CARRIER MATRIX VISCOSITY INFLUENCES MESENCHYMAL STEM CELL DELIVERY TO THE INTERVERTEBRAL DISC

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Introduction
Intervertebral disc (IVD) degeneration is an important and direct cause of spinal pathologies that account for most neck, mid-back and low back pain. Spinal pathology related to intervertebral disc degeneration is the most common cause of disability in patients age 20-50, and greatest healthcare cost for that age group. IVD repair has recently been the focus of much research, with techniques such as gene therapy and autologous disc cell injection being explored to retard or even reverse the degenerative process (1,2). Attempts to treat degenerated discs with allogenic pluripotent cells are currently underway. The purpose of this experiment is to identify problems particular to the delivery and retention of exogenous cells within the IVD. We questioned whether cells delivered into a pressurized site (like the IVD) require a viscous carrier to prevent post-delivery extrusion.

Methods
A. In-vitro experiment
Murine mesenchymal stem cells were harvested from the bone marrow of adult mice. The marrow was partially fractionated, then plated in Mesencult medium (Stem Cell Technologies) and incubated at 37°C for one day. The medium was then washed away, and the adherent cells (of mesenchymal origin) were resuspended in phosphate-buffered saline (PBS), agarose, sodium alginate, or thrombin-fibrinogen gel. The cell suspensions were injected into a transparent fixture containing pressurized 2% agarose to visualize their trajectory during the injection (Figure 1).

Figure 1: Injection Fixture

The test fixture consisted of two acrylic plates surrounding an agarose gel. It was pressurized by tightening the bolts or load application with a servo-hydraulic materials testing machine. The pressure was measured using a pressure-tip catheter (Medical Measurements). A table summarizing the conditions and matrices of the in-vitro experiments is shown below.

<table>
<thead>
<tr>
<th>Injection matrix</th>
<th>Fixture loading condition during injection</th>
<th>Loading after injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>0 MPa</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>0.03 MPa</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>0.1 MPa</td>
<td>none</td>
</tr>
<tr>
<td>0.5% agarose</td>
<td>0.03 MPa</td>
<td>none</td>
</tr>
<tr>
<td>1% agarose</td>
<td>0.03 MPa</td>
<td>none</td>
</tr>
<tr>
<td>2% agarose</td>
<td>0.03 MPa</td>
<td>none</td>
</tr>
<tr>
<td>Cured Na Alginate</td>
<td>0.03 MPa</td>
<td>1.0 MPa</td>
</tr>
<tr>
<td>Thrombin-fibrinogen gel</td>
<td>0.03 MPa</td>
<td>0.1 MPa</td>
</tr>
</tbody>
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Table I: In-vitro injection conditions

* The load within the native disc is approximately 0.3 MPa.

B. In-vivo experiment
A murine model of the degenerated intervertebral disc has been described previously by our laboratory (3). A system of intradiscal injection has also been described for the injection of growth factors (4). Eight 12-week old male Swiss Webster mice were used. A degenerated phenotype was induced in a single caudal disc of each mouse by statically compressing the disc for one week, followed by an unloading period of 3 months. An eight microliter injection of cell suspension (sodium alginate) was injected into each degenerated disc. Two sets of graft cells were used. The first set was harvested from mice heterogeneous for the green fluorescent protein (GFP) gene. Upon analysis of the histologic sections, autofluorescence of the native disc was encountered, making it difficult to distinguish graft from host. Consequently, the second set of cells was harvested from Swiss Webster mice, and transfected with red fluorescent protein (RFP) in order to increase the signal-to-noise ratio of graft to host. The partially fractionated marrow was electroporated with the RFP plasmid (Clontech) using an ElectroSquare Porator (BTX Instruments). The cells were grown in culture for 3 days. Flow cytometry was used for both sets of cells to separate them into live/dead and fluorescing/non-fluorescing fractions. The cells were then suspended in PBS or sodium alginate and injected into the degenerated discs.

Results
A. In-vitro experiment
Cells suspended in PBS and injected into the non-pressurized gel were retained in the gel after injection. Under loaded conditions, however, the cells infiltrated the gel, but then were extruded out of the gel along the needle track. This result was the same regardless of the applied load. When the cells were suspended in the agarose matrix, the cells were retained within the gel; an increasing percentage of cells were retained with increasing viscosity of the matrix (Figure 2).

Figure 2: In-vitro injection results in agarose matrix

The alginate and thrombin-fibrinogen matrices also retained the cells in the agarose at both 0.03 and 0.1 MPa.

B. In-vivo experiment
Three in-vivo injections with GFP cells suspended in PBS were performed; the mice were sacrificed immediately after the injection. Routine staining, fluorescence, and GFP immunohistochemistry did not identify injected cells in these discs. Five injections (4 GFP, 1 RFP) of the allogeneic suspension were performed; these mice were also sacrificed immediately. Routine staining identified scattered injected cells in 3 of the 4 GFP injections. In the RFP injection, visualization under a TRITC filter identified 4-10 injected cells per cross-section.

Discussion
These experiments demonstrate that the inherent swelling pressure of the disc poses a technical hurdle to the delivery of allogenic cells to the IVD. The conditions of the in-vitro experiment were not intended to exactly mimic the conditions of the disc. The pressure in the disc is higher than the pressure in the fixture, the material properties differ, and the disc is likely to be less compliant than the agarose. However, the fixture provided a means to visualize the cells during the injection, and demonstrated that high pressures can extrude the cells via the needle track. The in-vitro experiment also demonstrated that increasing the viscosity of the injection matrix increased the number of cells retained in the gel. This observation seems to extrapolate to the in-vivo condition. Cells in PBS were not retained after injection, while initial experiments show some success with retaining cells suspended in an alginate matrix. Experiments are currently underway to optimize the carrier matrix, as well as to assess the viability of the injected cells within the degenerated discs.

References
2. Gruber and Hanley (2002). ISSLS p.25
4. Walsh et al. (2001) 47th ORS, 8929