Non-Invasive Imaging of Therapeutic Cells Delivered Percutaneously to the Mouse Intervertebral Disc

Chenghao Zhang
Sun H. Peck
Justin R. Bendigo
Yian Kai A. Lau
Neil R. Malhotra
Lachlan J. Smith

Departments of Neurosurgery and Orthopaedic Surgery
University of Pennsylvania

Introduction

Intervertebral disc degeneration is strongly implicated as a cause of low back pain, and cell-based therapies hold significant promise for disc regeneration.[1] While large animal models are a requisite preclinical step prior to clinical translation of cell-based disc therapies, mouse models represent an important intermediate *in vivo* platform, as genetic manipulation permits mechanistic studies of cell-mediated regeneration. The small size of the mouse disc, however, poses technical challenges with respect to surgical technique and, consequently, experimental reproducibility. Our group recently described a percutaneous needle-injury model of moderate severity disc degeneration in the mouse caudal spine suitable for evaluating cell-based regenerative therapies.[2] As a prerequisite for using this model in studies of cell-based disc regeneration, the objectives of the current study were to: 1) establish a technique for percutaneous delivery of therapeutic cells to the mouse caudal disc; and 2) validate a technique for non-invasive quantitative tracking of injected cells.

Methods

With institutional IACUC approval, mesenchymal stem cells (MSCs) were isolated from the bone marrow of adult C57BL/6 mice using established techniques[3], expanded through 3 passages, and labeled with cell tracker red (Thermo Fisher). Using standard aseptic technique and under general anesthesia, and ~2-5x10^4 MSCs were suspended in saline were injected percutaneously into the nucleus pulposus (NP) of 3 caudal discs (C7-8, C9-10 and C11-12) of 5 adult male C57BL/6 mice (15 discs total) using a 33G needle under fluoroscopic guidance. Mice were euthanized at 1, 3, 5, 7 and 14 days post-injection. As a negative control, 1 additional mouse received injection of unlabeled MSCs into 3 caudal discs, while another served as an un-injected control, both euthanized after 1 day. Immediately following euthanasia, intact tails were imaged using an IVIS Spectrum (Perkin Elmer) imaging system. Mean fluorescence intensity was calculated for each injected disc for a 4mm diameter region of interest centered on the point of peak fluorescence intensity. Whole discs were isolated via sharp dissection and stained with Hoechst to visualize cell nuclei. Discs were then imaged through their complete thickness in the axial plane using confocal microscopy to assess both total cells (blue fluorescence) and injected cells (red fluorescence) in the NP. The NP boundary was identified using Photoshop (Adobe) and the relative area occupied by injected (red) cells was then determined using ImageJ (NIH) on a z-projection image. Linear correlation between the mean fluorescence intensity for each disc as determined via IVIS imaging, and NP area occupied by injected cells determined via confocal microscopy was evaluated ($p<0.05$).

Results

All animals successfully underwent surgery and survived to the study endpoints. MSCs labeled with cell-tracker could be detected using IVIS imaging (Figure 1) at all time points post injection, with mean fluorescence intensities for injected discs ranging from 0.50 to 6.02 $\times$ 10^7. No fluorescence signal was evident for either discs injected with unlabeled MSCs, or un-injected control discs (Figure 1). Confocal imaging clearly demonstrated the presence of injected MSCs in the NP (red fluorescence; Figure 2). The area occupied by injected MSCs ranged from 3.0 to 33.5% of the total NP area. There was a moderate, significant correlation between mean fluorescence intensity of injected MSCs measured non-invasively using IVIS imaging, and area occupied by injected MSCs measured using confocal microscopy ($r = 0.65$, $p = 0.008$; Figure 3).

Discussion

The small size of the mouse intervertebral disc makes surgical delivery of cells extremely challenging. The percutaneous technique applied here does not require an open incision and therefore minimizes local inflammation and scar tissue formation that could confound results. The non-invasive imaging technique validated here to track cells post-injection can be used to account for a variable number of cells injected by establishing a baseline against which longitudinal changes in cell survival and localization can be compared. Further, non-invasive imaging that does not require animal sacrifice may facilitate a reduction in animals

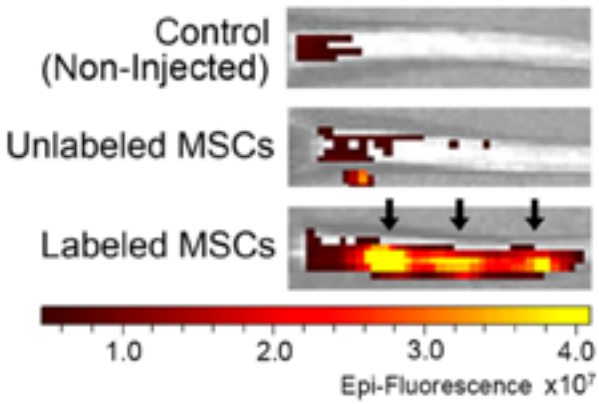

Figure 1. Non-invasive assessment of cell tracker-labeled MSCs delivered to the C7-8, C9-10 and C10-11 (arrows) mouse caudal intervertebral discs using the IVIS imaging system 1 day post-injection. No fluorescent signal is evident for control (non-injected) discs or discs injected with unlabeled MSCs.

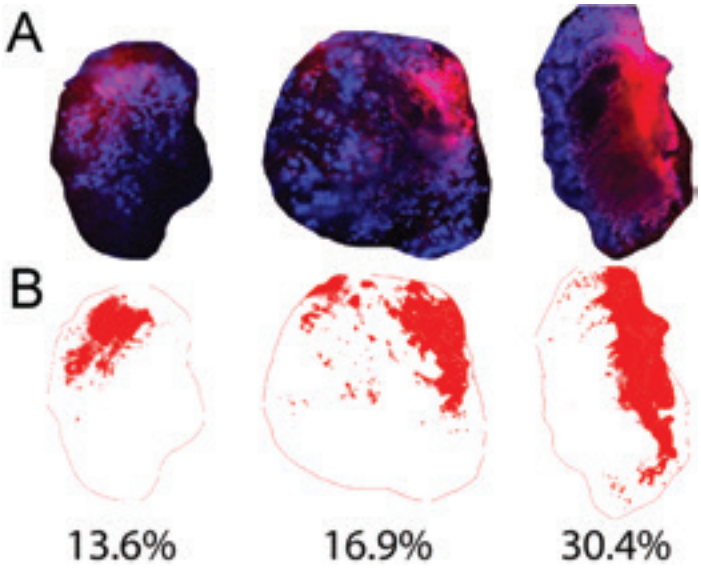

Figure 2. Nucleus pulposus area occupied by MSCs as determined by confocal microscopy of whole, isolated mouse intervertebral discs. **A.** Representative confocal images showing all cells (Hoechst, blue) relative to injected MSCs (cell tracker, red). **B.** Relative area occupied by injected cells determined using ImageJ.

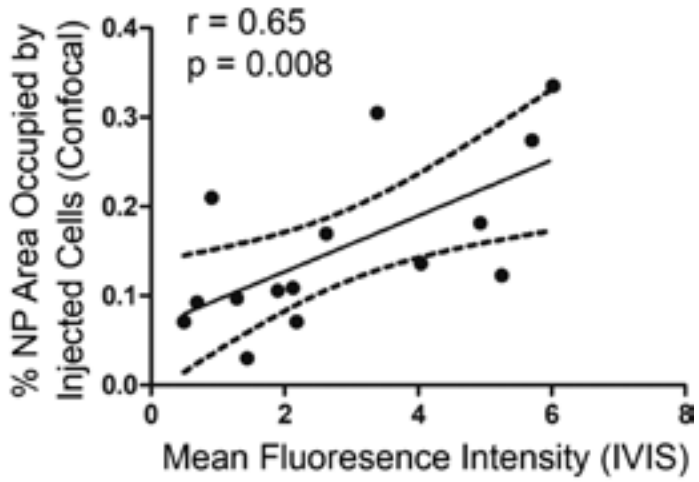

Figure 3. Correlation between mean fluorescence intensity of injected MSCs measured non-invasively using IVIS imaging, and NP area occupied by injected MSCs measured using confocal microscopy.

required for *in vivo* experimentation. Ongoing studies will apply these techniques to evaluate the regenerative effects of therapeutic cell types including MSCs and notochordal progenitor cells.

Significance

This study establishes techniques for percutaneous delivery of therapeutic cells to the mouse intervertebral disc and for subsequent non-invasive imaging of those cells.These techniques can be applied to conduct mechanistic studies of cell-based intervertebral disc regeneration in mice.

References

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3. Soleimani M and Nadri S. A protocol for isolation and culture of mesenchymal stem cells from mouse bone marrow. *Nat Protoc* 2009; 4(1): 102-6.