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REVIEW

Mesenchymal stem cell tracking in the intervertebral disc

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Abstract

Low back pain is a common clinical problem, which leads to significant social, economic and public health costs. Intervertebral disc (IVD) degeneration is accepted as a common cause of low back pain. Initially, this is characterized by a loss of proteoglycans from the nucleus pulposus resulting in loss of tissue hydration and hydrostatic pressure. Conservative management, including analgesia and physiotherapy often fails and surgical treatment, such as spinal fusion, is required. Stem cells offer an exciting possible regenerative approach to IVD disease. Preclinical research has demonstrated promising biochemical, histological and radiological results in restoring degenerate IVDs. Cell tracking provides an opportunity to develop an in-depth understanding of stem cell survival, differentiation and migration, enabling optimization of stem cell treatment. Magnetic Resonance Imaging (MRI) is a non-invasive, non-ionizing imaging modality with high spatial resolution, ideally suited for stem cell tracking. Furthermore, novel MRI sequences have the potential to quantitatively assess IVD disease, providing an improved method to review response to biological treatment. Superparamagnetic iron oxide nanoparticles have been extensively researched for the purpose of cell tracking. These particles are biocompatible, non-toxic and act as excellent MRI contrast agents. This review will explore recent advances and issues in stem cell tracking and molecular imaging in relation to the IVD.

Key words: Intervertebral disc; Stem cells; Cell tracking; Magnetic resonance imaging; Intervertebral disc degeneration

Core tip: Mesenchymal stem cell (MSC) transplantation shows exciting promise for the future regenerative approach to intervertebral disc (IVD) disease. Extensive preclinical research has demonstrated benefits from MSC treatment in disc degeneration. Cell tracking, with iron oxide nanoparticles and MRI, provides an opportunity to develop an in-depth understanding of stem cell survival, differentiation and migration, enabling optimization of stem cell treatment. This review summarizes the current literature relating to MSC tracking in the IVD, which is limited to short term monitoring. Medium to long-term cell tracking is required to accelerate translation of MSC treatment in the IVD to clinical practice.

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INTRODUCTION

Low back pain is the leading cause of disability in the developed world[1]. Lifetime prevalence is 75%-80%[2], with the annual cost in the United States alone estimated to be as high as $500 billion[3]. Low back pain is strongly linked to disc degeneration, with a two-fold increase in chronic lower back pain in patients with radiological evidence of degeneration[4,5].

Current treatments, including analgesia, physiotherapy and spinal fusion only address symptoms, not the underlying disease. Regenerative strategies, such as stem cell therapy, provide an exciting future in the treatment of intervertebral disc (IVD) disease. Tracking and long term monitoring of these cells is essential to develop an understanding of their survival, migration, proliferation and differentiation *in vivo,* which will enable optimization of this promising therapy. Magnetic resonance imaging (MRI) combined with contrast agents is the modality of choice for cell tracking. This review will summarize recent advances in stem cell tracking, current problems and their application to the treatment of IVD disease.

PATHOLOGY OF BACK PAIN

The intervertebral disc is composed of 3 main regions: the tough annulus fibrosis (AF), peripherally, the amorphous nucleus pulposus (NP), centrally, and cartilaginous endplates which bind the disc to the adjacent superior and inferior vertebral bodies[6]. With ageing and degeneration, significant cellular and matrix changes occur within the IVD. An early hallmark of disc degeneration is the loss of proteoglycans (PG), and associated water molecules, from the NP and AF, accompanied by structural changes to the lamellae of the AF[7-11]. With the loss of the water binding PGs from the disc, its functional capacity as a hydroelastic cushion is diminished, leading to additional mechanical stresses acting on the fibrocartilagenous AF. With time, these events can result in the presence of concentric and radiating tears in the lamellae of the AF that may eventually extend into the NP[7,8,11-13]. In addition, there is emerging evidence that damage to the cartilaginous endplate plays a role in the pathophysiology of degenerative disc disease, up-regulating matrix degrading enzymes and inflammatory cytokines in the NP[14]. Structural deficiencies in the NP are considered to provoke neovascularisation and growth of nerve fibers, normally confined to the periphery of the AF, to the deeper regions of the disc[13,15]. The establishment of these extended nerve fibers has been cited as a major cause of chronic lower back pain in degenerate discs[15-17].

CURRENT TREATMENT OF BACK PAIN

Current strategies to treat low back pain fail to regenerate the intervertebral disc or even reverse the degenerative process. Analgesics, non-steroidal anti-inflammatory drugs, physical therapies and other multimodal palliative modalities represent the mainstay of conservative therapy for low back pain[18-20]. There is no evidence, however, that any of these therapies provide long-term benefit by improving the underlying pathobiology of disc degeneration. In fact, recent research has demonstrated simple analgesia does not improve recovery time from acute low back pain[21]. When non-operative treatments fail, surgical interventions such as spinal fusion or total disc arthroplasty are commonly undertaken. These interventions aim to remove the pain generator, however, many patients remain with chronic pain and disability. Furthermore, spinal fusion has biomechanical consequences, which accelerates degeneration at adjacent levels[22-24].

REGENERATIVE STRATEGIES FOR THE INTERVERTEBRAL DISC

Recent research suggests novel biological therapies can provide restorative treatment of disc degeneration. Cell types investigated include NP cells[25-29], chondrocytes[30-33] and mesenchymal stem cells (MSC)[29,30,34-43]. MSCs, initially isolated from bone marrow, can also be derived from a range of tissues, including adipose and synovial[44,45]. In addition, MSCs are reported to be non-immunogenic and, in contrast to embryonic stem cells, cannot undergo malignant transformation[46,47]. Moreover, MSCs have the capacity for self-renewal, enabling maintenance of an undifferentiated phenotype in multiple subcultures[48]. However, in the appropriate environment, MSCs are capable of differentiating into multiple cell types including chondrocytes, osteocytes, tenocytes and adipose cells[44,45].

There are numerous preclinical studies investigating the use of MSCs in rat, rabbit, goat and porcine models[29,30,34-43]. MSC implantation in the rabbit model has resulted in an increase in PG content, partial restoration of disc height and disc hydration[42,43]. In the ovine model, intra-discal MSCs treatment has been shown to restore disc extracellular matrix, increase disc height and reduce radiological and histological grading scores 6 mo following injection[49,50]. How stem cells produce these effects remains unclear from current preclinical studies.

Early clinical research has been promising. Yoshikawa *et al*[51] reported two cases of autologous MSC implantation in markedly degenerate intervertebral discs. Symptomatic and radiological improvement was demonstrated without significant adverse effects[51]. A larger series of 10 patients treated with autologous MSCs demonstrated improvement in pain, disability and disc hydration[52]. Prior to translation to clinical practice, greater understanding of the mechanism of action of these cells is required.

NEED FOR CELL TRACKING

Longitudinal tracking of MSCs is necessary to ensure survival, evaluate distribution and assess if cells migrate to affected pathological sites in the IVD. Previous methods,

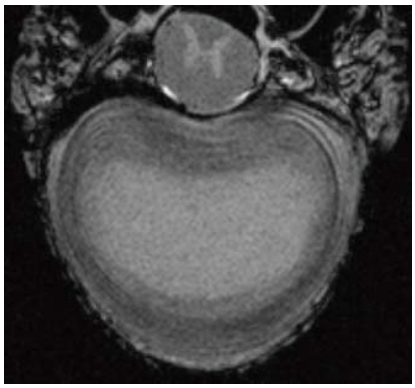

Figure 1 9.4 Tesla magnetic resonance imaging-Gradient Echo 2* weighted image of ovine lumbar intervertebral disc (normal control). Image obtained from our group 2014/02/18, not previously published.

based on histological analysis provide a snapshot view of the cells at a set time point. This is useful at the end of a scientific study but these methods cannot be translated to the clinical setting. Cellular labeling provides a non-invasive technique to assess the cells *in vivo* at any given time point. Thus, it can assess cell viability, track cell migration patterns and provide some information on efficacy. It may provide an understanding on mechanism of action, for example, potentially being able to determine whether cells differentiate into chondrocytic cells or act to modulate the resident native cell population through paracrine actions. In addition, cell tracking is required to ensure MSCs retention, as leakage of transplanted cells outside the disc has been reported to induce osteophyte formation[53].

CURRENT IMAGING TECHNIQUES

In vivo, non-invasive techniques are required for cell tracking in the research and clinical setting. Multiple imaging modalities have been utilized, including MRI[54-58], positron emission tomography (PET)[59-62], single photon emission computed tomography[63], bioluminescence imaging[64,65], fluorescence imaging[66-72] and computed tomography (CT)[73]. MRI has been accepted as the best modality given its high spatial resolution (< 100 microns), prolonged effective imaging window, lack of ionizing radiation and ability to provide detailed anatomical information[74,75]. There are now very high resolution MRI machines available such as 9.4 Tesla which provides high quality images (Figure 1).

CELL LABELING FOR MRI

The ideal contrast agent would be highly sensitive, biocompatible, non-toxic, easily taken up by the targeted cells and provide clear contrast between the labeled cells and surrounding tissue. Due to their superior sensitivity and excellent biocompatibility, iron oxide nanoparticles are the preferred contrast agent for cell labeling[76]. Superparamagnetic iron oxide nanoparticles (SPIONs) consist of an iron oxide core, usually magnetite (Fe_3O_4) or maghemite (γFe_2O_3) and a biocompatible coating. Coating substrates include dextran, carboxydextran, polyethylene glycol (PEG), polystyrene and silica[77]. Iron oxide nanoparticles can be subdivided into standard SPION, 60 to 150 nm in size, and ultrasmall superparamagnetic iron oxide nanoparticles (USPION) which measure 10 to 20 nm[78,79]. The type of coating, size and method of synthesis affect the SPIONs biocompatibility and magnetic properties[80].

MRI generates images by utilizing the differences in proton density and the local magnetic environment of hydrogen atoms[75]. There are two MR relaxation time constants, T1 and T2. Commonly used contrast agents, paramagnetic gadolinium analogues, alter longitudinal (T1) relaxation time of hydrogen protons and appear hyperintense. The abovementioned, superparamagnetic nanoparticles, affect the transverse (T2) relaxation time of hydrogen protons and appear hypointense. Due to their size, USPION demonstrate additional T1 effects[75]. Iron oxide nanoparticles can be detected at micromolar concentrations and offer sufficient sensitivity to be identified on T2* weighted imaging[81].

MR cell labeling requires the transfer of particles from extracellular to intracellular. The simplest method is spontaneous uptake of particles by phagocytic cells such as macrophages. While enhanced with an appropriate biocompatible coating, iron oxide nanoparticles are not efficiently taken up by stem cells[82,83]. However, labeling can be facilitated by incubation with cationic transfection agents, including poly-L-lysine and protamine sulfate[82,84-86]. If extended incubation time is not appropriate, other rapid techniques, magnetoelectroporation or magnetosonoporation, can be employed[87,88].

A number of contrast agents have been approved for clinical use in medical imaging. Previously, the most commonly used iron oxide nanoparticle for cell labeling was Feridex®, which contains an iron oxide core and dextran coating[77]. Dextran coated SPIONs have been shown to be biocompatible and biodegradable *via* iron metabolism through Kupffer cells, located in the liver[89]. Another widely used SPION, Resovist®, has a carboxydextran coating[90,91]. Both these products have been discontinued from production by the pharmaceutical companies[77,92]. Other commercial products continue to be utilized, such as SiMAG®, an SPIO with an unmodified silica surface. For example, Markides *et al*[93] labeled MSCs with SiMAG® in a rheumatoid arthritis mouse model.

Extensive research has been devoted to designing novel iron oxide nanoparticles for the purpose of stem cell labeling[92]. van Buul *et al*[94] demonstrated ferumoxides (Endorem®) complexed with protamine sulfate are superior to ferucarbotran particles for cell labeling. Subsequently, this group demonstrated safety and efficacy of the ferumoxide-protamine sulfate complex for MSC labeling in articular cartilage repair[95]. USPION have also been investigated recently. Coated with dextran and PEG and combined with protamine sulfate, USPIONs have been cultured with human Adipose Derived Stem Cells (hADSCs) within a three dimensional scaffold[96]. *In vitro*, no effect on cell viablilty or osteogenic differentiation was seen from cell

labeling. The USPIONs were effectively internalized by the hADSC and demonstrated T2* signal change. Hypointense regions, representing labeled cells, were seen *in vivo* 28 d following implantation[96]. Further research is required to optimize SPIONs for cell tracking.

ISSUES WITH CELL LABELING

Transfection agents are potentially toxic and, furthermore, there is capacity for iron oxide nanoparticles *in vivo* to cause toxicity to other organs, including liver and spleen[97,98]. Small polyhedral SPIONs with a silica coating have shown efficient MSC labeling without the need for a transfection agent and may offer a solution[99].

T2 signal change is due to the overall effect of magnetic nanoparticles rather than total number of cells[100]. Typically, a few hundred cells are required for detection with conventional MRI sequences[77]. Stem cells are known to proliferate following transplantation, leading to dilution of the iron oxide label and loss of MR signal over time[77]. If cells divide asymmetrically, with one daughter cell receiving the majority of nanoparticles, rapid dilution of signal can occur to an undetectable level[101]. Labeled cells could also become undetectable if they migrate in small rather than large groups. Sensitivity may be improved with post acquisition software analysis or a higher magnetic field strength.

A number of endogenous substances produce negative (or hypointense) MR signal, such as blood products containing haemosiderin or methaemoglobin. This leads to challenges differentiating blood product from labeled cells in an injured IVD. Novel MRI methodology has been adopted to help differentiate the labeled cells from endogenous substances, such as Inversion-Recovery With ON-Resonant Water Suppression, which delineates SPION labeled cells as positive contrast[102]. Further novel sequences are being developed to provide an exciting possibility to enhance non-invasive cell tracking.

Iron oxide nanoparticles fail to differentiate between live and dead cells. SPION signal has been demonstrated in the CNS long after cell death[103]. Multimodal imaging may be required to ensure cellular function, such as combining MRI with PET imaging. A study investigating iron oxide labeled stem cells in hemi-Parkinsonian rats used this multi modal technique. MRI visualized stem cells in the striatum and PET confirmed cellular viability[104].

CELL LABELING IN THE INTERVERTEBRAL DISC

To date, there is limited published research tracking MSCs in the IVD and this is summarized in Table 1. Saldanha *et al*[105] demonstrated feasibility by imaging MSCs labeled with SPION (Feridex®) *in vitro* to quantitatively characterize signal intensity loss using T1, T2 and T2* relaxation parameters. T2* weighted gradient echo (GRE) images demonstrated the most significant loss of signal intensity from labeled cells. Conversely, SPION labeled cells were indistinguishable from unlabeled cells on T1 weighted imaging[105]. This group also demonstrated SPION labeled cells, loaded in a fibrin gel and injected *via* fluoroscopic guidance, could be identified *ex vivo* within the IVD of excised rat tails[105]. Further research by Prologo *et al*[62] imaged MSCs labeled with a radioactive marker (iodine-124 2'fluoro-2'-deoxy-1β-D-arabinofuranosyl-5-iodouracil) using CT and PET. Four female pigs had approximately 100000 labeled MSCs injected under fluoroscopic guidance to the NP of discs which had degeneration induced 10 d prior. CT and PET were performed immediately following and three days after cell delivery. One animal was inaccurately injected. Results from the other three demonstrated accurate delivery and maintenance of labeled cells at three days[62]. While demonstrating useful preclinical results, using radioactive cell labeling in conjunction with CT and PET requires a significant amount of ionizing radiation, a consideration if used in clinical practice.

Recently, Barczewska *et al*[106] developed a technique for real time non-invasive monitoring of minimally invasive MSC delivery. IVD degeneration was induced in porcine discs of 3 animals *via* a fluoroscopic guided IVD vaporization procedure. Subsequently, the animals were placed in a 3T MRI scanner and 3×10^6 SPIO labeled MSCs were injected into the IVD through a plastic catheter in two divided injections. T2 weighted imaging was performed prior to, and following, each injection. Results demonstrated a statistically significant difference in signal intensity between both SPIO labeled MSCs compared with unlabeled MSCs and SPIO compared with control discs[106].

Although limited, current research has demonstrated MRI is a suitable modality for detecting SPION labeled MSCs in the IVD. Current data, utilizing both MRI and CT/PET, provides only short term tracking of labeled cells, demonstrating adequate initial placement of transplanted cells. Further research is required to monitor long-term retention of MSCs in the IVD. Moreover, longitudinal tracking of injected cells is required to improve understanding of the therapeutic mechanism of action of MSCs in IVD disease.

ADDITIONAL BENEFITS OF MRI IN CELL TRACKING

In addition to monitoring labeled MSCs, MRI has the potential to non-invasively measure response to biological therapies in IVD disease. Several techniques have been developed to classify disc degeneration by MR image criteria[107-110]. All are based on conventional T2 weighted sagittal imaging characteristics, which correspond to later morphological changes in IVD degeneration. The Pfirrmann classification system is the most widely used in the literature, utilizing an algorithm to generate five morphological grades[107]. Research has demonstrated acceptable intra and inter-observer variability for grading systems[107,109]. Ultimately, however, each is based on qualitative assessment and prone to inter-observer

Table 1 Review of stem cell tracking in the intervertebral disc in animal models reported in the literature

Ref.	Animal model	Cell type	Label	Imaging	Results
Saldanha *et al*[105], 2008	Rat tail (*ex vivo*)	Human MSCs (Loaded in fibrin gel-Tisseel)	FE-Pro (Feridex and Proatmine Sulphate)	3T MRI	No significant effect of labeling on MSC viability Hypointensity (signal loss) seen in discs injected with labeled cell
Prologo *et al*[62], 2012	Porcine $n = 4$	Human MSCs	Iodine-124 2′fluoro-2′-deoxy-1b-D-arabinofuranosyl-5-iodouracil	PET/CT	Inaccurate delivery in 1 animal Three animals showed persistence and containment of labeled cells 3 d following injection
Barczewska *et al*[106], 2013	Porcine $n = 1$ (*ex vivo*) $n = 3$ (*in vivo*)	Porcine MSCs	Molday ION USPION	3T MRI	*Ex vivo*-Injected labeled cells clearly visualized *In vivo*-hypointense regions identified immediately following injection of labeled cells. Histopathology performed 2 wk later confirmed the presence of MSCs in the disc

MSC: Mesenchymal stem cell; MRI: Magnetic resonance imaging; USPION: Ultrasmall superparagmetic iron oxide nanoparticle; PET/CT: Positron emission tomography/computed tomography.

variability. In addition, conventional MRI sequences lack sufficient sensitivity to identify early disc biochemical changes[111].

Novel MRI sequences have the potential to quantitatively assess early IVD degeneration. T1ρ MRI uses long duration, low power radiofrequency, or spin-lock (SL) pulses, applied on-resonance to lock magnetization in the transverse plane. Spin-locked magnetization relaxes with the time constant T1ρ, spin lattice relaxation in the rotating frame[112,113]. Post acquisition computer software analysis can be utilized to generate a quantitative T1ρ measurement for a region of interest, such as the NP. T1ρ has been shown to be sensitive to PG content in articular cartilage and the IVD[114,115]. Furthermore, Borthakur *et al*[112] demonstrated T1ρ values in IVDs correlate with low back pain. Another quantitative method recently developed is the NP voxel count, which has been shown *in vivo* to quantitatively assess disc degeneration. This assesses nuclear size and hydration with T2-relaxation time measurements[116]. These imaging techniques have the potential to non-invasively, quantitatively, monitor response to biological treatments, as well as diagnose early IVD degeneration.

SUMMARY AND EXPERT OPINION

MSC transplantation shows exciting promise for the future regenerative approach to IVD disease. Cell labeling techniques are integral to ongoing research, to ensure accurate delivery and long-term retention of cells. Current research is limited to only short term monitoring. A greater understanding of the mechanism of action is required, with longitudinal tracking of implanted MSCs. Further research is required to discover if cells remain in situ long term, if they remain viable and if they track to sites of pathology in the IVD. MRI, with SPIONs as contrast agents, provides an excellent imaging modality for cell tracking. Some issues remain, such as dilution of signal through cell division or migration and differentiating labeled cells from intrinsic negative signal. These may be addressed with stronger magnetic fields, post-processing analysis and novel MRI techniques. Furthermore, novel MRI sequences can non-invasively quantify early biochemical changes in disc degeneration. In conjunction with conventional MRI, this provides a mechanism for measuring response to biological therapy, in both the research and clinical setting. Ongoing effective research will facilitate the translation of MSC treatment for IVD disease to clinical practice.

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