Immunoselected STRO-3+ mesenchymal precursor cells and restoration of the extracellular matrix of degenerate intervertebral discs

Laboratory investigation

PETER GHOSH, D.SC.,1,2 ROBERT MOORE, PH.D.,3 BARRIE VERNON-ROBERTS, F.R.C.PATH.,3 TONY GOLDSCHLAGER, PH.D., F.R.A.C.S.,1,4 DIANE PASCOE, M.B.B.S., F.R.A.N.Z.C.R.,5 ANDREW ZANNETTINO, PH.D.,6 STAN GROMTHOS, PH.D.,7,8 AND SILVIU ITESCU, M.D.1

1Mesoblast Ltd.; 2Department of Neurosurgery, Monash Medical Centre; 3Department of Radiology, Royal Melbourne Hospital, Melbourne; 4Ritchie Centre, Monash Institute of Medical Research, Clayton, Victoria; 5Adelaide Centre for Spinal Research, SA Health; 6Department of Haematology, Centre for Cancer Biology and 7Mesenchymal Stem Cell Group, Department of Haematology, SA Pathology, Adelaide; and 8Centre for Stem Cell Research, Robinson Institute, University of Adelaide, South Australia, Australia

Object. Chronic low-back pain of discal origin is linked strongly to disc degeneration. Current nonsurgical treatments are palliative and fail to restore the disc extracellular matrix. In this study the authors examined the capacity of ovine mesenchymal precursor cells (MPCs) to restore the extracellular matrix of degenerate discs in an ovine model.

Methods. Three adjacent lumbar discs of 24 adult male sheep were injected intradiscally with chondroitinase-ABC (cABC) to initiate disc degeneration. The remaining lumbar discs were used as normal controls. Three months after cABC injection, the L3–4 discs of all animals were injected with either a high dose (4 × 10⁶ cells, in 12 sheep) or low dose (0.5 × 10⁶ cells, in 12 sheep) of MPCs suspended in hyaluronic acid (HA). The adjacent L4–5 degenerate discs remained untreated; the L5–6 discs were injected with HA only. The animals were euthanized at 3 or 6 months after MPC injections (6 sheep from each group at each time point), and histological sections of the lumbar discs were prepared. Radiographs and MR images were obtained prior to cABC injection (baseline), 3 months after cABC injection (pretreatment), and just prior to necropsy (posttreatment).

Results. Injection of cABC decreased the disc height index (DHI) of target discs by 45%–50%, confirming degeneration. Some recovery in DHI was observed 6 months after treatment in all cABC-injected discs, but the DHI increased to within baseline control values only in the MPC-injected discs. This improvement was accompanied by a reduction in MRI degeneration scores. The histopathology scores observed at 3 months posttreatment for the high-dose MPC–injected discs and at 6 months posttreatment for the low-dose MPC–injected discs were significantly different from those of the noninjected and HA-injected discs (p < 0.001) but not from the control disc scores.

Conclusions. On the basis of the findings of this study, the authors conclude that the injection of MPCs into degenerate intervertebral discs can contribute to the regeneration of a new extracellular matrix.

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KEY WORDS • STRO-3+ mesenchymal precursor cell • disc matrix • sheep • disc degeneration • degenerative disc disease • ovine model • restoration

Abbreviations used in this paper: AF = anulus fibrosus; cABC = chondroitinase-ABC; DHI = disc height index; HA = hyaluronic acid (hyaluron); MPC = mesenchymal precursor cell; MSC = mesenchymal stem cell; NP = nucleus pulposus.

LOW-BACK pain of discogenic origin is a significant cause of morbidity worldwide and represents an enormous economic burden to public health systems. Although the cause of discogenic low-back pain still remains the subject of debate, it appears to be strongly linked with disc degeneration. Disc degeneration is a disorder of multifactorial etiology, with aging and genetic and mechanical injury representing major determinants. Anterior rim lesions adjacent to the insertion of the AF into the vertebral body have been observed in young spines. Concentric (circumferential) and radiating tears extending into the NP are often present in older disc specimens. These latter types of annular injuries may reflect the actions of mechanical stresses incurred within the range of normal physical activities but acting on an inferior disc matrix. Disc cell senescence and loss of viability are very much age-determined and result in a decline in matrix integrity. Irrespective of the origin of these lesions, they are generally accompanied by the ingrowth of blood vessels and nerve fibers that in nondegenerate discs are normally confined to the periphery of the AF.
The presence of these extended nerve fiber terminals in degenerate discs has been implicated as a major cause of chronic low-back pain. Moreover, the situation may be exacerbated by the loss of cell viability and matrix degeneration that accompanies decreased disc height. Attempts to resolve discogenic low-back pain by nonsurgical methods have only been moderately successful. Intradiscal injection of corticosteroid agents is reported to provide short-term pain relief, and injection of hypertonic dextrose is claimed to provide rapid pain relief that is maintained in 70% of patients for 18 months. More recently, in a randomized placebo-controlled trial of intradiscal methylene blue, a significant reduction in disability index scores and Oswestry Disability Index scores, relative to results seen with placebo, was reported over a 24-month follow-up period.

In recent years, new biological approaches for restoring the extracellular matrix of degenerate discs have been investigated using animal models. The methods employed to induce degeneration include complete removal of the AF, removal of the NP by aspiration, or injection of the enzyme chondroitinase-ABC (cABC, which degrades the chondroitin sulfate chains of the proteoglycans) into the NP. While these models fail to faithfully reproduce the complex temporal pathological changes characteristic of human disc degeneration, they nevertheless have facilitated the evaluation of novel biological modalities in terms of their safety and ability to regenerate a cartilaginous matrix. Of particular interest has been the use of plastic-adherent derived MSCs because these are readily available from bone marrow and adipose tissue. The encouraging results obtained using MSCs in experimental animal models have been followed by a recent human clinical study in which autologous bone marrow–derived MSCs were used to reconstitute degenerate discs.

Preparation of Ovine STRO-3+ MPCs

The ovine STRO-3+ MPCs used for this study were prepared according to a procedure described previously. Briefly, approximately 1 × 10^6 ovine bone marrow mononuclear cells, isolated from a bone marrow aspirate by means of Ficoll gradient density centrifugation (1.077 g/ml Lymphoprep, Axis- Shield) at 400 G for 30 minutes, were incubated with 20 mg/ml purified STRO-3 murine IgG1 anti–human bone-specific alkaline phosphatase) for 1 hour on ice. The cells were washed twice with phosphate-buffered saline/1% bovine serum albumin and subsequently incubated with sheep anti–mouse IgG-conjugated magnetic Dynabeads (4 beads per cell; Invitrogen Dynal AS) for 40 minutes on a rotary mixer at 4°C. Cells binding to beads were isolated using a magnetic particle concentrator (MPC-1, Invitrogen Dynal AS) according to the manufacturer’s recommended protocol. These cells were then expanded in culture to passage P5, released with trypsin, collected by low-speed centrifugation, washed, and aliquoted into vials with the cryopreservant ProFreeze NAO Freezing Medium (Lonza) at concentrations of 40 × 10^6 and 5 × 10^6 MPCs/ml per vial. The authenticity of these MPCs was confirmed using multilineage differentiation assays as previously described.

Methods

This study was reviewed and approved by the Animal Ethics Committee of the Institute of Medical and Veterinary Science, Frome Road, Adelaide, South Australia, Australia.

The ovine STRO-3+ MPCs retained the highest clonogenic capacity of the bone marrow cells and were free of contaminating hematopoietic accessory cells such as monocytes/macrophages, lymphocytes, and erythrocyte progenitors, which can influence the growth and development of MPCs in unfractionated cultures isolated by plastic adherence. The STRO-3+ MPCs were injected with an HA vehicle into the NP of degenerate ovine discs and their ability to improve matrix reconstitution, relative to non-MPC-injected controls, was assessed by changes in DHI, histopathological characteristics, and MRI degeneration scores determined after 3 and 6 months.
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scribed and by flow cytometry, which showed that the MPCs expressed the MSC markers CD29, CD44, CD146, CD166, and HSP-90 [39] and lacked expression of the hematopoietic and vascular endothelial markers CD31, CD14, and CD45. These preparations were stored in the vapor phase of liquid nitrogen until required.

Animal Protocol and Imaging

Lateral radiographs were made of the lumbar spines of 24 2-year-old anesthetized Merino wethers (55–80 kg) to confirm the absence of disc degeneration and anatomical abnormalities. The DHI for each of the lumbar disc spaces was calculated from these radiographs using a standard method of measurement. These values, determined by a blinded observer (R.M.), were recorded as baseline DHI for each animal. Using an open anterior surgical approach, the lumbar spines were exposed and 3 adjacent lumbar discs (L3–4, L4–5, and L5–6) of each animal were injected with 1.0 IU cABC (Seikagaku Corporation) via a 29-gauge, 0.5-in syringe to initiate progressive disc degeneration. This concentration of cABC was selected on the basis of previous unpublished studies in which we found that this dose consistently produced moderately degenerate discs (deemed to be an appropriate clinical target) over a period of 3 months. The remaining lumbar discs (L1–2 and L2–3) were not injected with cABC and were used as normal controls.

Three months (± 3 weeks) following administration of cABC, the animals were again anesthetized and radiographs and MR images of the lumbar spine were obtained. (The MR images were obtained with a 1.5-T Siemens Vision MRI scanner with Numaris 33G software or a 1.5-T Siemens Avantro MRI scanner with Syngo B13 software.) The DHI for each disc space was recorded and the data identified as pretreatment DHI.

The T1-weighted, T1-weighted gradient echo, T2-weighted, and STIR image sequences of the L6–S1, L5–6, L4–5, L3–4, L2–3, L1–2 discs were captured on CDs as 12 sagittal sections. The MR images were scored from the CD by 2 blinded observers (T.G. and D.P.) using the classification of Pfirrmann et al. The mean scores for this time point are referred to as the pretreatment MRI disc degeneration scores.

The anesthetized animals were radiographed and the degenerate cABC-injected lumbar discs identified. The DHI values were determined (pretreatment DHI), and animals were returned to the operating theater, where the lumbar spine was exposed on the side opposite to that used for the first surgery. The NPs of the most cranial degenerate lumbar discs, as identified on the lateral radiographs, were injected with a high or low dose of STRO-3+ MPCs which were prepared as previously described.

High-dose MPC–injected discs (n = 12) received 100 μl ProFreeze NAO Freezing Medium (Lonza) containing 4 × 10^6 MPCs mixed with an equal volume of Euflexxa, a high–molecular weight (2.4–3.6 × 10^8 D) noncross-linked HA (Ferring Pharmaceuticals). Low-dose MPC–injected discs (n = 12) received 100 μl ProFreeze containing 0.5 × 10^6 MPCs mixed with an equal volume of HA. The low dose was selected on the basis of previous studies that confirmed chondrogenesis in pellet cultures with 0.5 × 10^6 MPCs. The higher dose was used as an 8-fold multiple of the lower dose. The high- and low-dose MPC+HA and HA-alone injections were administered using a 19-gauge, 0.5-in BD needle and 1.0-ml calibrated BD Luer-Lok syringe. A preliminary (unpublished) study had shown that MPC viability and cell counts were preserved using this brand of syringe and needle.

In each animal, the lumbar degenerate disc just caudal to the MPC-injected discs was not treated, while the next caudal degenerate disc was injected with HA alone (100 μl Profreeze with 100 μl Euflexxa).

Three months and 6 months after MPC injection, DHIs and MRI degeneration scores were again determined for discs of the lumbar spines. Immediately thereafter, the animals (6 from each group at each time point) were euthanized by intravenous administration of pentobarbital (Virbac [Australia] Pty Ltd.) and spinal columns removed en-bloc.

Histopathological Analysis

The individual lumbar discal segments to be processed for histological examination were dissected from the spinal columns by cutting through the adjacent cranial and caudal vertebral bodies close to the growth plates with a bone saw. These discs with attached vertebral bodies were fixed in Histochoice (Amresco, Inc.) for 56 hours and decalcified in several changes of 10% formic acid in 5% neutral buffered formalin for 2 weeks with constant agitation until complete decalcification was confirmed using a Faxitron HP43855A x-ray cabinet (Hewlett Packard). The individual fixed and decalcified spinal segments were sliced in the sagittal plane with a band saw and processed using the standard paraffin-embedding and sectioning methods. One section from all blocks prepared from the sagittal slices was stained with H & E. The H & E–stained histological sections were coded and then scored in a blinded fashion by an experienced pathologist (B.V.R.) using a published 4-point (0 = normal, 4 = worst) semiquantitative disc degeneration grading system. In each disc, a separate score was assigned for each of the following 4 regions: NP, AF, cartilage end plate, and bony end plate. The mean overall scores for each experimental group are the means of these individual scores. Additional sections were stained with Alcian blue and counterstained with neutral red and used for illustrative purposes.

Statistical Analysis of Data

The statistical analysis of the mean DHI for the high- and low-dose MPC–injected discs; normal, untreated controls; and HA-injected discs radiographed at baseline, pretreatment, and 3 and 6 months posttreatment was undertaken using 1-way ANOVA, together with the Bartlett test for group variance and Tukey multiple comparison test. For the histopathological and aggregate MRI disc degeneration scores, comparison between the various treatments was undertaken using the Kruskal-Wallis or Friedman test (nonparametric repeated-measures ANOVA) with the Dunn multiple comparison post hoc. Between-groups differences were considered statistically significant at p < 0.05.
Results

Confirmation of the Degenerative Status in the Ovine Model

Comparison of the MRI studies of lumbar spines taken prior to and 3 months after the injection of cABC into the target discs revealed a marked decline in brightness of the T2-weighted signal of the NP (Fig. 1). The individual MRI degeneration scores assessed for the control and each of the 3 lumbar discs destined to receive the respective treatments for the 4 experimental groups (low- and high-dose MPC to be euthanized at 3 months; low- and high-dose MPC euthanized at 6 months) are shown in Fig. 2. For all the pretreatment groups the mean aggregate MRI scores for these discs were highly significantly different (p < 0.001) from the mean scores of the adjacent nondegenerate control discs (Fig. 2). The degenerative disc changes induced by the cABC injections and identified by MRI (Fig. 2) were consistent with the radiographically determined mean DHI scores, which showed a 40%-50% loss in disc height from baseline to pretreatment (p < 0.0001) for all experimental groups (Figs. 3 and 4).

Disc Height Index Changes Following Treatment

The mean DHI scores for the noninjected, HA-injected, and low-dose MPC–injected degenerate discs at 3 months posttreatment were all significantly lower than their respective baseline DHI values (p < 0.0001) (Fig. 3B–D). However, by 6 months the mean DHI score of the low-dose MPC–injected discs was statistically equivalent to the baseline values (Fig. 3B). A similar situation was observed for the high-dose MPC–injected group 6 months posttreatment (Fig. 4B). However, the mean DHI value for the noninjected discs also showed a similar recovery to a value that was not statistically different from baseline (Fig. 4C).

Magnetic Resonance Imaging Disc Degeneration Scores

The MRI-assessed aggregate disc degeneration scores for the low-dose MPC–injected discs evaluated 3 months posttreatment and the high-dose MPC–injected discs evaluated 6 months posttreatment were statistically equivalent to the scores for the control discs (Fig. 5A, B, and D). Moreover, the scores of these MPC-injected discs were significantly different from those of the noninjected (p < 0.01) and HA-injected (p < 0.01) discs (Fig. 5A and D).

Disc Histopathology Scores

As shown in Fig. 6 the histopathological grading system used for this study encompassed matrix and cellular changes that were observed in all compartments of the disc. Using this semiquantitative grading system the score for each disc section was determined as described in Methods and the overall mean for each experimental group was examined. This method of blinded assessment showed that only the 3-month score for the high-dose MPC–injected discs and the 6-month score for the low-dose MPC–injected discs were significantly lower than the scores of the untreated and HA-injected discs (p < 0.001) (Fig. 7). Furthermore the mean histopathological score for the discs of animals euthanized 3 months after receiving a low-dose MPC injection, although showing a decline in disc integrity, still remained significantly different from the mean control disc score (p < 0.0001) (Fig. 7A). However, by 6 months the mean score of the low-dose MPC–injected discs had declined to a level that was statistically indistinguishable from the mean control disc score (Fig. 7C). In addition, the mean 6-month score for the low-dose MPC–injected disc was significantly different from the untreated and HA-injected disc scores (p < 0.001) (Fig. 7C). Although the mean 3-month score for the high-dose MPC–injected discs was also found to be equivalent to the mean control disc score (p < 0.0001) and lower than the scores for the HA-injected and untreated discs (p < 0.001) (Fig. 7B and D), this difference was lost by 6 months (Fig. 7D).

Discussion

The results of the present study show that 6 months following intradiscal administration of MPC+HA into degenerate discs, the DHI and structural integrity improved to a greater extent than degenerate discs injected with HA alone. The increase in disc height (DHI) observed for the MPC-injected discs 6 months after administration may be explained by the deposition of a partially reconstituted extracellular matrix within the degenerate discs.

In the healthy spinal column the disc height is maintained by the presence within the NP and inner AF of high concentrations of proteoglycans and their bound water molecules. These entities confer a high swelling pressure to the disc but also allow it to recover from deformation after axial loading. Indeed, the use of cABC to induce disc degeneration in this ovine model was predicated on the ability of this enzyme to degrade and thus remove the majority of the proteoglycans from the NP. Although the histopathology scores were assessed on disc sections stained with H & E, adjacent sections were stained with Alcian blue and neutral red, which bind to high concentrations of proteoglycans and their bound water molecules. These entities confer a high swelling pressure to the disc but also allow it to recover from deformation after axial loading. Indeed, the use of cABC to induce disc degeneration in this ovine model was predicated on the ability of this enzyme to degrade and thus remove the majority of the proteoglycans from the NP.

Although the histopathology scores were assessed on disc sections stained with H & E, adjacent sections were stained with Alcian blue and neutral red, which bind to the negatively charged proteoglycans, and provided a representation of their distribution. However, the intensity of staining can vary with the processing of the sections and therefore provides only a semiquantitative marker of proteoglycan concentration. Nevertheless, histological examination of these stained sections suggested that discs injected with MPC+HA contained more uniformly distributed PGs in the NP than nontreated discs or discs injected with cABC+HA (data not shown). Moreover, the disruption of the structural integrity of the cartilage endplate noted in some histological disc sections was most likely a secondary event caused by the weakening of the endplate following the initial cABC injection, accompanied by subsequent supraphysiological mechanical stresses arising as a result of the diminished resilience of the NP due to its depleted proteoglycan content.

There was some indication from these studies that recovery of discs from the degenerative state was more sustained over 6 months with the lower dose of MPCs than with the higher dose. A possible explanation for this observation may be related to the poor nutritional supply to the NP of the disc that is dependent on the diffusion of nutrients from the blood vessels within the vertebral bod-
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Fig. 1. Left: Baseline MR image obtained in a normal ovine lumbar spine showing the strong water signal for the NP of all discs prior to injection with cABC. Right: Pretreatment MR image obtained 3 months after cABC injection showing marked reduction in signal in the injected discs, confirming that cABC induced disc degeneration by initially degrading the water-binding proteoglycans of the NP. The nondegenerate control discs and the relative spinal levels of the 3 degenerate (cABC-injected) discs that were untreated or injected with either MPC+HA or HA alone are also indicated.

Fig. 2. Graphs showing the mean MRI aggregate degeneration scores determined 3 months after cABC injection for the uninjected control discs and the cABC-injected discs that were to remain untreated (cABC), were to be injected with HA, or were to be injected with low-dose (A and C) or high-dose (B and D) MPC+HA. Before treatment, the values for all categories of cABC-injected discs were significantly different from those of the uninjected controls within each group. mths = months. Error bars indicate SEM. **p < 0.001, ***p < 0.0001 relative to control disc values.

ies across the cartilage endplate. Loss of cartilage endplate integrity arising from the degradative effects of the injected cABC on the cartilage matrix could, as already noted, adversely influence the normal solute exchange between the NP and the blood supply of the vertebrae. This nutritional deficiency in the degenerate discs could present an upper limit to the survival of the number of MPCs injected into the NP. There is some support for
Fig. 3. Mean DHI values determined at baseline, pretreatment, and 3 and 6 months posttreatment for control discs (A), MPC+HA–injected discs (B), untreated cABC-injected discs (cABC) (C), and HA-injected discs (D) for spines from animals that received the low-dose MPC injections. Only the degenerate discs injected with MPCs (B) showed recovery in DHI after 6 months as indicated by the nonsignificant difference (NSD) in mean values relative to baseline. The DHI values for the untreated degenerate discs (C) and HA-injected degenerate discs (D) remained significantly different from baseline at 3 and 6 months posttreatment. Error bars indicate SEM. *p < 0.01, ***p < 0.0001 relative to baseline.

Fig. 4. Mean DHI values determined at baseline, pretreatment, and 3 and 6 months posttreatment for control discs (A), MPC+HA–injected discs (B), untreated cABC-injected discs ("nil injected") (C), and HA-injected discs (D) from animals that received the high-dose MPC injections. The degenerate discs injected with a high dose of MPCs (B) as well as those that were left untreated (C) showed a recovery in DHI to a value that was not significantly different from baseline. In contrast the DHI of the HA-injected discs (D) remained significantly different from baseline. Error bars indicate SEM. **p < 0.001, ***p < 0.0001 relative to baseline.
this view from a recent study using a canine model of disc degeneration where it was shown that transplantation of $10^6$ autologous bone marrow–derived MSCs produced a better outcome, in terms of recovery of disc height and production of extracellular matrix, than when $10^7$ MSCs were used. Significantly, 3 months after implantation of the $10^7$ MPCs, more apoptotic cells were detected than when the lower dose was used. Although the disc degeneration model studied in the aforementioned study was different from that employed here, we believe it highlights the need to recognize the poor nutritional status of the NP and how this could affect the survival of cells implanted within this tissue.

The mechanisms responsible for the recovery of disc integrity following administration of MPCs in our animal model have yet to be resolved, but it is known that MPCs express an array of antiinflammatory cytokines and growth factors including members of the TGF (transforming growth factor) superfamily. The local release of these trophic factors into a degenerate NP could not only suppress catabolic events triggered by mechanically mediated disc cell injury but also enhance resident cell biosynthesis of a new extracellular matrix. Apart from the known paracrine effects of the MPCs on injured host cells, it is possible that some of the injected MPCs may engraft and undergo differentiation into NP-like chondrocytes or even fibrochondrocytes of the AF. Indeed, previous studies have provided evidence that MSCs possess the capacity, in vitro and in vivo, to differentiate into NP-like cells capable of synthesizing a proteoglycan-rich disc matrix. Further investigations will be required to resolve these important questions.

Conclusions

Although this investigation was undertaken in an ovine model of disc degeneration induced by enzymatic depolymerization of the proteoglycans of the NP, it does serve to highlight the capacity of injected MPCs to reconstitute a new disc matrix. Whether a proportion of this new matrix was synthesized by those injected MPCs that differentiated into disc-like cells or was due to the trophic effects of MPCs on resident NP cells has not been resolved by the present investigation. Irrespective of the mechanism(s) of action, we nevertheless conclude that intradiscal administration of MPCs into degenerate discs does offer a potential modality for their repair.

Disclosure

Peter Ghosh is the senior vice president for Orthopaedic Research Programs, Mesoblast Ltd. Silviu Itescu is the chief executive officer of Mesoblast Ltd. and a stockholder. Andrew Zannettino is a consultant for Mesoblast Ltd. Tony Goldschlager has received

Fig. 5. Mean MRI aggregate degeneration scores for control, untreated (cABC), HA-injected, and low- (A and C) and high-dose (B and D) MPC+HA–injected discs, 3 (A and B) and 6 (C and D) months posttreatment. The scores for the low-dose MPC–injected discs at 3 months and the scores for the high-dose MPC–injected discs at 3 and 6 months were all significantly lower than those of the untreated (cABC-injected) and HA-injected discs at those time points and were not significantly different from the control values. In contrast, the scores for the untreated and HA-injected discs were significantly higher than the control values. Error bars indicate SEM. *p < 0.01, **p < 0.001, ***p < 0.0001 relative to control values; #p < 0.01 relative to MPC-injected discs.
The authors gratefully acknowledge the competent technical assistance of the Adelaide Centre for Spinal Research staff members in the preparation of histological disc sections and Dr. David Ardem, Mr. Martin Wilby, Mr. Simon Tizzard, Mr. Bernard LaRue, and Dr. Martin Wilby of the Adelaide Centre for Spinal Research.

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Fig. 6. Criteria used to grade the histopathological changes observed in sections of discs from the various experimental groups. The system was based on a previous publication (Sasaki et al.42) that produces an overall score derived from pathological changes in all tissues of the disc. The photomicrographs show Alcian blue/neutral red–stained sections of the NP, AF, and cartilage endplate (CEP) of a normal disc highlighting the respective structural variations for these disc regions. BEP = bony endplate; PG = proteoglycan.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Anulus Fibrosus</th>
<th>Nucleus Pulposus</th>
<th>Cartilage Endplate</th>
<th>Margins/Subchondral Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>intact lamellae, narrow interlamellar matrix, intact anulus attachment, vessels only in outer 1/3</td>
<td>homogeneity, absence of clefing</td>
<td>uniform thickness, intact attachment to bone, uniform calcification &lt;1/5 of depth, uniform cell distribution</td>
<td>even thickness of BEP, lamellar bone only, distinct junction w/ CEP, few vascular invasions into CEP</td>
</tr>
<tr>
<td>2</td>
<td>minor lamellar splitting &amp; disorganization, minor widening of matrix, minor disorganization of attachment, rim lesion w/o reparative reaction</td>
<td>minor clefing, minor cell necrosis, minor posterior displacement of annulus, minor chondrone formation</td>
<td>minor cartilage thinning, small transverse fissures, irregular thickening of calcified zone, few invading vascular channels, small chondrones</td>
<td>slightly uneven BEP, Schmorl nodes, minimal remodeling of BEP, small marginal osteophytes</td>
</tr>
<tr>
<td>3</td>
<td>moderate widening of matrix, moderate fissuring of attachment, radiating tears not involving outer 1/3, minimal chondroid metaplasia, cystic degeneration, vessels in outer &amp; middle 1/3, rim lesion w/ minor reparative reaction</td>
<td>moderate clefing, moderate cell necrosis, cystic degeneration, posterior displacement w/in annulus, centripetal extension of collagen, moderate chondrone formation</td>
<td>marked cartilage thinning, marked thickening of calcified zone, many transverse fissures, many vascular channels, many chondrones</td>
<td>moderately uneven BEP, vascularized Schmorl nodes, moderate trabecular thickening, defect in bone lamellae, minimal fibrosis tissue in marrow spaces, medium-size osteophytes</td>
</tr>
<tr>
<td>4</td>
<td>extensive lamellar disorganization, radiating tears extending into outer 1/3, extensive chondroid metaplasia, vessels in all zones, rim lesion w/ marked reparative reaction</td>
<td>complete loss of nucleus, loose body formation, marked chondrone formation</td>
<td>total loss of cartilage, calcification of residual cartilage, widespread fissuring</td>
<td>marked uneven BEP, ossified Schmorl nodes, large osteophytes, marked trabecular thickening, marked fibrosis of marrow spaces, cartilage formation</td>
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travel support from Mesoblast Ltd. The other authors report that they have no competing financial interests related to this study. This project was financially supported by funds from an Australian government Commercial Ready Grant and Mesoblast Ltd.
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Penny Tisdall for undertaking the ovine spinal surgery and assistance in the preparation of animals for imaging.

References


**Fig. 7.** Mean histopathology scores for control, untreated (cABC), HA-injected, and low- and high-dose MPC+HA–injected discs obtained 3 (A and B) and 6 (C and D) months posttreatment. The 3-month score for the high-dose (B) and 6-month score for the low-dose (C) MPC–injected discs were not significantly different from the scores of control discs but were lower than those of the untreated discs (p < 0.001). The scores of the untreated (cABC) and HA-injected discs were significantly higher than the control disc scores at 3 and 6 months for both high- and low-dose MPC groups. Error bars indicate SEM.

**p < 0.001, ***p < 0.0001 relative to control values; ##p < 0.001 relative to the MPC-injected discs.**


