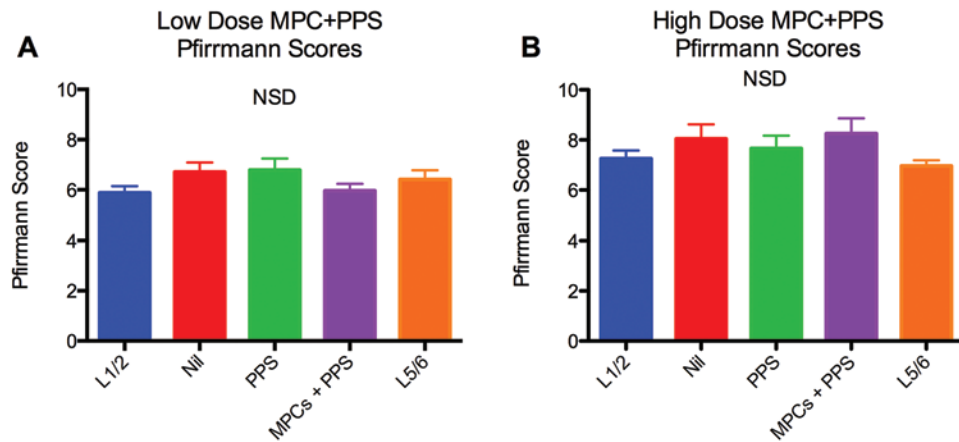


FIG. 3. Pfirmann MRI disc degeneration scores for the low-dose (A) and high-dose (B) MPC treatment groups. No statistically significant differences were detected between treatment groups or controls. Figure is available in color online only.

dose MPC+PPS-injected groups is shown in Fig. 5. Within the low-dose MPC+PPS group, the mean levels of GAG in the MPC+PPS-injected discs were significantly higher ($p < 0.05$) than those in the PPS-injected discs and equivalent to the levels in the L5–6 control discs but not to those in the L1–2 discs (Fig. 5A). Moreover, the GAG levels in the nil- and PPS-injected discs were significantly lower than those in the L5–6 control discs ($p < 0.005$) (Fig. 5A).

The corresponding GAG analysis of the high-dose MPC+PPS groups failed to find significant differences between treatments, but the mean NP GAG value of the MPC+PPS-injected discs was lower than that in the other discs (Fig. 5B). Statistical analysis of the NP collagen content data from all the low- and high-dose MPC formulations and their non-MPC component-injected discs failed to find any significant differences between treatments or normal controls (data not shown).

Although the GAG contents of the corresponding AF1 and AF2 regions for the low- and high-dose MPC+PPS

groups were not significantly different between the groups (data not shown), the collagen contents of the injured segment of the discs (AF1) were generally lower than those of the normal control discs ($p < 0.05$) (Fig. 5C and D). The exceptions were for the AF1 collagen content of discs injected with high-dose MPC+PPS, in which the levels were noted to be equivalent to the normal control levels (Fig. 5D), and low-dose MPC+PPS group analysis, for which only the PPS-injected disc collagen levels were indistinguishable from those of the control discs. The collagen contents of the AF2 disc region were similar for all groups examined (data not shown).

Analysis of the NP and AF regions for their DNA failed to reveal any significant difference between the treatment groups and normal controls (data not shown).

Histological Analysis

Attempts to analyze the mean scores of each of the treatment groups by ANOVA were unsuccessful because

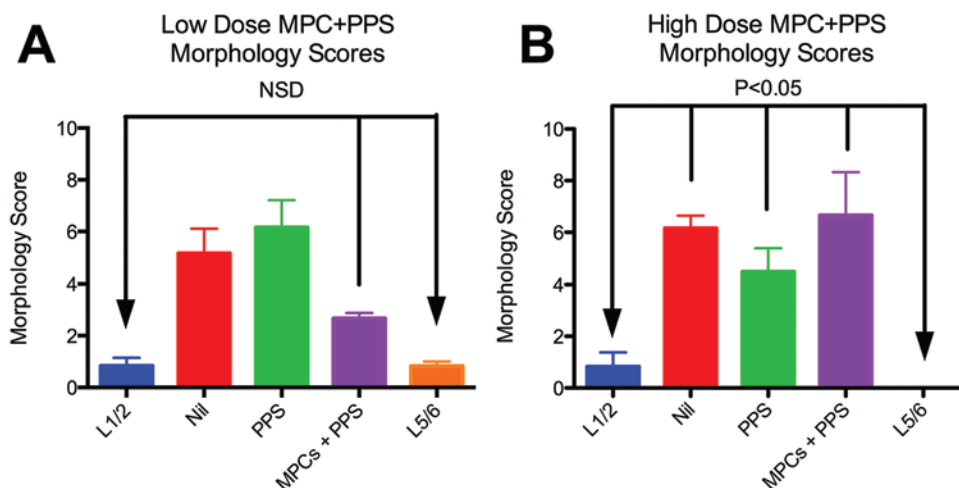


FIG. 4. A: In the low-dose MPC+PPS group, the morphology scores of the nil- and PPS-injected discs were significantly higher than those of the controls ($p < 0.05$), but the MPC and PPS-injected discs were not. **B:** All high-dose-injected discs had higher scores than the control discs. Figure is available in color online only.

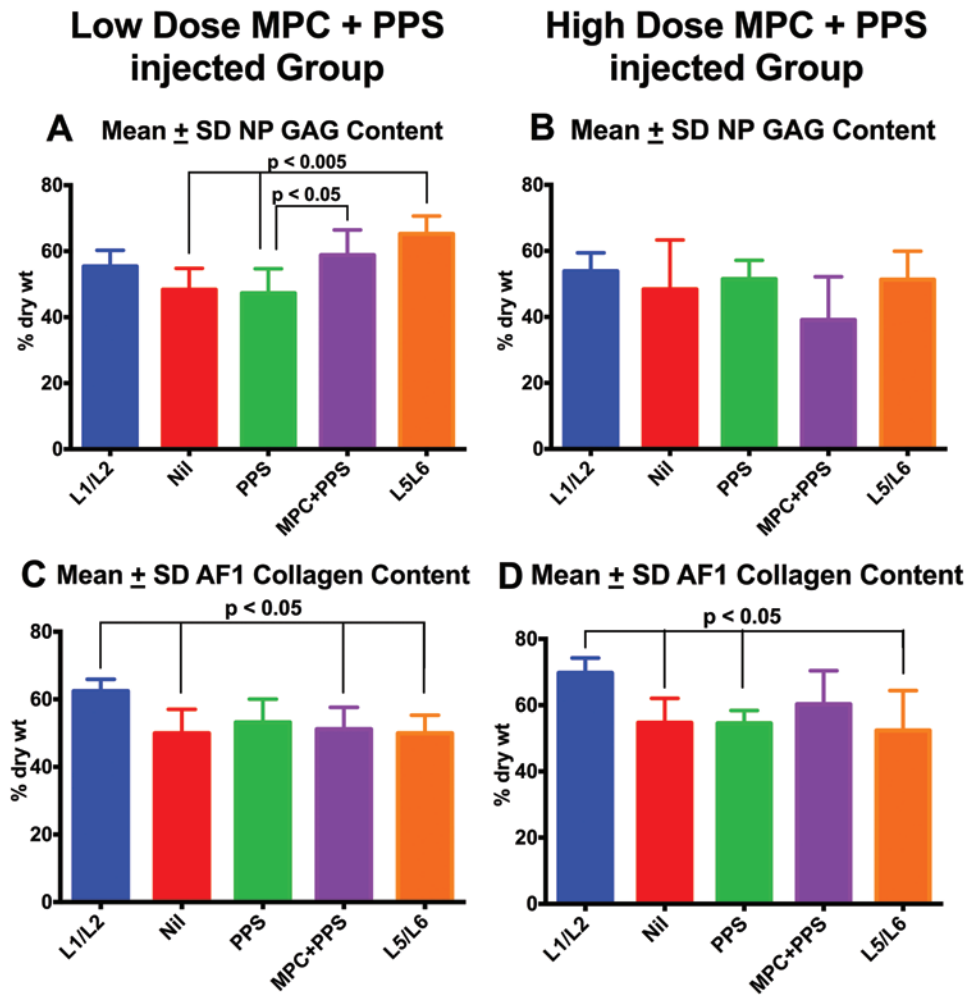


FIG. 5. A: Levels of GAG in the NP region of the low-dose MPC+PPS group; higher levels were found in the MPC+PPS-injected discs relative to those in the nil controls ($p < 0.05$). **B:** No significant differences in GAG levels were observed for the high-dose MPC+PPS group. **C and D:** Collagen contents of the injured (AF1) segment of the disc AFs. In the low-dose MPC+PPS group analysis (C), only the PPS-injected disc collagen levels were indistinguishable from control disc levels, and the same result was found for the MPC+PPS discs from the high-dose groups (D). dry wt = dry weight. Figure is available in color online only.

of the magnitude of the standard deviations that arose as a consequence of the high intragroup animal variations and the limited number of animals ($n = 6$ per group). Notwithstanding these shortcomings, it was evident on inspection of the decoded raw data that differences existed between the groups in the distribution of their individual histological scores that ranged from the least degenerated (scores 0–6) to the most degenerated (scores 13–19). On the basis of these observations, it was decided to represent the outcome of the histological analysis graphically in the form of frequency diagrams that show the range of scores for each disc. These results are shown in Fig. 6, in which it can be seen that for all animals, the normal control discs had the lowest scores. For the Nil-injected and low-dose MPC+PPS-injected discs, the degeneration scores roughly followed a normal distribution pattern; only 1 disc scored within the range of 13–19. However, 2 MPC+PPS-injected discs exhibited low scores (1–6) compared with only 1 for the nil-injected disc. The highest scores were observed

with the PPS-injected discs; 4 discs had a score equal to or greater than 7–12. These results suggest that, although the overall scores were similar to those of the nil-injected discs, the low-dose MPC+PPS-injected discs were less degenerated than the PPS-injected discs.

Photomicrographs of histological sections of the AF1 region of an L4–5 disc that had been injected with low-dose MPC+PPS are shown in Fig. 7. Although the outer region is filled completely with collagen, the inner region adjacent to the NP shows minimal healing (Fig. 7A). However, evidence of deposition of PGs and healing in the areas surrounding the annular defect is evident in the section stained with Safranin-O/Fast Green (Fig. 7B).

The frequency distribution of score patterns for the high-dose MPC+PPS group was found to be quite different than the corresponding profile obtained for the low-dose group (Fig. 6). First, none of the treatment groups had scores that fell within the low degeneration score range (1–6). Moreover, the highest degeneration scores (13–19) were

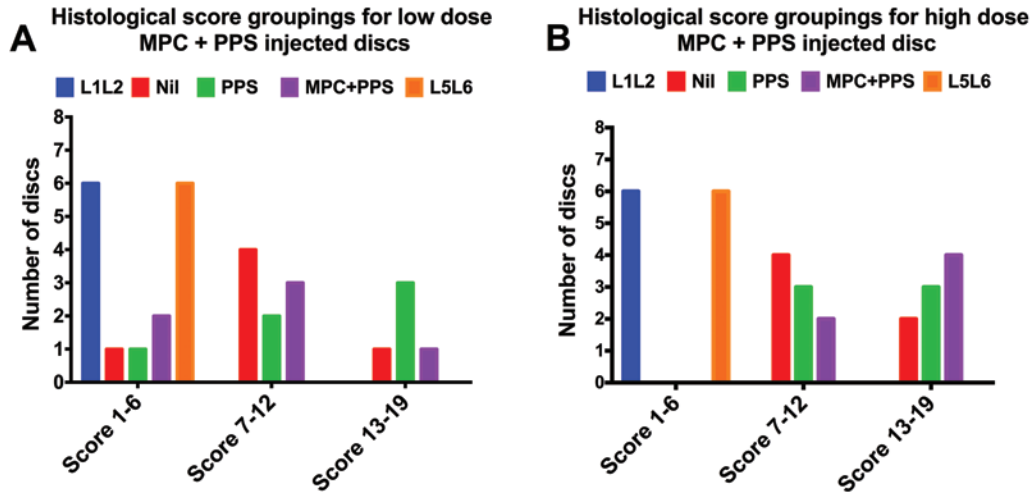


FIG. 6. Frequency distribution of histopathological scores for the low-dose (A) and high-dose (B) MPC+PPS-injected groups. Note the absence of low-score discs (scores 1–6) in the high-dose MPC+PPS group and the normal distribution of scores in the nil- and MPC+PPS-injected discs from the low-dose group. Figure is available in color online only.

recorded for 4 of 6 discs from the high-dose MPC+PPS-injected disc group, whereas only 2 of the nil-injected discs were identified within this high score interval.

Discussion

In this study, we found that a low-dose formulation of MPC+PPS, when injected into the NPs of degenerated ovine lumbar discs induced by a controlled annular incision, can achieve partial matrix restoration 6 months after administration. The lower dose of 0.1 million MPCs formulated with 100 µg of PPS was more effective for supporting disc recovery than a higher dose of 0.5 million MPCs + 500 µg of PPS.

An explanation for the observed efficacy of the low MPC dose compared with that of the high MPC dose remains unresolved; however, the survival of the MPCs when injected into the NP may be limited by the precarious nutritional supply to this region of the disc.^{18,25,70} Al-

though a contributing role of the higher levels of PPS in this formulation cannot be discounted, many other pre-clinical and clinical studies that used this agent administered as multiple systemic or intraarticular injections at concentrations as high as 100 mg/ml have not reported induction of endogenous cell toxicities.¹⁷ On the contrary, PPS has been shown to enhance cell viability and reduce MPC apoptosis.¹⁹

Loss of disc height is a hallmark of disc degeneration in human studies. Moreover, any therapeutic modality that restores the disc height of a degenerated disc is considered to represent a successful outcome of treatment, because it implies restoration of the disc's extracellular matrix. Over the 24 weeks after administration of the test substances, the low-dose MPC+PPS formulation resulted in a significant recovery of DHI compared to the ProFreeze+PPS- and nil-injected discs. In contrast, disc height in the high-dose MPC groups continued to diminish after injection, albeit at a slower rate than the controls that received the formulation components. This finding was also consistent with the biochemical studies conducted on tissues collected during the necropsies, which revealed levels of GAG, and thus PGs and associated water, in the NPs of discs injected with the low-dose MPC+PPS formulation that were higher than those of the nil-injected and PPS-injected discs.

The Pfirrmann MRI disc degeneration scores determined for spines in the high- and low-dose MPC-injected disc groups, including the controls, failed to demonstrate significant differences between the sheep that received treatment and the controls. However, because of the restriction on undertaking MRI on live sheep in our institution at the time of commencing the study, we were unable to determine the Pfirrmann scores for any of the ovine discs before surgery. The absence of baseline MRI data represents a major limitation of the study.

The morphological assessment of the discs before biochemical analysis correlated well with the DHI results. Again, the aggregate scores of the low-dose MPC+PPS

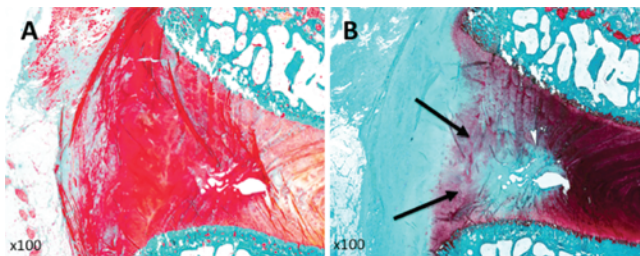


FIG. 7. Photomicrographs of histological sections of injured AF and NP of disc L4–5 of animal 9745, 6 months after the injection of low-dose MPC+PPS into the NP. **A:** Section stained with Goldner's trichrome. **B:** Section stained with Safranin-O/Fast Green. Note that the reconstituted outer AF1 consists essentially of granulation tissue, which is heavily stained for collagen with Goldner's trichrome, but the deposition of proteoglycans with the Safranin-O/Fast Green stain around the defect can also be seen (arrows). The injury extends to the NP that still retains its proteoglycan content. Figure is available in color online only.

group were not significantly different from those of the normal controls, whereas the nil-injected and PPS-injected discs were significantly worse ($p < 0.05$).

Loss of PGs from the NP is a hallmark feature of disc degeneration, and the finding of GAG levels in the NPs of the low-dose MPC+PPS-injected discs that were higher than those of the PPS-injected discs is supportive of the restoration of extracellular matrix in this region. Moreover, the lower histopathological scores for the low-dose MPC+PPS-injected discs relative to those of the PPS-injected discs, the improved DHI, and the lower morphology scores are all consistent with the interpretation of a more rapid recovery from the surgically induced degenerative changes in the presence of the low-dose MPC+PPS formulation.

Although details of the mechanism(s) of action of MPCs in promoting recovery of DHI and reconstitution of the extracellular matrix in degenerated discs remains the subject of investigation, there are several avenues by which MPCs could mediate their regenerative effects. First, after implantation into the NP, cells may engraft and undergo chondrogenic differentiation to disc-like cells that subsequently produce new extracellular matrix, as has been reported for autologous MSCs.^{30,58} Second, the transplanted MPCs may release growth factors, cytokines, and other soluble factors that stimulate resident disc cells to proliferate and synthesize additional extracellular matrix while also suppressing the levels of tumor necrosis factor α , ADAMTS-4, and ADAMTS-5, which are reported to be elevated in degenerated discs and have been implicated in their degradation.^{44,54,56,60,72} MPCs could also act by suppressing mechanically mediated apoptosis⁴⁶ and macrophage and inflammatory cell migration into the disc from the capillaries proliferating at the periphery of the injured AF.^{52,61,65}

Formulations that contain low-dose MPCs in combination with PPS have been shown to enhance disc regeneration in other animal models,^{20,50} but the mechanism(s) of action remains speculative. In vitro studies have found that a combination of MPCs and PPS increases MPC viability and reduces apoptosis and TACE (ADAM-17) production while supporting proliferation and chondrogenic differentiation.²³ Furthermore, apart from its known antiinflammatory and anticomplement activities,^{4–6,8,31,63} PPS can abrogate aggrecanase (ADAMTS-4 and ADAMTS-5) activity by increasing the extracellular levels of tissue inhibitor of matrix metalloproteinase 3 (TIMP-3).^{66–69}

There are several important limitations to this study. First, the inability to perform preoperative MRI means that we did not have baseline Pfirrmann scores for the treated discs and instead relied on comparisons with adjacent-level normal controls. In an attempt to overcome this limitation, the animals were matched for age, weight, and breed to normalize baseline disc degeneration status. We also used preoperative baseline radiographs to calculate the DHI at multiple time points, which is a well-accepted method of assessing disc degeneration in animal models.

Moreover, notwithstanding the recognized limitations of using animal models of human disease, the sheep used in this study were healthy adults with no overt clinical symptoms of spinal problems before treatment with the

test substances, a situation that is clearly inconsistent with the human clinical situation.

Despite these unavoidable limitations, the findings from this preclinical study strongly suggest that the combination of low-dose MPCs with PPS may act synergistically in achieving its positive effects on restoration of the matrix of the degenerated discs used in this animal model.

Conclusions

The outcomes of the various assessments used in this study collectively support the conclusion that a single injection of 0.1 million allogeneic ovine STRO-3-positive MPCs when combined with 100 μ g of PPS and administered directly into the NP of a degenerated lumbar disc can, after 6 months, improve its proteoglycan content and restores disc height. However, it is yet to be determined if these promising preclinical findings can be translated to the clinical setting.

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Disclosures

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Author Contributions

Conception and design: Oehme, Ghosh, Goldschlager, Itescu, Rosenfeld, Jenkin. Acquisition of data: Oehme, Shimon, Wu, McDonald, Troupis. Analysis and interpretation of data: Oehme, Ghosh, Goldschlager, Shimon, Wu, McDonald, Troupis, Jenkin. Drafting the article: Oehme, Ghosh, Goldschlager, Jenkin. Critically revising the article: Oehme, Ghosh, Goldschlager, Rosenfeld, Jenkin. Reviewed submitted version of manuscript: Oehme, Ghosh, Goldschlager, Itescu, Shimon, Wu, Troupis, Rosenfeld, Jenkin. Approved the final version of the manuscript on behalf of all authors: Oehme. Statistical analysis: Oehme, Ghosh, McDonald. Administrative/technical/material support: McDonald. Study supervision: Jenkin.

Supplemental Information

Previous Presentations

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Correspondence

David Oehme, P.O. Box 6178, South Yarra, Victoria 3141, Australia. email: drdavidoehme@mac.com.