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Cell Therapy for the Degenerating Intervertebral Disc

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Clinical Significance

The annual costs of spinal conditions related to intervertebral disc (IVD) degeneration exceed $190 billion in the US.(1) In industrialized countries, low back pain is extremely common, with a prevalence of 60–90%.(2) Despite this prevalence and soaring cost, there is no specific treatment that restores the physiological function of the degenerate IVD. Thus, developing new treatment strategies to repair the degenerating IVD is vital.

Current treatments for disc-related pain include surgical and non-surgical approaches,(3) and often result in incomplete symptomatic relief. A key limitation of current treatments for disc degeneration is that they do not maintain or restore native tissue structure and mechanical function. Therefore, there is a pressing need for new therapies to treat disc degeneration that

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retain and/or restore disc structure and mechanical function by directly addressing the underlying causes and mechanisms.

The IVD is an elegant structure, with a gelatinous inner core (the nucleus pulposus (NP)) that functions as a shock absorber, converting axial loads into radial forces. The concentric outer rings (annulus fibrosus (AF)) enclose the inner core (Figure 1). The elegance and complexity of the IVD structure is illustrated in Figure 1. Specifically, tamoxifen was used at postnatal day 6 to induce the expression of Cre-recombinase driven by the type II collagen promoter (*Col2CreER*). The lumbar spine was examined at postnatal day 28 (Figure 1A). The motion segment consists of an IVD with the adjacent vertebral bodies (VB) (Figure 1B). Cell nuclei, stained with DAPI, are shown in blue (Figure 1A). Inner AF cells express type II collagen (col2) and have been highlighted by red fluorescent protein variant (tdTomato; Figure 1A). The cartilaginous endplate (CEP), growth plate (GP) and cancellous bone adjacent to the GP also express col2, and thus also expressed tdTomato (shown in red in Figure 1A&B).

The IVD progressively degenerates with age in humans (Figure 2), and strategies to repair the IVD depend on stage of degeneration.(4, 5) Cell therapy and cell-based gene therapy aim to address moderate IVD degeneration (Figure 2). In early disc degeneration, an attractive strategy is to encourage resident progenitor cells to proliferate. At this stage, various protein factors such as growth factors might be effective since resident cells may respond to the stimuli and produce additional extracellular matrix. However, the number and functional capacity of endogenous stem cells tend to become reduced with aging and degeneration.(6) Therefore, in moderate stages of degeneration, when fewer viable cells remain in the diseased IVD, cell or gene therapy is likely to be required to repopulate the disc and provide additional trophic factors. In advanced stages of degeneration when both cell and extracellular matrix loss is severe, tissue-engineering approaches may be needed.(7, 8)

The quality of life for people with chronic pain is often irreversibly affected due to rewiring of brain circuitry,(9) supporting the notion that early treatments will result in better outcomes. The ideal therapy for moderate IVD degeneration would: 1) be minimally invasive; 2) attenuate local inflammation; and 3) restore tissue structure and biomechanical function. In order to achieve success, transplantation of cells into the degenerate IVD must overcome several hurdles. First, transplanted cells must survive in the harsh IVD environment that is low in nutrients, oxygen and pH, exhibits elevated inflammatory cytokine expression, and experiences fluctuations in mechanical stress. Secondly, to achieve therapeutic efficacy, cells must remain viable and in place, produce extracellular matrix (ECM) rich in proteoglycans and type II collagen, or secrete trophic factors that stimulate resident cells to do so.

Patient selection

Most people with IVD degeneration do not have back pain,(10) and thus do not require any intervention. For patients with intractable back pain due to internal disc disruption, cell therapy may help to repair the structure and modulate inflammation, thus reducing pain. When selecting which disc is most symptomatic, the best tool available may be discography,

with patient response of severe concordant pain. Discography is a valuable tool since positive findings during discography correlate with levels of cytokines/chemokines in the tissue, thus providing a pathophysiological basis for discogenic back pain.(11, 12) Discography, however, may cause further IVD degeneration,(13) due to needle puncture damage and the toxicity of injected anesthetics and contrast media.(14–16) Based on these observations, only patients with severe axial back pain that is suspected to be due to degenerative disc disease are clinical candidates for discography. Patients with severe back pain confirmed to be related to IVD degeneration should undergo cell therapy. Therefore, after patient-confirmed severe concordant pain with provocation, cells could be injected during the same procedure to avoid puncturing the IVD multiple times.

Patients undergoing partial discectomy are another group of individuals who could benefit from cell therapy, since disc degeneration accelerates after partial removal of the disc.(17) Cells could be injected during surgery, after removal of the disc fragment(s) impinging on the nerve roots. The above indications for cell therapy require that cells are ready to use before the procedure.

Cell Sources and Types

Autologous and allogeneic cells have been used in clinical trials, but xenogeneic cells have only been used in animal studies. Autologous cells are ideal, due to concerns over disease transmission and immune responses. Autologous mesenchymal stromal cells can be harvested from bone marrow or adipose tissue. The main limitation is that most patients with back pain are middle aged, and their stem cells have limited expansion potential. A small clinical study (10 patients) examining the possible efficacy of hematopoietic stem cells in disc repair did not show any treatment effect.(18) A more recent study using bone marrow concentrate cells did show pain reduction.(19) The EuroDISC study is the largest (112 patients) prospective multicenter randomized controlled trial comparing patients who had discectomy with or without subsequent treatment with expanded autologous IVD cells; this treatment led to moderate success in preserving the disc structure.(20, 21) The limitation of this study is that patients underwent two procedures, and the cells expanded may have included fibroblasts and inflammatory cells.

Allogeneic cells could be isolated from umbilical cord blood,(22) umbilical tissue,(23) or articular surface.(24, 25) Allogeneic cells from younger donors have higher expansion potential than most autologous cells. There is less ethical concern using these cells than with embryonic stem cells. Only one clinical trial using allogeneic young articular chondrocyte transplantation has been completed. This trial recruited 15 patients and showed promising results.(26)

Xenogeneic cells have only been tested in animal models; most of these studies used various human cells to repair injured animal IVDs.(17)

The main cells used in animal studies include stem cells, IVD cells and articular chondrocytes of autologous, allogeneic and xenogeneic sources (Table 1). Thirty-seven of the animal studies reviewed utilized stem cells. Among these, 10 studies used autologous

stem cells,(27–36) 15 studies used allogeneic cells,(25, 37–50) and 12 used xenogeneic cells (23, 51–61) (Table 1). Studies involving allogeneic and xenogeneic cells have shown good survival of these cells in the IVD, confirming that the disc niche is a relatively immunologically privileged site. In the intact IVD, there is limited blood supply to the outer 1/3 of the posterior annulus fibrosus.(62) With injury and degeneration, there is nerve and blood vessel ingrowth,(63) possibly allowing immune cells to migrate into the diseased tissues. In fact, we have observed macrophages in both the injured IVD, and injured IVD injected with allogeneic articular chondrocytes, but did not find significant differences in macrophage infiltration between the two groups.(24) The infiltration of macrophages did not appear to result in elimination of allogeneic or xenogeneic cells implanted into the disc space.

Differentiated cells used in animal studies include IVD cells and articular chondrocytes. Among the 13 animal studies using IVD cells to repair the degenerating IVD, 6 used autologous cells.(64–69) Five studies used allogeneic cells,(70–74) and 2 used xenogeneic cells.(51, 54) Among the studies using articular chondrocytes, 2 studies used allogeneic cells,(24, 25) and one study used xenogeneic chondrocytes (54) to repair the injured IVD. All studies reported some improvement of the disc structure, while allogeneic articular chondrocyte transplantation was reported to attenuate local inflammation.(24)

Mesenchymal stem cells (MSCs) from various sources (e.g., bone marrow, fat,(28) umbilical cord blood,(22) Wharton's jelly,(23, 75) olfactory stem cells(42)) or induced *pluripotent stem cells* (76) have also been investigated for repairing the degenerate IVD. While readily available, MSCs may suffer from overt cell loss when implanted in the undifferentiated state, due to inability to survive in the harsh, nutrient-poor environment.(77) Induced pluripotent stem cells from autologous or allogeneic sources are very attractive.(78, 79) There are concerns over teratoma formation in the disc space, a consideration that needs further examination.

There are only limited direct comparisons between the outcomes of IVDs treated with stem cells, differentiated disc cells or articular chondrocytes; the differentiated cells seem to be superior in producing more cartilage-like matrix.(25, 51) Further work is needed to directly compare the survival of undifferentiated stem cells, stem cells pre-conditioned to the IVD environment with biomechanical stress and hypoxia, and differentiated cells. Likewise, direct comparison of the cells' ability to attenuate local inflammation, and to improve disc structure and biomechanical function is needed.

Scaffolds

There are concerns over bone spur formation due to injury to the IVD during cell injection or leakage of the cells.(45) We have observed non-calcified cartilaginous protrusion(s) that contain injected articular chondrocytes at the needle insertion site (Figure 3). Using a scaffold seems to reduce cell leakage and osteophyte formation.(31) Among the 50 studies reviewed here, 25 used some form of scaffold. Of these, fibrin gel has been reported to reduce cell leakage.(71) Use of collagen microspheres has been shown to reduce osteophyte formation (which may be consequent on cell leakage).(31) Our preliminary data have shown

that young allogeneic articular chondrocytes (AC) injected into the center of the injured rabbit IVD survive and reduce host inflammation, but also can leak at the injection site (Figure 3). To reduce leakage and support cell growth, our group has developed hyaluronic acid (HA)-based hydrogels that preserve the chondrocytic phenotype and growth;(80–83) these materials solidify at body temperature and are thus ideal for injection-based therapies. We have also shown that hyaluronic acid hydrogels promote NP cell phenotype stability.(84) We have further tested a tripleinterpenetrating-network (TIN) hydrogel that enhances biomechanical properties of the repaired IVD and supports cell delivery.(85) Other natural and synthetic scaffold materials, including laminin,(86) pig bone gelatin and cartilage extracellular matrix,(87) collagen,(88) composite of collagen with alginate,(89) and carboxymethylcellulose (90) have been tested for engineering IVD by seeding with cells in vitro, followed by implantation into the disc space. All the above scaffolds could be modified into injectable form and used in minimally invasive cell therapy. Thus, these hydrogels should be assessed as scaffolds to prevent this leakage, and to enhance biomechanical properties of the repaired IVD in the future.

Animal models

A critical step towards the clinical translation of new therapies for IVD degeneration is testing in an appropriate *in vivo* model. Disc degeneration is a complex process involving both mechanical and biochemical factors. When considering the appropriate animal model for disc degeneration studies, the choice of species represents a balance between size, morphology, mechanical properties, nutrient diffusion, repair potential and logistical concerns. Rabbits are the most frequently used among the studies reviewed, likely reflecting the cost and size of the IVDs (Table 2). Larger animals such as the sheep, goat or pig have bigger IVDs, with shapes more similar to that of humans. However, the costs would be higher and therefore are more appropriate for definitive pre-clinical studies. Among the 50 animal studies reviewed here, 28 studies used rabbits, 7 used rats, 5 used dogs, 6 pigs, and there were 1 goat, 2 sheep and 1 mouse study (Table 2). Sheep and goat have IVDs resembling those of humans in size and absence of notochordal cells,(91) but only 3 studies out of 50 used these models. Although the authors agree that a large animal model is critical given the risk of implant ejection and subsequent catastrophic injury, large animal studies are not consistently conducted before clinical trials. However, it is our belief that study in a large animal without persisting notochord (i.e., goat or sheep) should be considered before clinical trials.

The mouse model has the advantage of opportunities for genetic manipulations (as illustrated in Figure 1), and is less costly than using larger animals. Challenges of working with the mouse IVD mainly reflect its small size: surgical precision is crucial, and the amount of tissue for molecular and biochemical assays is limited. Our group and others have developed the mouse injury model.(92, 93) In addition, we have developed microinjection methods, and use cell tracing methods to confirm precision injection (Figure 4). Various volumes of protein labeled with infrared (IR) dye have been injected into the degenerating IVD in the mouse tail. Tail IVD degeneration has been induced with a needle puncture. It is worth noting that injecting into the intact IVD is exceedingly difficult, due to the positive

pressure within the disc. Further refinement of the mouse model is our priority and an important future direction.

Role of inflammation in IVD degeneration and back pain

IVD degeneration is a slowly progressing cascade mediated in part by inflammation.(94) Inflammatory stimulation directly alters the mechanobiology of NP cells (95) and inhibits cell extracellular matrix (ECM) production.(96, 97) Inflammation in the IVD tissues is also increasingly recognized to be associated with back pain.(11, 12, 98–100) More recently, serum biomarkers have been reported that vary with diagnosis.(101, 101, 101, 101, 102, 102) In particular, serum levels of IL-6 were significantly higher in subjects with LBP compared with control subjects.(101, 102) Novel treatments targeting inflammation are being pursued.(103, 104) The high levels of proinflammatory mediators found in disc tissue from patients undergoing fusion for discogenic back pain suggest that production of proinflammatory mediators within the IVD may be a major factor in the genesis of a painful lumbar disc.(11, 12, 98, 99) These findings strongly suggest that in addition to the commonly used histology and extracellular matrix composition, inflammatory markers may be used as outcome measures in response to cell therapy. However, among the 50 studies, only 2 examined local inflammation.(24, 41) All the clinical trials used patient symptoms as outcome measures. Future work should focus on identification of inflammatory mediators as outcome measures.

Benchmark for success

None of the above animal studies or clinical trials completely restored IVD structure. Although mechanical function changes in degenerative discs have been well documented, (105) whether cell therapy can restore biomechanical function has not been previously determined. In light of the fact that patients primarily seek medical care for back pain, attenuating local inflammation should be a priority amongst benchmarks for success. The ideal therapy should also be minimally invasive, and concurrent with other procedures such as discography or discectomy. Restoration of tissue structure and biomechanical function are important, and preservation of spinal motion is also desirable.

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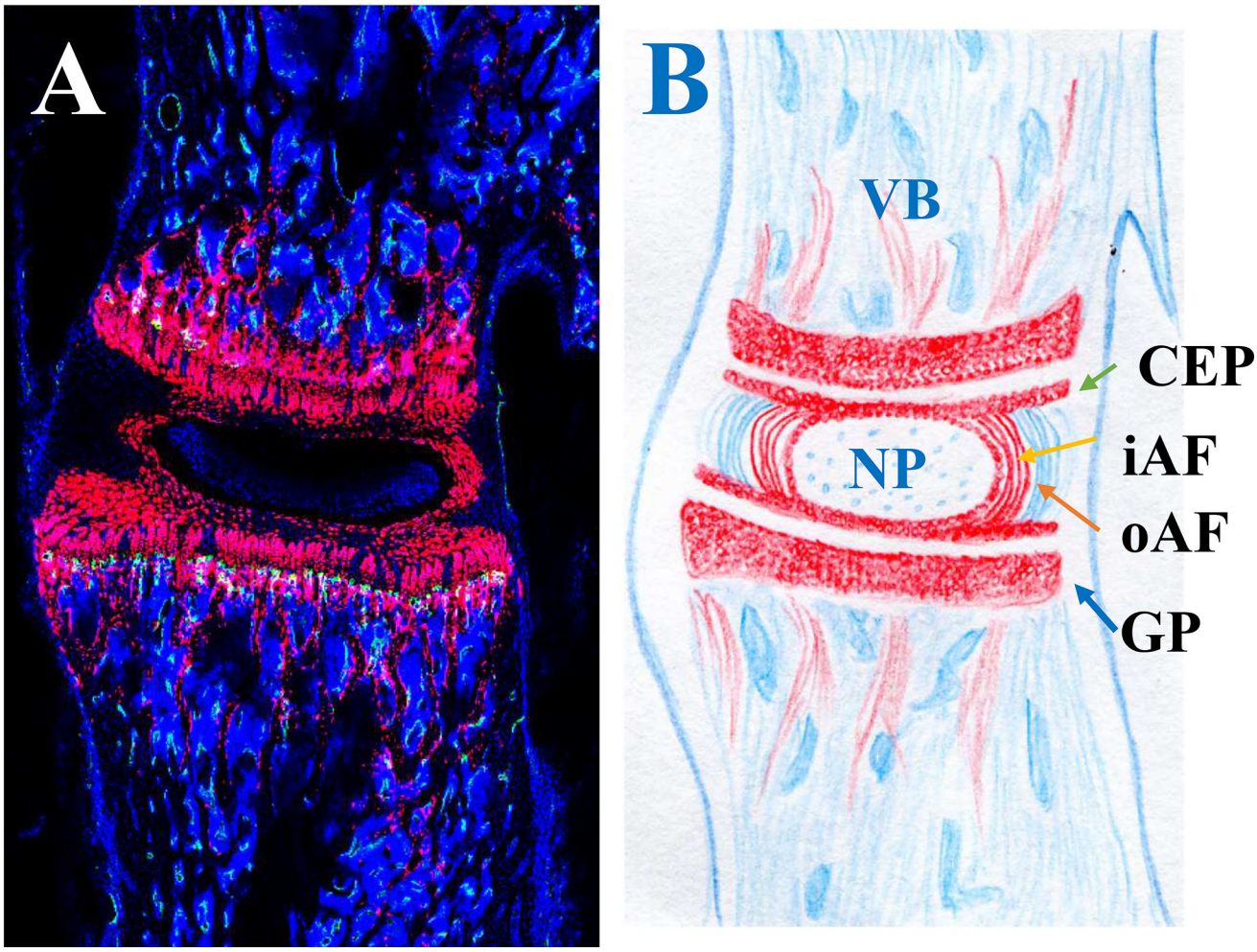

Figure 1. Mouse lumbar intervertebral disc (IVD)

A. Sagittal section of a *Col2CreER;R26- tdTomato* mouse IVD. **B**: schematic drawing of the vertebral body (VB)-IVD-VB motion segment. Red: type II collagen expressing cells; Blue: cell nuclei stained with DAPI. NP: nucleus pulposus; CEP: cartilaginous endplate; iAF: inner annulus fibrosus (AF); oAF: outer AF; GP: growth plate.

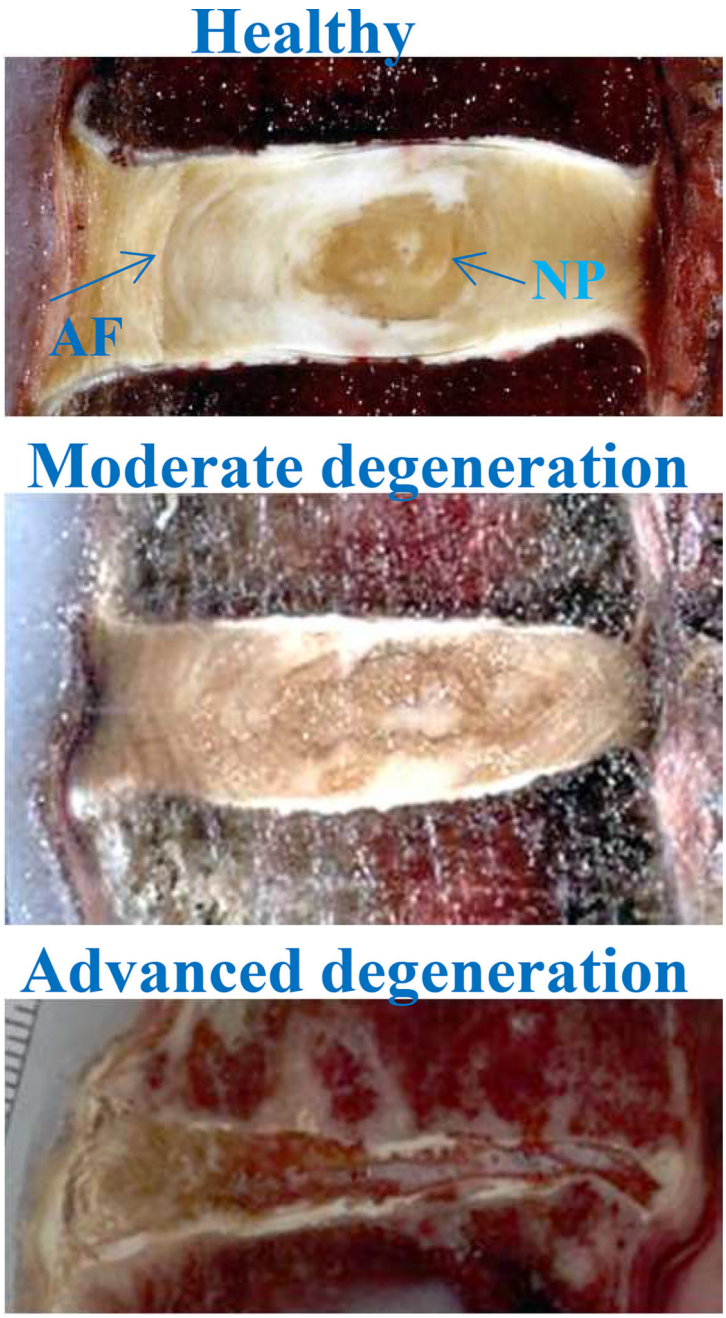

Figure 2. Gross morphology illustrating progressive human disc degeneration
NP: nucleus pulposus; AF: annulus fibrosus.

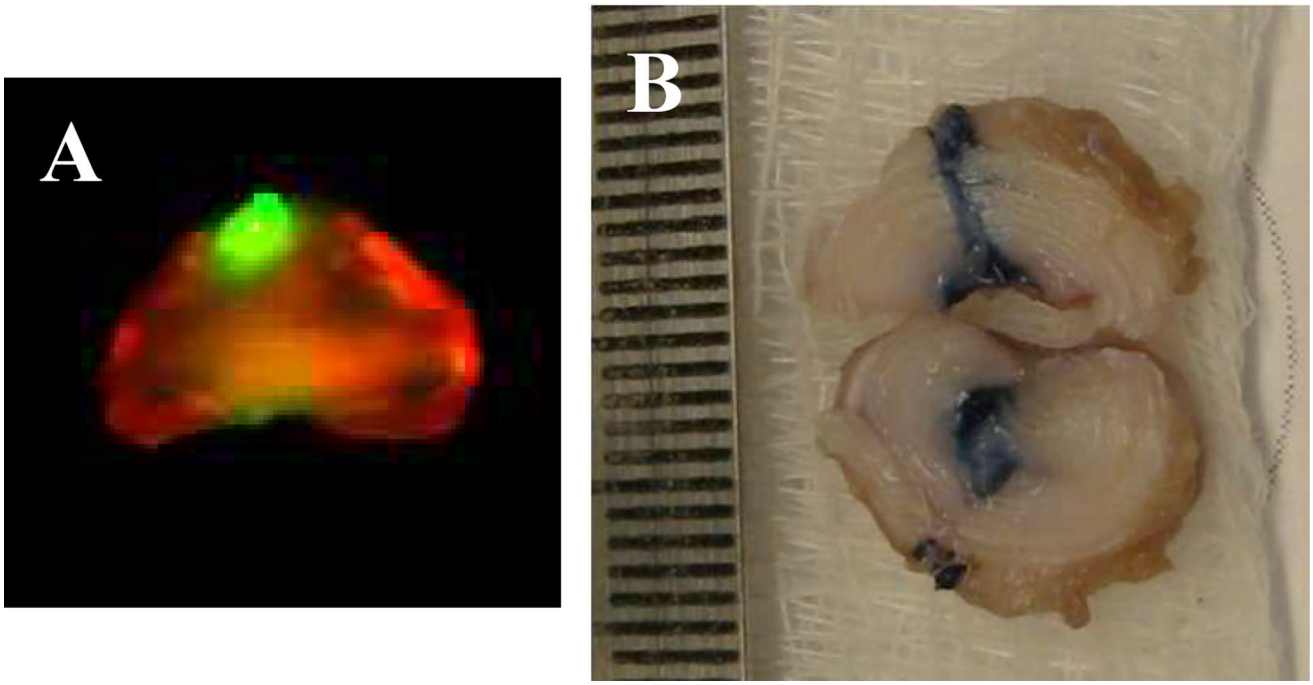

Figure 3. Rabbit intervertebral disc injected with chondrocytes labeled with infrared dye and transduced with adenovirus expressing β-galactosidase

A: infrared scan; B: X-gal stain. Ruler in right panel is 1mm/space.

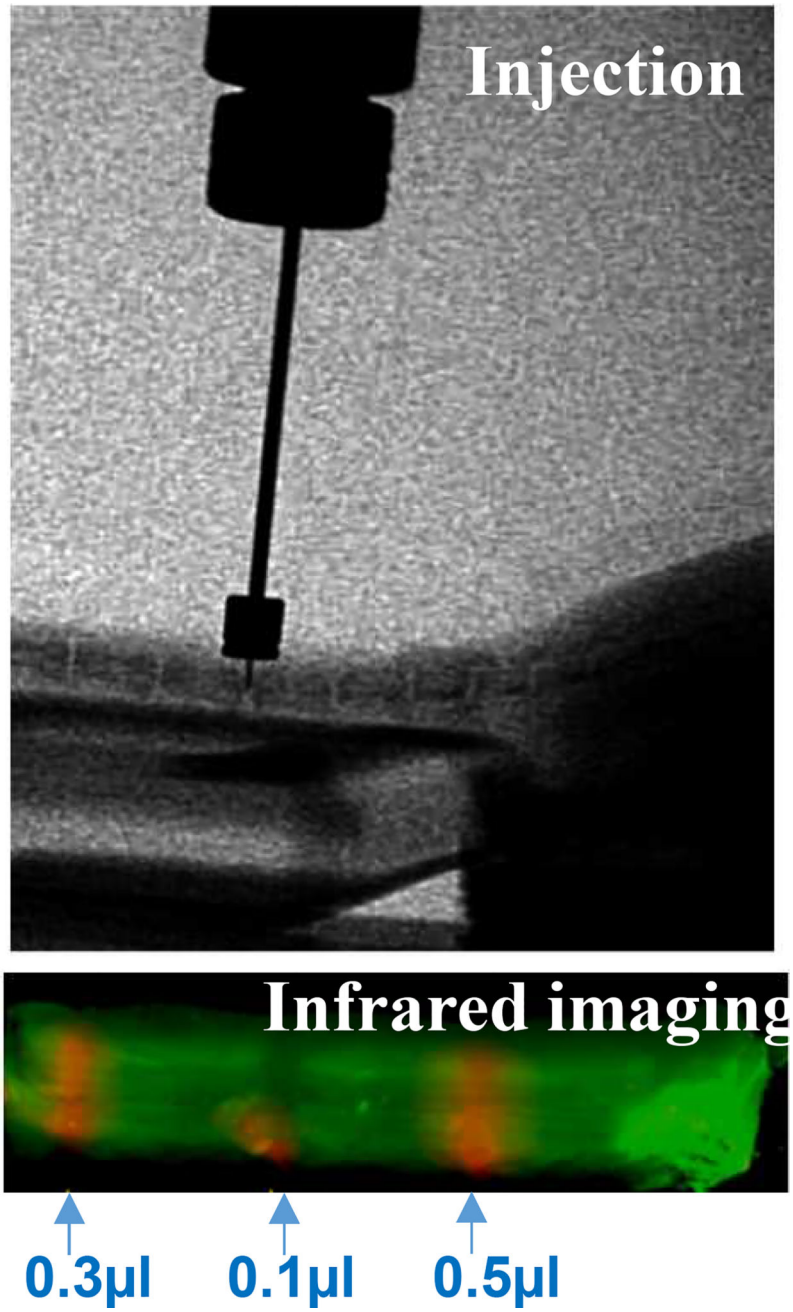

Figure 4. Protein labeled with infrared (IR) dye was injected into the degenerating mouse tail IVD

Table 1

Cell Sources and Types Used in Animal Models.

Cell Source	Autologous	Allogeneic	Xenogeneic	Total Studies
Stem Cells	10	15	12	37
Intervertebral Disc Cells	6	5	2	13
Articular Chondrocytes	0	2	1	3

Table 2

Animal Models.

Animal Models	Mouse	Rat	Rabbit	Pig	Dog	Goat	Sheep
Number of Studies	1	7	28	6	5	1	2