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Emerging technologies for molecular therapy for intervertebral disc degeneration

Won C. Bae, PhD[a] **[Assistant Professor]** and **Koichi Masuda, MD**[b] **[Professor]**

[a]Department of Radiology, University of California, San Diego, San Diego, California

[b]Department of Orthopaedic Surgery, University of California, San Diego, La Jolla, California

Abstract

Intervertebral discs are biologically regulated by the maintenance of a balance between the anabolic and catabolic activities of disc cells. Therapeutic agents, initially evaluated using *in vitro* studies on disc cells and explants, have been used as intradiscal injections in preclinical settings to test *in vivo* efficacy. These include anabolic growth factors and other biostimulatory agents as well as antagonistic agents against matrix-degrading enzymes and cytokines. Additional work is needed to identify suitable patient populations, using methods such as MRI, and to better understand the mechanism of healing. Clinical trials are currently underway for a few of these agents, while many other promising candidates are on the horizon.

Keywords

back pain; animal model; disc injection; growth factor; cytokine; MRI

Intervertebral Disc Degeneration and Homeostasis of the Extracellular Matrix

Intervertebral disc (IVD) degeneration is one of the major causes of low back pain and lumbar disc herniation. Biologically, the cells in the disc actively regulate the homeostasis of the IVD extracellular matrix by maintaining a balance between anabolism and catabolism. The modulation of disc cell metabolism involves a variety of molecules (e.g., cytokines, enzymes, enzyme inhibitors, and growth factors) that act in a paracrine and/or autocrine fashion. The degeneration of an IVD may result from the loss of steady state metabolism, maintained in the normal discs, due to an imbalance between the anabolic and catabolic processes. The proteoglycan (PG) content of the extracellular matrix (ECM) and the rate of PG synthesis decreased markedly with age and degeneration, similar to the case in articular cartilage.[1–9] The anabolic regulators include polypeptide growth factors, such as insulin-like growth factor (IGF-1), transforming growth factor-β (TGF-β), and the bone morphogenetic

[b]Corresponding author for proof and reprints: Koichi Masuda, M.D., Department of Orthopedic Surgery, University of California, San Diego, 9500 Gilman Dr, La Jolla, CA 92093-0863, Tel: (858) 246-0426, Fax: (858) 534-5304, koichimasuda@ucsd.edu.
[a]Coauthor address: Won C. Bae, Ph.D., Department o f Radiology, University of California, San Diego, 408 Dickinson Street, San Diego, CA 92103-8226, Tel: (619) 543-5507, Fax: (866) 387-1895, wbae@ucsd.edu

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proteins (BMPs).[10,11] Catabolic regulators include many cytokines, notably interleukin-1 (IL-1)[12,13] and tumor necrosis factor (TNF-α),[12–14] which influenced the synthesis of matrix-degrading enzymes, such as the matrix metalloproteinases (MMPs).[15–20] Alterations in both anabolic and catabolic processes are thought to play key roles in the onset and progression of IVD degeneration; the biochemical processes that regulate these changes are important to understand for the development of effective treatments of disc degeneration.

Catabolic Mediators and Enzymes in Disc Degeneration

Under pathological conditions, including IVD degeneration,[21] physical injury (e.g., puncture or stab wounds)[15,22] and abnormal mechanical loading,[23,24] the IVD expresses cytokines and proteinases. While macrophages that infiltrate herniated tissue or granulation tissue seem to be the major source of these cytokines,[25–27] recent studies clearly indicate that IVD cells may also synthesize these molecules in an autocrine fashion.[28] In particular, increases in both protein and mRNA levels of IL-1 and its major regulator, TNF-α, have been observed in degenerated or herniated IVD tissues and both are spontaneously expressed by these tissues in culture.[29–36]

The catabolic processes induced by cytokines are mediated by various enzymes, such as the MMPs[13,31,37–39] or the aggrecanases.[40] IL-1 has been shown to stimulate matrix degradation as well as to inhibit the synthesis of ECM macromolecules[41–44] with minimum effect on cell proliferation.[41] IL-1 induced the production of collagenase,[45] cycloxygenase-2 (COX-2[46]), prostaglandin E2,[13,47] nitric oxide,[45,44] MMP-1,[48] total[46] and active[43] MMP-3, MMP-13,[20,44] the aggrecanase, a disintegrin and metalloproteinase with thrombospondin motifs-4 (ADAMTS-4),[21,44] IL-6,[46] and monocyte chemoattractant protein-1 (MCP-1).[49,50] Recently, IL-1β was shown to increase the sensitivity of IVD cells to shear stress,[51] suggesting that this cytokine is involved in the acceleration of degradation processes in IVDs subjected to biomechanical stress. Although IL-1 affects both synthetic and degradative processes, it is worth noting that, at lower concentrations, IL-1 was much more effective in inhibiting the synthesis of aggrecan than in stimulating its degradation in articular cartilage.[52–54] An epidemiological study has shown that IL-1 gene cluster polymorphisms significantly increased the risk of disc degeneration; this has shed more light on the possible involvement of IL-1 in IVD degeneration.[55]

The effects of the inhibition of catabolic cytokines have been evaluated in a number of studies. IL-1 receptor antagonist (IL-1ra), applied *in vitro* to degenerated[56] and herniated[57] human disc tissues, reduced the expression of MMP-3.[56,57] IL-1ra pretreatment of nucleus pulposus (NP) cells from moderately degenerate human discs reduced the expression of ADAMTS-4 and MMP-3 in subsequent treatment with IL-1.[58] In another human cell culture study, the addition of IL-1ra and soluble TNF receptor significantly upregulated PG synthesis, suggesting that IL-1 and TNF suppress PG *de novo* synthesis.[59] IL-1ra, and other agents, may benefit from administration of slow-release agents, such as IL-1ra mixed with thermally responsive elastin-like polypeptide (ELP)[58] or with gelatin hydrogel.[60] In addition to IL-1, TNF-α has gained attention in association with disc herniation.[33,34,36,61] TNF-α inhibition, using a monoclonal antibody in herniated human disc explants, showed suppression of MMP-3 levels[57]; other anti-TNF agents are beginning to be applied to reduce

pain in patients with sciatica[62–64] and discogenic pain.[65] Other anti-cytokine therapeutics include the p38 mitogen-activated protein kinase (MAPK) inhibitor, which hindered the catabolic effects of IL-1[66] as well as nuclear factor-kappaB (NF-κB) decoy, which reduced pain in a rat lumbar disc herniation model.[67]

Anabolic Effects of Cytokines and Growth Factors on IVD Cells

A variety of growth factors and cytokines (Table 1) can alter IVD homeostasis and stimulate ECM synthesis by shifting cellular metabolism to a more anabolic state.[68] The effects of TGF-β on PG synthesis[10,69] and cell proliferation[69] were noted early in the literature. Similar effects of IGF-1 have also been reported.[11] IGF-1 and platelet-derived growth factor (PDGF) were also shown to reduce the percentage of apoptotic anulus fibrosus (AF) cells induced by serum depletion in culture.[70]

Osteogenic protein-1 (OP-1),[71] which is a member of the BMP family, upregulates the PG metabolism of IVD cells. OP-1 strongly stimulated the production and formation of the ECM by rabbit IVD cells;[71] similar effects have been noted using human IVD cells.[72] OP-1 also replenished PGs and collagens after depletion of the ECM following exposure of IVD cells to IL-1[42] or chondroitinase ABC (C-ABC).[73]

Growth and differentiation factor-5 (GDF-5), originally found to be a factor responsible for skeletal alterations in brachypodism mice,[74] is another member of the BMP family that has anabolic effects on IVD cells. GDF-5 stimulated PG and type II collagen expression by mouse IVD cells.[75] Recombinant human GDF-5 (rhGDF-5) enhanced cell proliferation and matrix synthesis and accumulation by cells from bovine NP, and to lesser extent, AF cells.[76]

Because biological molecules, prepared in an autologous fashion, avoid certain regulatory complications, they may be useful clinically. Autologous IL-1ra has been used, along with IGF- 1 and PDGF proteins, to reduce apoptosis of disc cells and their production of IL-1 and IL-6.[77] Platelet-rich plasma (PRP) can be produced by centrifugal separation of a patient's own blood in the operating room; it contains multiple growth factors concentrated at high levels. *In vitro*, PRP stimulated cell proliferation and matrix synthesis, as shown using porcine disc cells.[78] PRP induced cell proliferation and differentiation, and facilitated NP-like tissue formation by human disc cells.[79]

Intradiscal Therapeutics: Animal Studies

To study the ability of growth factors and other therapeutic agents to stimulate repair *in vivo* (Table 2), small animal models, in which the degree of degeneration can be controlled, are useful. Rabbit lumbar[76,80–83] and rat caudal discs[84–87] have been used extensively, although other animal models exist.[85,86] Both physical injury, such as stab wound[6,88] or controlled needle puncture,[89,90] and chemical degradation[83–87] approaches have been used.

The *in vivo* efficacy of OP-1 injection has been evaluated in a number of animal studies. In adolescent rabbits, an injection of recombinant human OP-1, but not the lactose vehicle, reversed the reduction in disc height and worsened the magnetic resonance imaging (MRI) grade caused by an anular needle-puncture.[80] In another study with the same experimental

design, the injection of OP-1 restored dynamic viscoelastic biomechanical properties, such as elastic and viscous moduli, of puncture-degenerated IVDs.[81] OP-1 treatment was also effective in restoring discs that have been chemically degraded. C-ABC has been used as an alternative to chymopapain for chemonucleolysis,[91–98] as well as an animal model of disc degeneration in the rat tail[84–87] and goat[85,86] disc. When OP-1 or vehicle was injected into rabbit discs degraded with C-ABC for 4 weeks, the disc height initially decreased (~34%), then recovered and gradually approached the level of the normal control.[82]

rhGDF-5 is another promising growth factor whose efficacy has been evaluated in animal models. The efficacy of injecting a single dose of GDF-5 has been reported in the mouse caudal disc with degeneration induced by static compression.[99] In that study, GDF-5 induced an increase in disc height and the expansion of the inner AF fibrochondrocytic population into the NP. In a needle-puncture model of disc degeneration (4 weeks) in adolescent (5–6 months-old) rabbits, a single injection of rhGDF-5 restored disc height (Figure 1A) and improved MRI and histological grading scores within approximately 6 weeks.[76] There are concerns that aged or degenerated discs might have lower cellularity, especially in the number of notochordal cells often found in normal adolescent rabbit discs,[100,101] and might respond poorly to growth factor treatment. Despite this, a study using 2-year old rabbits showed that rhGDF-5 effectively recovered disc height (Figure 1B) as well as MRI and histologic grades after a 12-week observation period.[102] Biomechanical analyses indicated that the viscous and elastic moduli of the IVDs in the rhGDF-5-injected discs were significantly higher than those in the PBS-injected discs. Additionally, rhGDF-5 has shown efficacy in rabbit discs that have been degraded using thrombin,[83] a serine proteinase that results in cleavage of PGs and decreased disc height.[103,104] In this study, discs of adolescent rabbits were injected with thrombin (100 U/10 μl) and the rabbits were maintained for 4 weeks. A single injection of rhGDF-5 (10 μg/10 μl) or saline was then given, and endpoint measures (MRI and gene expression) were determined 12 weeks later. Quantitative MRI was used to evaluate T2 and T1rho[105,106] MR properties. T2 (Figure 2AC) and T1rho (Figure 2BD) MRI maps showed maintenance of NP morphology and MR values in rhGDF-5-treated discs. Both T2 (Figure 3A) and T1rho (Figure 3B) values of the NP were higher in the rhGDF-5 group, approaching those of the unoperated control. Treatment with rhGDF-5 also reduced the level of expression for ADAMTS-5 (Figure 4A), ADAMTS-4 (Figure 4B), COX-2 (Figure 4C), and vascular endothelial growth factor (VEGF) (Figure 4D), molecules related to PG degradation, pain, and neovascularization seen in degenerated IVDs.[107]

PRP is another agent that has been evaluated in animal models. Using a nucleotomy rabbit model, the effects of allograft PRP with or without gelatin hydrogel microspheres (to provide slow release and mechanical support) and phosphate buffered-saline (PBS)-only injectates were evaluated.[108] The PRP+microspheres group had markedly suppressed degeneration, compared to the PBS- and PRP-only groups. A recent study by the same group reported additional findings that microspheres alone without PRP did not have therapeutic value, and that the PRP+microsphere group benefited from increased disc height, water content, expression of PG core protein and collagen II, and fewer apoptotic cells in the NP.[60] While the use of a sustained delivery system has been effective in nucleotomy models in a less severe anular puncture model, an injection of PRP alone was sufficient to induce

restoration of disc height and T2 MRI values.[109] Interestingly, the use of fibrin glue alone had a positive effect in a porcine nucleotomy model, where the fibrin glue resulted in the suppression of IL-6 and TNF expression, while restoring mechanical properties and glycosaminoglycan (GAG) content.[110]

A recent study has developed and evaluated the efficacy of an inhibitor for ADAMTS-5 expression using small interference RNA (siRNA).[111] First, the ability of siRNA to reduce ADAMTS-5 expression was determined using rabbit NP cells *in vitro*. Adolescent rabbit discs, punctured in the anulus to induce degeneration, were injected with either ADAMTS-5 or control siRNA. After 8 weeks, the discs receiving ADAMTS-5 siRNA had markedly better MRI and histology results (Figure 5). The control group exhibited complete loss of NP tissues (Figure 5ac) that had been replaced by a fibrocartilaginous tissue (Figure 5eg). In contrast, the ADAMTS siRNA group had maintained disc structure (Figure 5bd), including a clearly distinguishable NP. Both MRI grade (Figure 6A) and overall histology grade (Figure 6B) were significantly better for the ADAMTS siRNA group. The inhibition of degradative enzymes and catabolic cytokines provides a complimentary approach to treat degenerated discs at different levels of the modulation mechanism.

Several possible mechanisms governing the long-term effects of growth factor injection warrant further discussion. First, the residence time of an injected protein in the disc has not been solidly established. Some authors have suggested a short half-life in the order of minutes,[112] while others, using radiolabeling, have noted much greater times, likely over 1 month (Figure 7).[113] Both the structural integrity of the disc and injection location may affect the movement of injected materials in the disc. Second, it has been suggested that OP-1 binds to collagen molecules, which can explain its long acting effects.[114] Finally, the duration of the anabolic effect resulting from a single exposure to a growth factor needs to determined.

Injection Therapeutics: Effect on Pain

In addition to the structural-modifying effects of injection therapy seen in many preclinical animal studies, there is growing evidence that suggests its pain-relieving effects. In a rat model of pain in which degenerated disc tissue was applied to lumbar nerve roots, mechanical hyperalgesia was observed in the sham- and saline-injected groups, but not in the OP-1-treated group.[115] The pain relief may have been due to the ability of OP-1 treatment to result in a reduction of immunohistologic staining for aggrecanase, MMP-13, substance P, TNF-α and IL-1β.[116] These changes are consistent with the suppression by OP-1 treatment of the expression of IL-1β, TNF-α, IL-6, MMP-3 and aggrecanse-1 in IL-1 insulted human disc cells.[117] These results suggest that OP-1, in addition to being an anabolic mediator, is a catabolic regulator of the metabolism of IVD cells. OP-1 suppression of pro-inflammatory factors can also suppress a variety of pain markers, such as nerve growth factor (NGF), and can lead to pain reduction.

Considerations for Injectable Therapeutics and their Limitations

While the clinical application of injectable therapeutics is being actively pursued, several important limitations need to be considered. First, the target population of this therapeutic

approach is mainly an aged population, which has fewer cells in the IVD than the normal population. IVD cell density is also lower in discs with advanced degeneration.[118] Because injection therapy seeks to influence existing cells in the disc, the appropriate stage of disc degeneration and age of the patients may need to be defined. For those with very low cellularity, it may be possible to utilize a tissue-engineering approach. For example, a small number of functional cells recovered from herniated tissues or mesenchymal stem cells may be expanded for later use.[119–123]

In addition to cellular changes, nutritional supply and removal of metabolic wastes may be compromised by changes in the endplate, such as calcification seen in sclerosis. Hindrance of the nutritional pathway may render an injection therapy ineffective because the lack of nutrition may impair the synthesis of the disc matrix needed for repair. Similarly, when tissue-engineered cells are added to discs, they may not survive without a proper supply of nutrients to meet the demands of increased energy consumption. Thus, a non-invasive means to assess environmental conditions (e.g., changes in cartilage endplate) in the IVD is essential. Using a contrast-enhanced MRI method, the diffusion function of the endplate has been assessed indirectly in human subjects.[124,125] Recent advances in MRI, such as the ultrashort time-to-echo (UTE) technique,[125–127] allow for direct visualization (Figure 8A) of regions of the cartilage endplate with high contrast, unlike conventional MRI sequences (Figure 8B). Abnormalities (Figure 8C) of the cartilage endplate in UTE MRI have been significantly associated with disc degeneration ($p<0.01$ chi-square test), as evaluated by Pfirrman grading,[128] in approximately 30 cadaveric human lumbar spines. It would be useful to determine if the appearance of the cartilage endplate by MRI provides information on the time course of subsequent disc degeneration, as well as the outcome of therapeutic treatments or the proper selection of patients.

Although much evidence of structural modifications in animal disc degeneration models has been demonstrated in this review, there are significant limitations to applying these results to human patients. Studies using large animals with discs of a size similar to those of the human that do not contain notochordal cells may be needed to answer the remaining issues of nutritional supply and cellular composition. However, the cost of studies using large animals and the ethical problems associated with these animals are some of the issues that delay their use. In addition, pain measures have not been well-established for large animals, making pain research difficult. However, once a therapeutic agent is proven safe, a clinical trial on carefully selected patients (e.g., those patients who would otherwise undergo spinal fusion) may be a good approach for testing therapeutic efficacy.

Injection Therapeutics in Clinical Trials

Several clinical trials involving intradiscal injection therapeutics are underway. These studies typically select patients exhibiting chronic moderate-to-severe discogenic pain, without involvement of nerve compression or facet joints, for which non-surgical therapy has not been effective. Sponsored by Advanced Technologies and Regenerative Medicine and DePuy Spine, multi-center studies in the United States (ClinicalTrials.gov identifier: NCT01124006) and in Korea and Australia (NCT01158924 and NCT01182337) are currently (as of Nov 2010) recruiting patients to evaluate safety, tolerability and

effectiveness of single injection of rhGDF-5 vs. placebo, with outcome measures including safety outcomes, pain and MRI at 12 mo.

Another study sponsored by Spinal Restoration (NCT01011816) will be evaluating the effects of injecting Biostat fibrin sealant on the time course of pain reduction (visual analog scale) and function restoration (Roland-Morris Disability score) through 78 weeks, along with safety measures, on 260 patients. This study seeks to achieve a 30% decrease in pain and 30% improvement in function. Once safety concerns are addressed in the current studies, it is hoped that later stage studies with additional measures and a greater number of patients will reveal the mechanism of treatment as well as suitable patient selection.

Conclusion

Abundant evidence for the efficacy of injectable therapeutics, including many growth factors, for the treatment of IVD degeneration has been presented in studies using IVD cells *in vitro* as well as in preclinical *in vivo* animal studies. For animal studies, outcomes focused mainly on structural modification, and the effects of injection therapy on pain generation are not well known. Recent data obtained from small animal studies suggest that injection therapy can lead to modification of pain behaviors, as well as changes in cytokine expression. Such results offer great potential for patients with chronic discogenic low back pain. Multiple clinical trials for injection therapy are now underway, and their results will be useful for establishing safety and efficacy injection treatments. In addition, for discs with advanced stages of degeneration, the prophylactic use of growth factor injection therapy, such as its application to discs adjacent to a fusion level, may be an alternative approach. Quantitative studies on the effects of a growth factor injection on pain reduction and on long-term cell survival using large animals are desirable. Diagnostic techniques to evaluate the disc environment, such as the MRI evaluation of the cartilage endplate, may also become a desirable tool for patient selection. Furthermore, comprehensive studies looking at the muscles, facet joints and vasculature that comprise the whole spinal structure are important to seek new innovative diagnostic and therapeutic approaches, encompassing surgical and conservative approaches.

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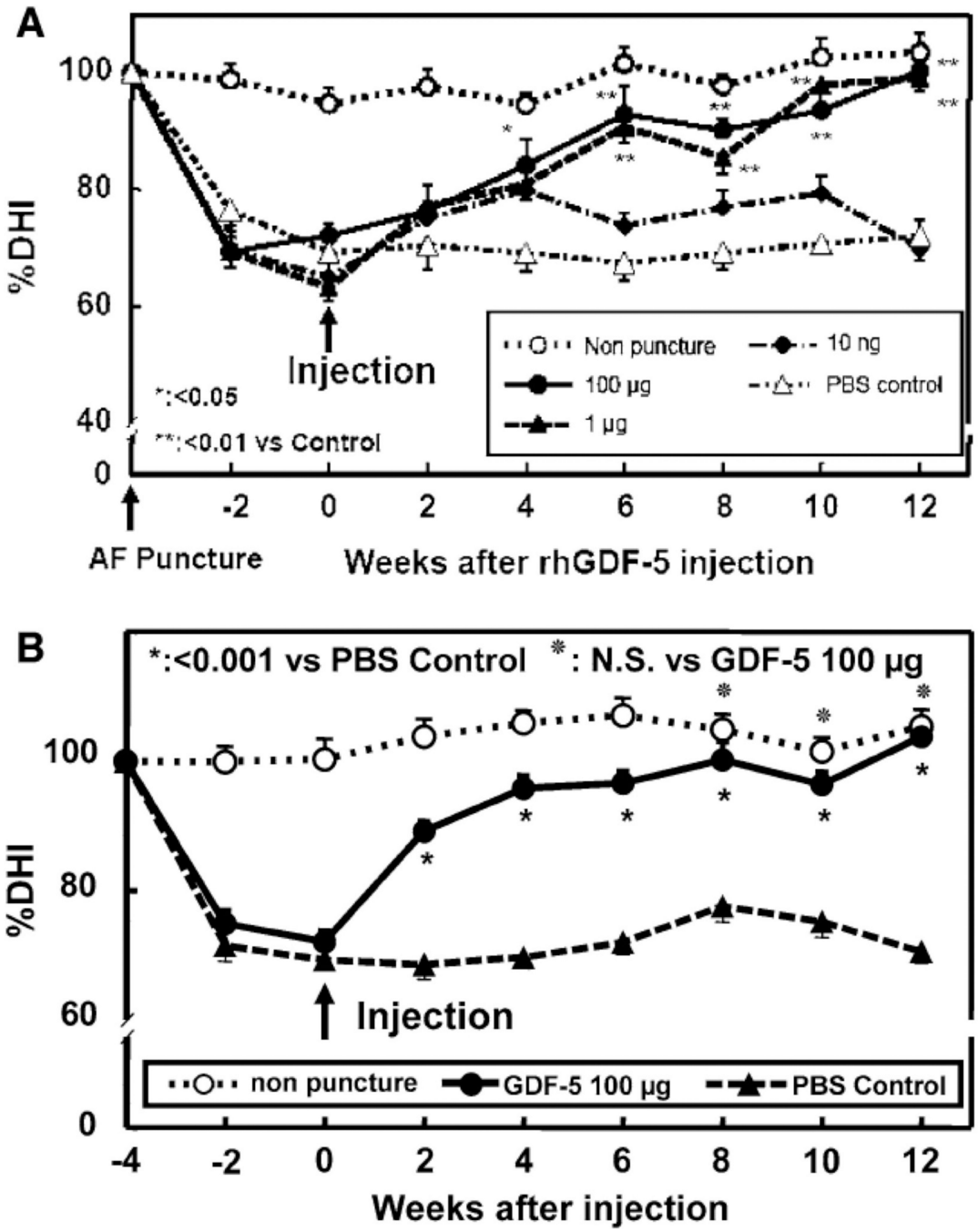

Figure 1. Changes in the intervertebral disc (IVD) height index (DHI) after anular puncture and recombinant human growth and differentiation factor-5 (rhGDF-5) injection

In a needle-puncture model of disc degeneration (4 weeks) in adolescent (5–6 months-old) rabbits, a single injection of rhGDF-5 restored disc height (**A**). In a study using 2-year old rabbits, rhGDF-5 disc height was also effectively recovered (**B**). (Modified from Chujo T, et al. Effects of growth differentiation factor-5 on the intervertebral disc- *in vitro* bovine study and *in vivo* rabbit disc degeneration model study. Spine 2006;31(25):2909-17 and Chujo T, et al. *In vivo* effects of recombinant human growth and differentiation factor-5 on the repair

of the mature rabbit intervertebral disc. Spine J, 6(5):23S–24S, 2006 (North American Spine Society, 21st Annual Meeting Proceeding, abstract). AF: annulus fibrosus; PBS: phosphate-buffered saline.

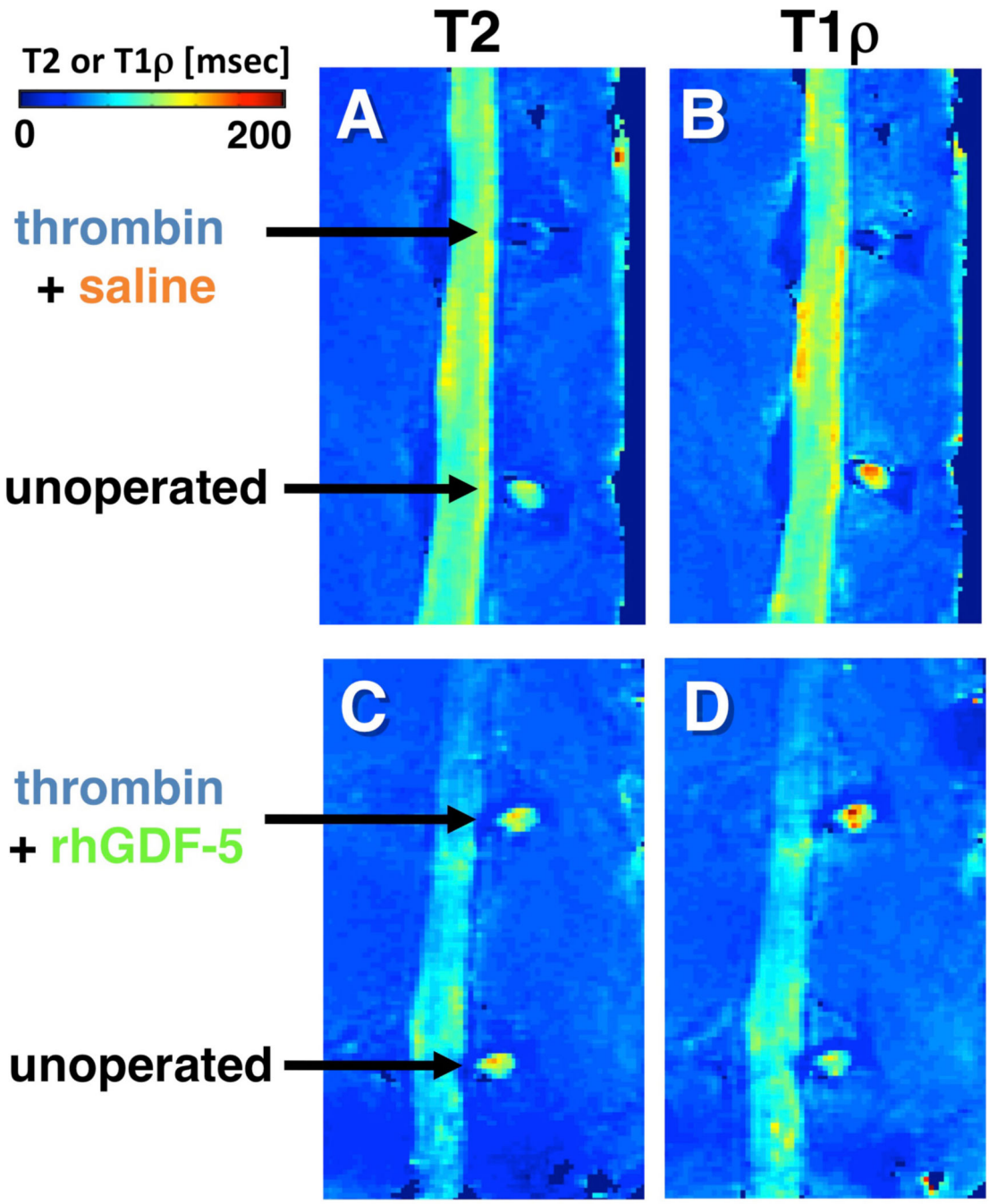

Figure 2. Quantitative magnetic resonance (MR) parameter maps of thrombin-induced degraded rabbit lumbar spines after injection with the growth factor, recombinant human growth and differentiation factor-5 (rhGDF-5)

Four weeks after thrombin injection (100 U/10 μl) into adolescent rabbit discs, a single injection of saline (10 μl) (**A, B**) or rhGDF-5 (10 μg/10 μl) (**C, D**) was given. T2 (**A, C**) and T1rho (**B, D**) MRI maps were obtained 12 weeks later. High MR values in the nucleus pulposus regions are apparent in the samples treated with the growth factor (**C, D**). (Modified from Bae WC, et al. Effect of rhGDF-5 on the thrombin model of rabbit

intervertebral disc degeneration: T1rho quantification using 3T MRI. Rad Soc North Am 2009;95:SSE14-02, abstract)

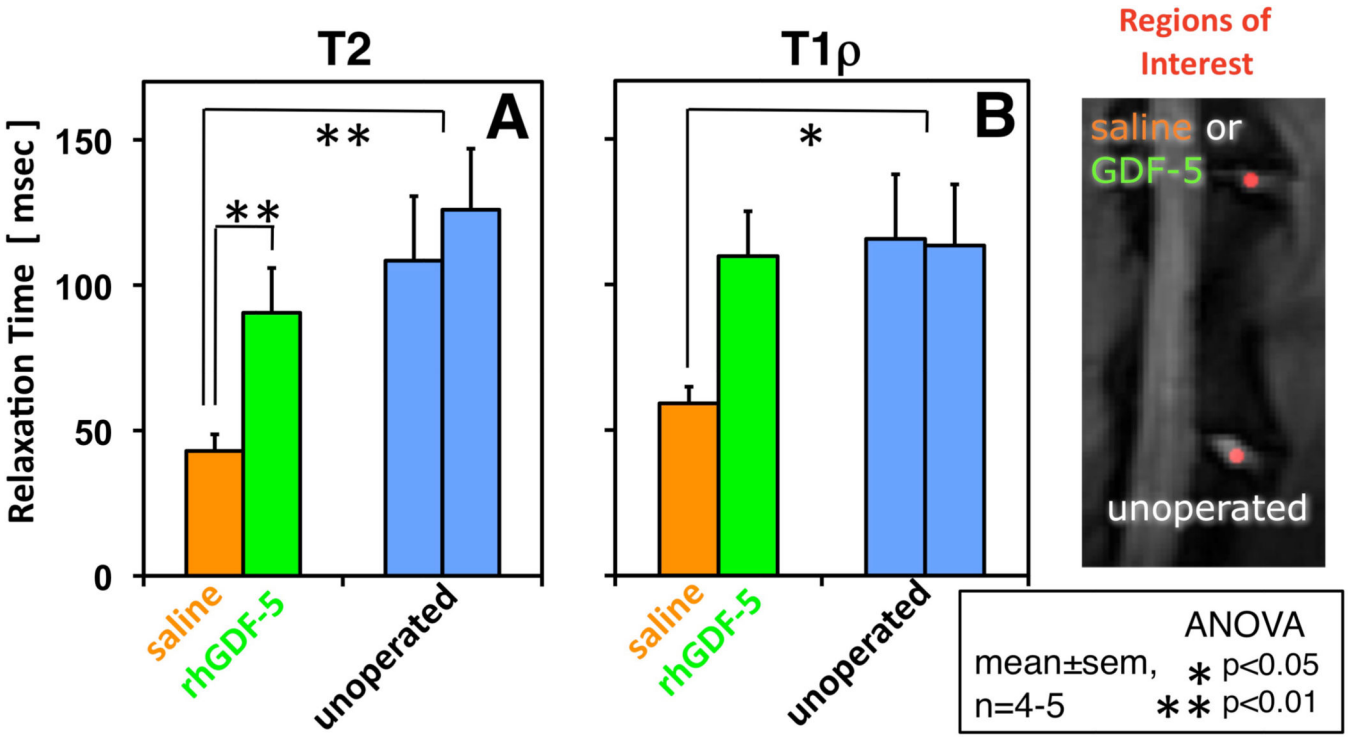

Figure 3. Treatment with recombinant human growth and differentiation factor-5 (rhGDF-5) restored T2 and T1rho values after thrombin-induced degeneration in adolescent rabbit discs

Four weeks after thrombin injection (100 U/10 μl) into adolescent rabbit discs, a single injection of saline (10 μl) or rhGDF-5 (10 μg/10 μl) was given. T2 and T1rho MRI maps were obtained 12 weeks later. Regions of interest were selected to determine T2 and T1rho values in the nucleus pulposus (NP). Both T2 (**A**) and T1rho (**B**) values of the NP were higher in the rhGDF-5-treated group, approaching those of the unoperated group. The saline group had significantly lower values compared to the unoperated control. (Modified from Bae WC, et al. Effect of rhGDF-5 on the thrombin model of rabbit intervertebral disc degeneration: T1rho quantification using 3T MRI. Rad Soc North Am 2009;95:SSE14-02, abstract)

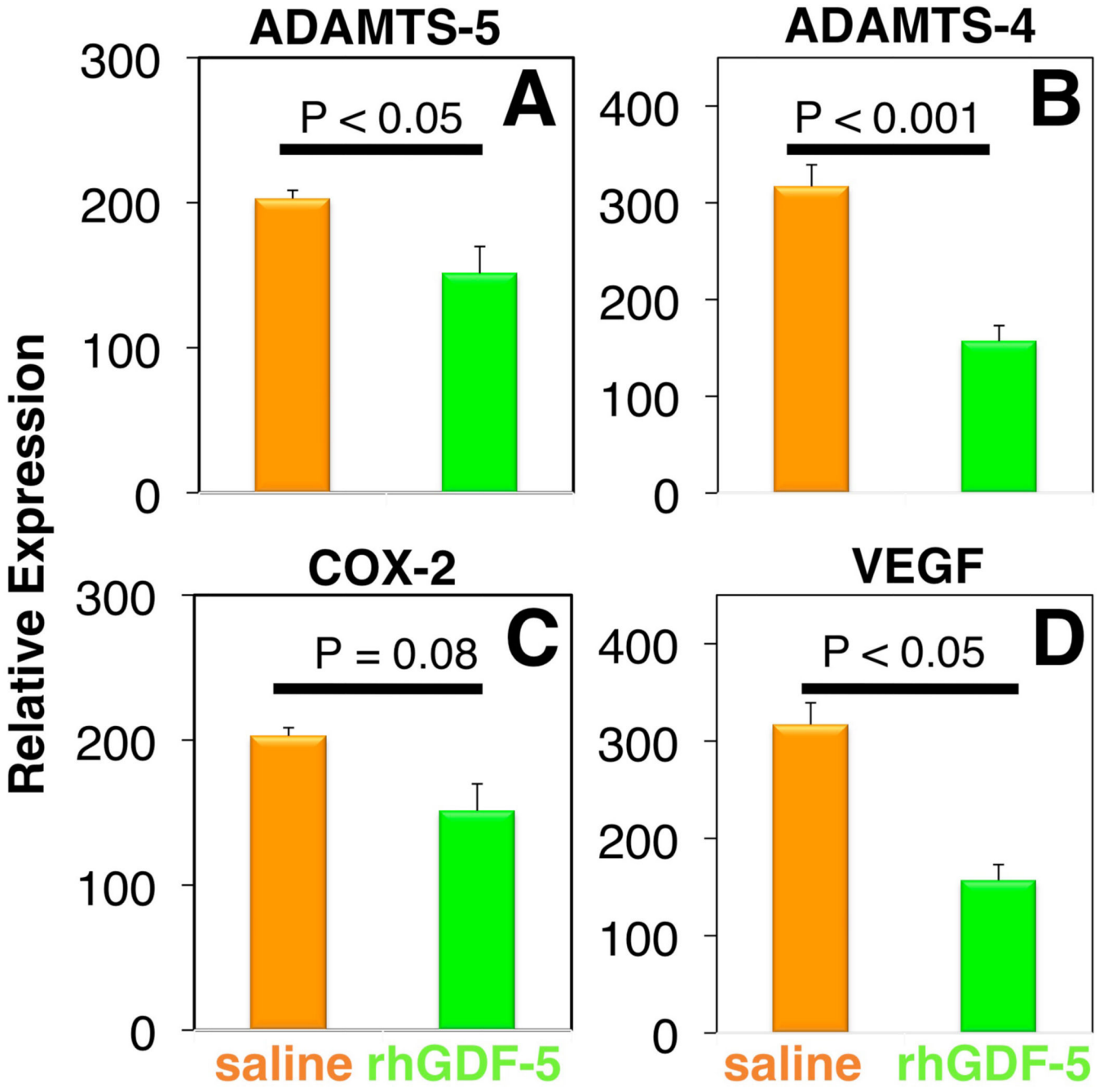

Figure 4. Treatment with recombinant human growth and differentiation factor-5 (rhGDF-5) inhibits the expression of molecules related to disc degradation, pain, and neovascularization in thrombin-degenerated adolescent rabbit discs

Four weeks after thrombin injection (100 U/10 μl) into adolescent rabbit discs, a single injection of saline (10 μl) or rhGDF-5 (10 μg/10 μl) was given. Twelve weeks later, mRNA expression levels for ADAMTS-5 (**A**), ADAMTS-4 (**B**), COX-2 (**C**) and VEGF (**D**), in saline-and rhGDF-5-treated discs showed a trend or significantly lower levels in the rhGDF-5-treated samples. (Modified from Masuda K, et al. Intradiscal injection of recombinant human growth and differentiation factor-5 significantly suppressed the

expression of cytokines, catabolic enzymes and pain markers in the rabbit anular puncture model. Paper presented at: The 35th Annual Meeting of the International Society for the Study of the Lumbar Spine 2009:47, abstract) ADAMTS: a disintegrin and metalloproteinase with a thrombospondin type 1 motif; COX-2: cyclooxygenase-2; VEGF: vascular endothelial growth factor.

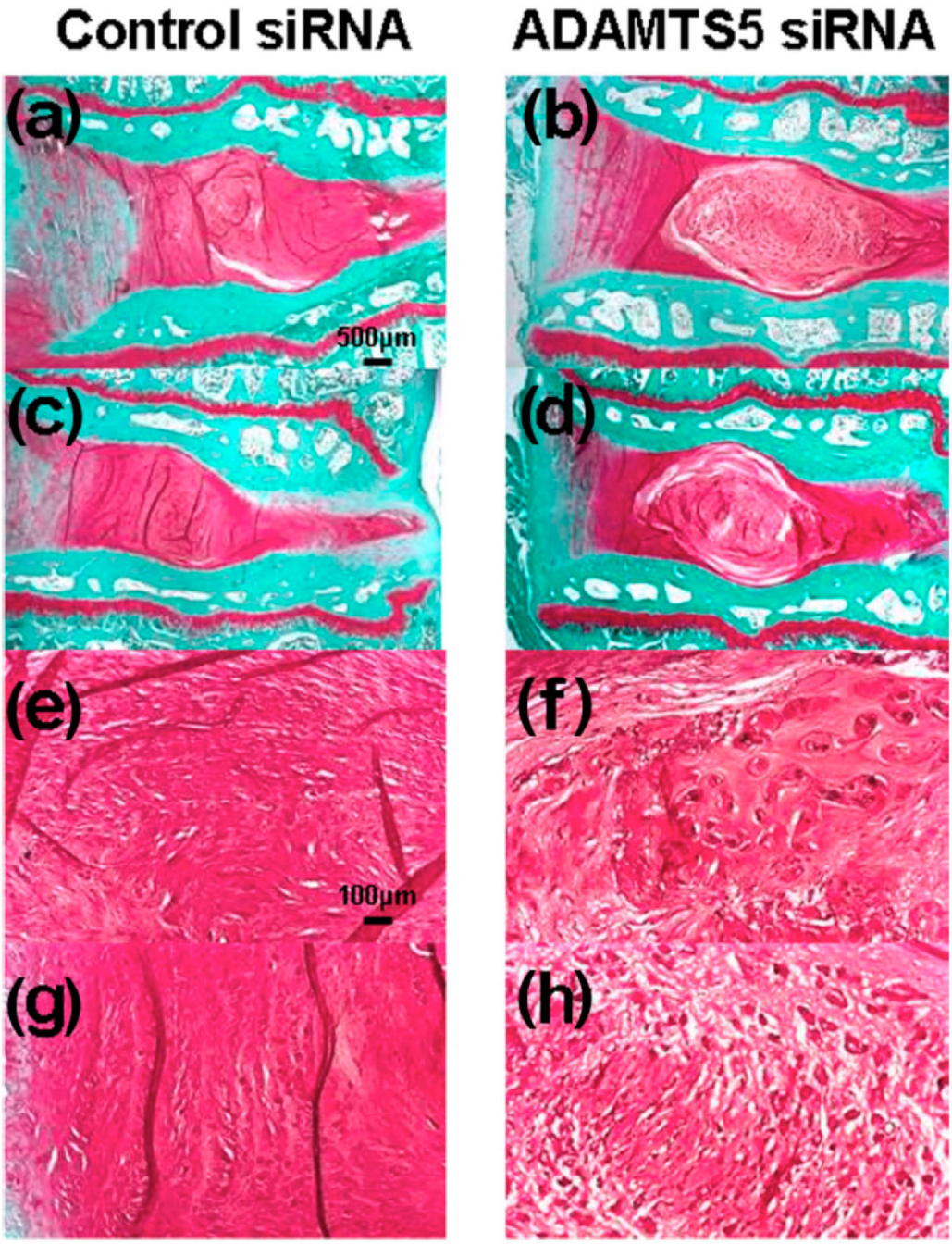

Figure 5. Histology of anular puncture-induced degenerated adolescent rabbit discs injected with control small interference RNA (siRNA) or ADAMTS-5 siRNA

Representative safranin-O-stained sections after injection of ADAMTS-5 siRNA or control siRNA in the rabbit anular puncture model of disc degeneration. Eight weeks after the siRNA injections, the control siRNA group displayed a complete loss of nucleus pulposus (NP) tissues that had been replaced by a fibrocartilaginous tissue (**a, c**). The severely degenerated discs that had received the control siRNA showed a loss of proteoglycans and the collapsed, wavy fibrocartilage lamellae typical of the anulus fibrosus (AF), with

associated fibrochondrocytes (**e, g**). In the ADAMTS5 siRNA-injected discs, safranin-O staining demonstrated the maintenance of intervertebral disc structure with a lightly stained matrix and large cells (**b, d**); the NP was rounded and bloated in appearance, and consisted of numerous large, vacuolated cells and smaller chondrocyte-like cells (**f, h**). A clear demarcation was seen between the NP and inner anulus in the ADAMTS5 siRNA-injected discs. (Magnification is 20× (a–d) or 100× (e–h)). The level in **a, b, e**, and **f** is L2/3, and in **c, d, g**, and **h** is L4/5. (Reproduced from Seki S, et al. Effect of small interference RNA (siRNA) for ADAMTS5 on intervertebral disc degeneration in the rabbit anular needle-puncture model. Arthritis Research & Therapy 2009;11:R166). ADAMTS: a disintegrin and metalloproteinase with a thrombospondin type 1 motif.

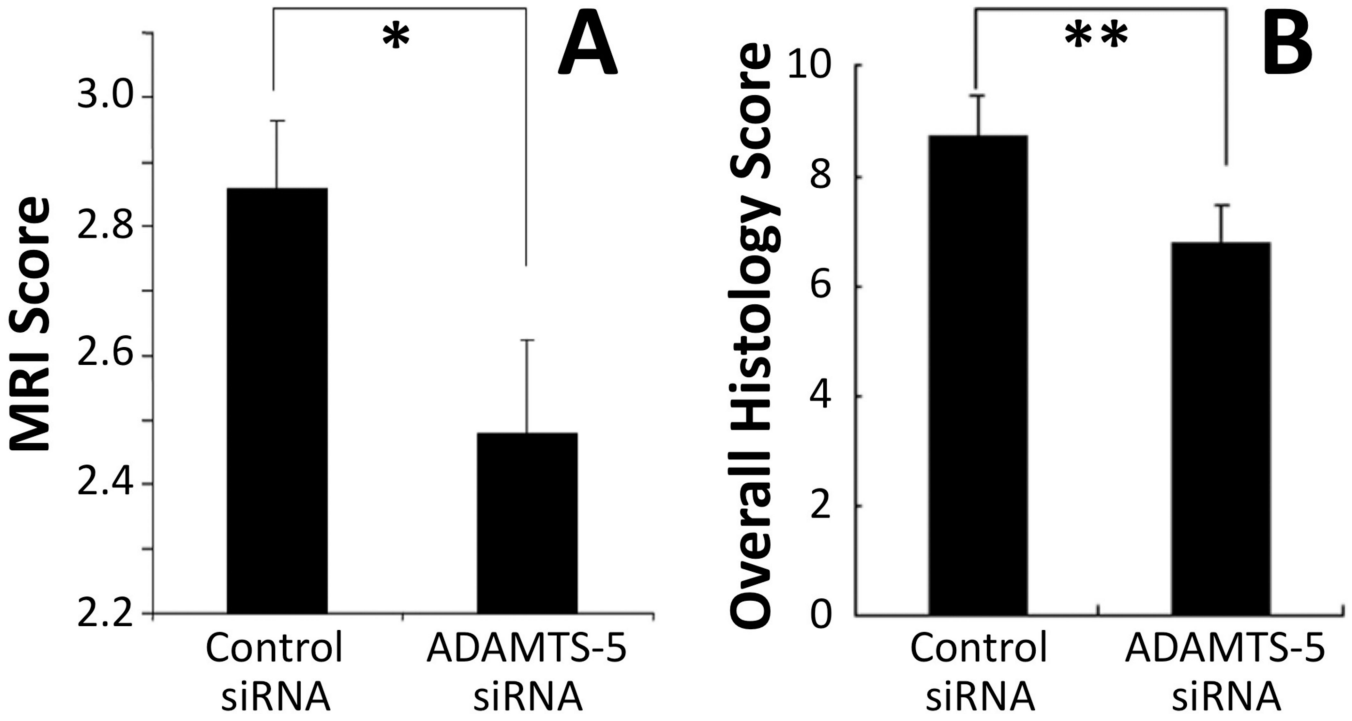

Figure 6. Magnetic resonance imaging (MRI) and histology grading scores of anular puncture-induced degenerated adolescent rabbit discs injected with control small interference RNA (siRNA) or ADAMTS-5 siRNA

(**A**) MRI assessment 8 weeks after siRNA injection, using a modified Thompson scale (1–4). The MRI grade in the ADAMTS-5 siRNA-treated discs show significantly better (lower) MRI grade compared to control siRNA-treated discs. (**B**) Histological assessment for structure, cellularity and matrix staining showed significantly better (lower) overall score (range 0–12) for ADAMTS-5 siRNA-treated samples. Mean ± S.E.M., n=6. * $p<0.05$, ** $p<0.01$ Mann-Whitney U test. (Modified from Seki S, et al. Effect of small interference RNA (siRNA) for ADAMTS5 on intervertebral disc degeneration in the rabbit anular needle-puncture model. Arthritis Research & Therapy 2009;11:R166).

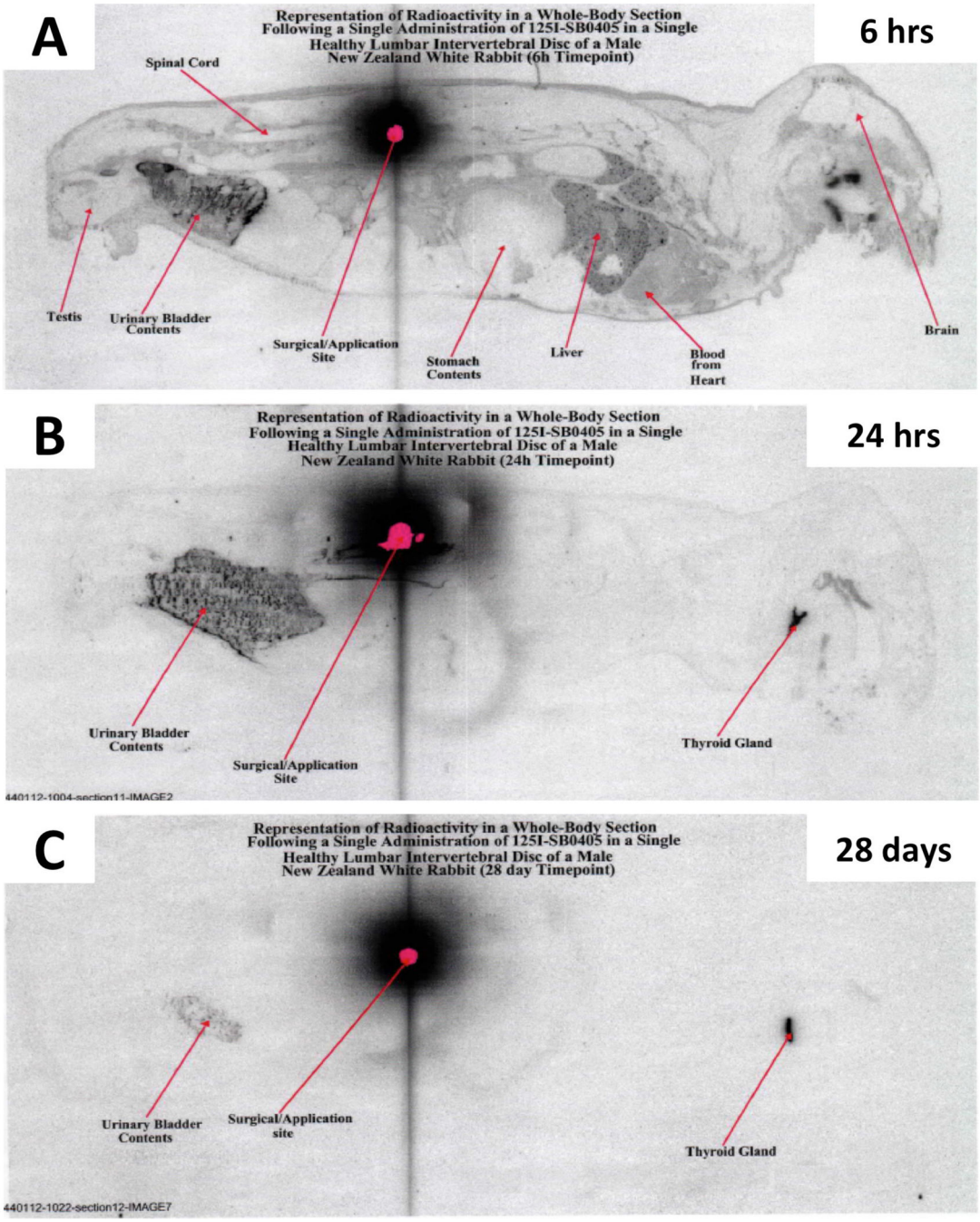

Figure 7. Retention of radiolabeled bone morphogenetic-7 (BMP-7) in a rabbit disc

Normal rabbits that received a single intradiscal injection of ^{125}I-labeled BMP-7 (otherwise known as osteogenic protein-1) were imaged using autoradiography (**A**) 6 hours, (**B**) 24 hours or (**C**) 28 days after the injection. The signal from the radiolabeled BMP-7 is prominent even after 14 days. (Modified from Pierce, A. et al. Distribution, pharmacokinetics and excretion of 125-Iodine labeled BMP-7 (OP-1) following a single dose administration in lumbar IVD or knee joint of NZW rabbits. Paper presented at: The sixth International conference on bone morphogenetic protein 2006; Cavtat, Croatia.)

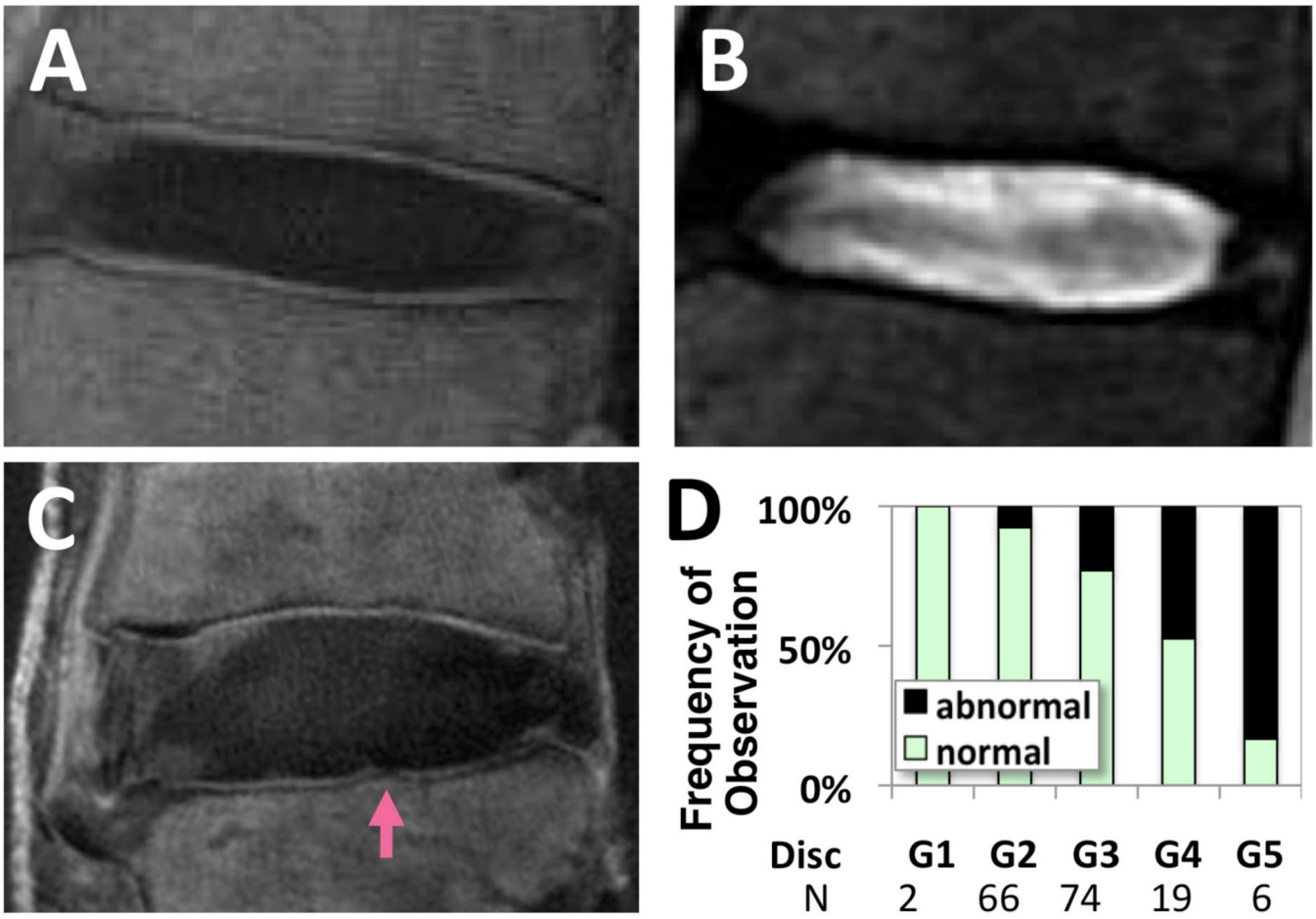

Figure 8. Evaluation of region of cartilage endplate using ultrashort time-to-echo (UTE) magnetic resonance imaging (MRI)

A normal lumbar disc imaged using UTE MRI (**A**) and conventional T2-weighted spin echo MRI (**B**). Note the characteristic high-intensity lines (**A**) near the regions of cartilage endplate. The same regions appear dark in conventional MRI (**B**). Abnormalities such as focal signal loss have been observed (arrow, **C**) and correlated with the grade of the adjacent disc (**D**). (Modified from Bae WC, et al. Ultrashort time-to-echo MRI of human intervertebral disc endplate: association with disc degeneration. Proc Int'l Soc Magn Reson Med 2010;18:534)

Table 1

The *in vitro* effects of therapeutic agents.

Agent	Target	Dose	Effect	Author
TGF-β	mature canine IVD	1 ng/ml	PG synthesis increased up to 5×; higher in NP than AF.	Thompson 1991[10]
IGF-1	mature canine IVD	20 ng/ml	PG synthesis marginally increased in NP.	Thompson 1991[10]
TGF-β	human anulus cells, 3D culture	0.25 to 5 ng/ml	Cell proliferation and PG synthesis increased; reduced apoptosis with serum depletion.	Gruber 1997[69]
IGF-1	young and old bovine NP cells	0 to 1000 ng/ml	Cell proliferation and matrix synthesis stimulated; more IGF-1 receptors.	Osada 1996[11]
OP-1	young rabbit NP and AF cells; alginate beads	0, 100, 200 ng/ml	Increased PG and collagen production and content.	Masuda 2003[71]
BMP-2	rat IVD cells monolayer	0 to 1000 ng/ml	Increased cell number, GAG, expression for collagen, aggrecan at higher doses.	Yoon 2003[129]
OP-1	human NP and AF cells, alginate beads	0, 100, 200 ng/ml	Maintained cell density, increased PG synthesis and accumulation.	Imai 2007[72]
OP-1	rabbit IVD cells; alginate beads: IL-1a pre-exposure	200 ng/ml	IL-1 decreased PG and collagen; reversed and exceeded with OP-1.	Takegami 2002[42]
OP-1	rabbit IVD cells; alginate bead: C-ABC pre-exposure	200 ng/ml	OP-1 upregulated PG synthesis. Greater effect on C-ABC pre-exposure than control.	Takegami 2005[73]
BMP-2	human IVD cells	300, 1500 ng/ml	Increased PG synthesis, expression of aggrecan, types I and II collagen; no bone formation.	Kim 2003[130]
rhBMP-2 & 12	human IVD cells in monolayer	25 to 300 ng/ml	PG, collagen synthesis increased in NP cells; minimal effect on AF cells.	Gilbertson 2008[131]
GDF-5	bovine IVD cells; alginate bead	100, 200 ng/ml	Increased DNA and PG content; at higher dose, PG and collagen synthesis increased.	Chujo 2006[76]
PRP	porcine IVD cells; alginate bead	10% PRP	Mild increase in cell proliferation; marked increase in PG and collagen synthesis and PG accumulation.	Akeda 2006[78]
TGF-β1 and PRP	human NP cells	1 ng/mL TGF-β1 in PRP	NP cell proliferation and aggregation; increase in mRNA of SOX-9, type II collagen, aggrecan.	Chen 2006[79]
Ad-TIMP-1, Ad-BMP-2	IVD cells from human degenerated IVD	50–150 MOI	2000 pg/ml production of TIMP w/100 MOI at day-4. PG synthesis increased with both Ad-TIMP-1 and Ad-BMP-2.	Wallach 2003[132]
Dexa-methasone	human disc herniation tissue explants	0.01 mM	Decreased MMP-1 and -3 levels.	Genevay 2009[57]
IL-1ra	human disc herniation tissue explants	100 ng/ml	Decreased MMP-3 levels.	Genevay 2009[57]
IL-1ra	human normal and degenerated disc tissues *in situ* with IL-1 treatment	100 ng/ml	IL-1ra reduced cytokine levels (MMP-3, -7, -13) and matrix degradation in all tissue types.	Le Maitre 2007[56]
IL-1ra/ELP	human IVD cells (grade 2–3); alginate beads: IL-1ra pre-Tx then IL-1β insult	4–8000 pM	Reduced ADAMTS-4, MMP-3 transcription.	Shamji 2007[58]

Agent	Target	Dose	Effect	Author
p38 MAPK inhibitor (SB 202190)	rabbit NP cells pre-treated with IL-1	1 μM	Decreased message for collagen, aggrecan, IGF-1. Increased message for iNOS, COX-2, MMP-3, IL-6.	Studer 2008[66]
TNF inhibitor mAb	human IVD herniation tissue explants	10 μg/ml	Decreased MMP-3 levels	Fujita 1993[38]

TGF-β: transforming growth factor-β; PG: proteoglycan; NP: nucleus pulposus: AF: annulus fibrosus; IGF-1 insulin-like growth factor-1; 3D: three-dimensional; OP-1 osteogenic protein-1; BMP-2: bone morphogenetic protein-2; IVD: intervertebral disc; GAG: glycosaminoglycan; C-ABC: chondroitinase-ABC; GDF-5 growth differentiation factor-5; PRP: platelet-rich plasma; Ad-TIMP-1: adenoviral vector delivering cDNA of tissue inhibitor of matrix metalloproteinases-1; Ad-BMP-2: adenoviral vector delivering cDNA of BMP-2; MOI: multiplicity of infection; IL-1ra: IL-1 receptor antagonist; ELP: elastin-like polypeptide; Tx: treatment; ADAMTS: a disintegrin and metalloproteinase with thrombospondin motifs; MAPK: mitogen activated protein kinase; NP: nucleus pulposus; iNOS: inducible nitric oxide synthase; COX-2: cyclooxygenase-2; TNF: tumor necrosis factor; mAb monoclonal antibody.

Table 2

The in vivo effects of intradiscal injection treatments.

Agent	Species	Site	Model	Dose per Disc	Effect	Author
IGF-1	Rat	Tail	Static compression	8 ng/8 μl	Clustering of inner anulus cells after single injection	Walsh 2004[99]
GDF-5	Rat	Tail	Static compression	8 ng/8 μl	Clustering of cells, increase in disc height (single injection)	Walsh 2004[99]
TFG-β	Rat	Tail	Static compression	1.6 ng/8 μl	Proliferation of cells (multiple injections)	Walsh 2004[99]
bFGF	Rat	Tail	Static compression	8 ng/8 μl	No response	Walsh 2004[99]
OP-1	Rabbit	Lumbar	None (normal)	2 μg/10 μl	Increased disc height and PG content in NP	An 2005[133]
OP-1	Rabbit	Lumbar	C-ABC: Co-injection	100 μg/10 μl	Increased disc height and PG content in NP	Imai 2003[134]
OP-1	Rabbit	Lumbar	Needle puncture: Tx 4 wks later	100 μg/10 μl	Increased disc height and PG content in NP and AF, improvement of MRI and histology grades	Masuda 2006[80]
OP-1	Rabbit	Lumbar	Needle puncture	100 μg/10 μl	Increased disc height and viscoelastic properties	Miyamoto 2006[81]
OP-1	Rabbit	Lumbar	C-ABC: Tx 4 wks later	100 μg/10 μl	Increased disc height, PG content in NP and AF	Imai 2007[82]
GDF-5	Rabbit	Lumbar	Needle puncture: Tx 4 wks later	1, 100 ng/10 μl; 1, 100 μg/10 μl	Increased disc height, improvement of MRI and histology grades	Chujo 2006[76]
GDF-5	Rabbit	Lumbar	Thrombin-degraded: Tx 4 wks later	10 μg/10 μl	Increased disc height, improved T1rho and T2 values. Decreased ADAMTS-4, -5, COX9 expression	Bae 2009[83]
BMP-2	Rabbit	Lumbar	Anular stab (5×7 mm)	100 μg/100 μl	More degeneration, vascularity and fibroblast	Hwang 2007[135]
PRP	Rabbit	Lumbar	Nucleotomy, immediate Tx	PRP+GHM(20 μl) or PRP(5μl) +PBS(15μl)	PRP+GHM group had less degeneration and increased PG; PRP+PBS group showed no differences	Nagae 2007[108]
PRP	Rabbit	Lumbar	Nucleotomy, immediate Tx	20 μl of PRP+GHM, PBS +GHM, PRP+PBS; puncture-only	PRP+GHM had greater disc height, water content, mRNA for PG core protein and type II collagen; fewer apoptotic cells in NP	Sawamura 2009[60]
BMP-17	Sheep	Lumbar	Anular stab (3×6 mm), immediate Tx	300 μg/70 μl	BMP-17 maintained disc height, MRI and histology scores, NP cell density; increased PG and collagen synthesis.	Wei 2009[136]
ADAMTS -5 siRNA	Rabbit	Lumbar	Needle puncture: Tx 4 wks later	10 μg/10 μl	Improved MRI and histology scores	Seki 2009[111]

IGF-1: insulin-like growth factor-1; GDF-5 growth differentiation factor-5; TGF-β: transforming growth factor-β; bFGF: basic fibroblast growth factor; OP-1: osteogenic protein-1; PG: proteoglycan; NP: nucleus pulposus; C-ABC: chondroitinase-ABC; Tx: treatment; MRI: magnetic resonance imaging; AF: annulus fibrosus; ADAMTS: a disintegrin and metalloproteinase with thrombospondin motifs; COX: cyclooxygenase; BMP-2: bone morphogenetic protein-2; PRP: platelet-rich plasma; GHM: gelatin hydrogel microspheres; PBS: phosphate-buffered saline; siRNA: small interference RNA (siRNA).