Differential MHC Class Receptor Expression in *In Vitro* Human Mesenchymal Stem Cells is Mediated by Properties of Scaffold Material

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Tissue engineering

• Certain cell sources present many issues such as
  • cell sources being difficult to procure
  • Cells lacking in providing viable mechanical properties
  • Cells triggering immune responses

[Links]
http://www.pitt.edu/~ajr138/engineering_trends.html
Mesenchymal Stem Cells (MSCs)

• Mesenchymal stem cells, or MSCs, are multipotent stromal cells that can differentiate into a variety of cell types

• These cells can be found throughout the body, including the bone marrow and visceral fat tissue

• MSCs are thought to generate a local immunosuppressive microenvironment

[1] Ryan, 2005

http://www.eurostemcell.org/de/image/mesenchymal-stem-cell-differentiation
MHC class receptor expression

• MHC class receptors are cell antigens that are vital in the immune system of animals.

• There are three classes of these receptors
  • Class Ia – immunogenic
  • Class II- immunogenic
  • And Class Ib – immunosuppressive

• In a previous study (Hudson OSA 2015), these cells were found to be expressing the immunogenic antigens in some Intervertebral disk constructs.

http://www.zo.utexas.edu/faculty/sjasper/bio301l/cells.html

http://www.pathologystudent.com/?p=1494
Hypothesis

The material properties of alginate and type 1 collagen affect the expression of the MHC class receptors.
Methods

Scaffold Material

Alginate
3% alginate
cells/ml

Collagen
4 mg/ml collagen
cells/ml

Cell Culture
Construct incubation
2 Weeks
4 days - 2 Weeks

Constructs Harvested at days 4, 7, 9, 11, 14

Immunohistochemistry
Immunohistochemistry (IHC)

Using the ABC System:

1. Add Primary Antibody
2. Add Biotinylated Secondary Antibody
3. Add Avidin/Biotinylated Enzyme Complex (ABC)
4. Add Enzyme Substrate

• Antibodies are used to identify particular receptors on the cells

Collage has a greater up regulatory affect on immunogenic receptors compared to alginate.

<table>
<thead>
<tr>
<th>Scaffold material</th>
<th>Class IA</th>
<th>Class II</th>
<th>Class IB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alginate</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
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<tr>
<td>Collage</td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
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</tbody>
</table>
Collagen up regulates immunogenic receptors

Expression of class I ABC MHC Receptors in Collagen

Day 4  Day 7  Day 9  Day 11  Day 14

Expression of Class DR-DP-DQ in Collagen

Day 4  Day 7  Day 9  Day 11  Day 14
Collagen down regulates immunosuppressive receptors

Changes Class G MHC antigen in Collagen

Day 4  Day 7  Day 9  Day 11  Day 14
Less Regulation of Receptor Expression in Alginate
Conclusions

• The material in which MSCs are cultured have an effect on the expression of the MHC class receptors

• Collagen, in comparison to alginate, had a greater up regulation of immunogenic receptors, and down regulation of immunosuppressive receptors

• Cells had increased expression of immunogenic receptors the longer they were in the collagen
Future Directions

- Analyze cells using, flow cytometry, a method that allows for the cells expressing certain receptors to be quantified
- Continue Immunohistochemistry analysis
- Explore effects of varying cell concentrations, scaffold material concentrations, and cell adhesion domains
- Explore the hypothesis that the expression of these antigens is controlled by either adhesion to the scaffold material, or the cell shape they form in the scaffold material.
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