The Influence of Scaffoldings on C2C12 Cell Behaviors

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Regenerative Medicine

Replacing injured tissues with tissue constructs fabricated for the needs of each patient.
Tissue Engineering Principles

Defect -> Cells -> ECM -> Blood Supply -> Regeneration
Tissue Scaffolds

Provide temporary framework for cell proliferation, migration, and differentiation

Type/composition varies with purpose, seeded cells, and host tissue compartment

Many different materials (natural and synthetic) have been investigated for use in tissue regeneration
Scaffold Types

- **PLA Scaffolds** - popularly used in tissue engineering because of its biodegradability
- **PCL Scaffolds** - A biodegradable scaffold material (neural engineering)
- **UBM Scaffolds** - Urinary Bladder Matrix is an extracellular matrix (ECM) scaffold. It is now used in wound care management of partial and full-thickness wounds where conventional methods for wound care usually fail to give satisfactory results.
Stem Cells

Cell that can produce lineages more specialized than themselves and can renew itself
Spectrum of Stem Cell Behavior:
  - totipotent embryonic cells
  - pluripotent cells
  - multipotent cells
  - unipotent cells.

Un-specialized cells that can differentiate into various body tissues and organs
Have potential to cure or treat diseases such as heart disease, and diabetes
C2C12 Cell Line

- Subclone of the *mus musculus* (mouse) myoblast cell line.
- A common TE experimental model
- Differentiates rapidly, forming contractile myotomes and produces characteristic muscle proteins.
- Useful model to study the differentiation of nonmuscle cells (stem cells) to skeletal muscle cells.
Objective

- To compare stem cell behaviors on a synthetic and natural scaffold
The decellularized matrix will promote the cell behaviors of attachment, proliferation, and differentiation. A secondary hypothesis is that cells will have a greater response to a growth factor cue on the decellularized matrix.
## Materials

<table>
<thead>
<tr>
<th>Item</th>
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</thead>
<tbody>
<tr>
<td>PLA Scaffold Material</td>
<td>DMEM Media (10% Serum)</td>
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<tr>
<td>C2C12 Cell Line</td>
<td>DMEM Media (2% Serum)</td>
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<tr>
<td>Microscope</td>
<td>Ethanol</td>
</tr>
<tr>
<td>Flasks</td>
<td>Trypsin</td>
</tr>
<tr>
<td>Pipet</td>
<td>Nikon inverted scope/computer interface</td>
</tr>
<tr>
<td>Water</td>
<td>Growth Factor, FGF2</td>
</tr>
<tr>
<td>Rubber Gloves</td>
<td>Test Tube Rack</td>
</tr>
<tr>
<td>Sterile Pipet Tips</td>
<td>100 mL Graduated Cylinder</td>
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<td>Well Plates</td>
<td>Decellularized Extracellular Matrix</td>
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<tr>
<td>Laboratory Aspirator</td>
<td>Tongs</td>
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</tbody>
</table>
Procedure

A 1 mL aliquot of C2C12 cells* from a Cryotank was used to inoculate 30 mL of 10% serum DMEM media in a 75mm² culture flask yielding a cell density of approximately $10^6$ to $2 \times 10^6$ cells. *Between 200,000 and 300,000 cells are in 1mL.

The media was replaced with 15 mL of fresh media to remove cryo-freezing fluid and incubated (37°C, 5% CO₂) for 2 days until a cell density of approximately $4 \times 10^6$ to $5 \times 10^6$ cells/mL was reached.

The culture was passed into 2 flasks in preparation for experiment and incubated for 2 days at 37°C, 5% CO₂.

After trypsinization, cells from both of the flasks were pooled into 1 common 75mm² flask (cell density of approximately 1 million cells/mL).

1 ml of the cell suspension and 3 ml of media were added to 9 well plates, creating a cell density of approximately $5.0 \times 10^5$ cells per flask.

1 ml of the cell suspension and 3 ml of media containing FGF-2 growth factor were added to 9 well plates, creating a cell density of approximately $5.0 \times 10^5$ cells per flask.

The cells were incubated (37°C, 5% CO₂) for the remainder of the study.
Images were taken on day 4

Before the experiment the UBM and PLA scaffolds were created and sterilized.

The UBM was spin coated in pepsin a technique that had never been done before.

After the experiment, the well plates were imaged using a Nikon computer interface on three areas on the well plate.
Results
ECM Coverslip with differentiation media with FGF2

200 μm
ECM Coverslip differentiation media without FGF2
PLA chip with differentiation media with FGF2

200 μm
PLA chip with differentiation media with FGF2
Tissue culture well with differentiation media without FGF2
Tissue culture well with differentiation media with FGF2
UBM Scaffold with FGF2
UBM Scaffold without FGF2
Control with FGF2
Control without FGF2
With FGF2

Without FGF2

Cells have not formed nearly as many myotubes

Myotube Formation
At Carnegie Mellon University during imaging.
Conclusion

The results indicate the following...

- The use of bladder decellularized extracellular matrix digested with pepsin to make into a hydrogel then spin coated onto coverslips had not been done before.
- In differentiation media the C2C12 readily differentiated. In the presence of FGF2 there was significant cell growth, but not myoblast differentiation.
- The growth factor affected the cells.
- There was not a significant difference in cell growth or differentiation between UBM or PLA matrix types.
- As predicted in the hypothesis, well plates that were not treated with the FGF2 growth factor formed greater myotubes than the samples treated with the growth factor in the media.
- The hypothesis that stated that each scaffold was able to promote cell attachment, proliferation and a response to an outside growth factor will be supported.
Possible Limitations

Due to the irregular 3D nature of the PLA construct, it could not be properly imaged.

The incubator was shared so there is some risk of contamination.

No cell counts were performed.

Only imaged on one day.

Scaffold areas not identical

No precise differentiation marker

Cell images may not reflect the cells overall.
Extensions

More consistent compensation and area of scaffold
Employ other types of stem cells
Other growth factors (synergistic effects)
Use of a differentiation marker
References

A

B


C

D


Y

Interviews

Campbell, P, Ph.D. Interviewed by E. Mullen. Central Catholic High School.
   2:00 P.M., November 3, 2015

Hawranko, B. Interviewed by E. Mullen. Shadyside Place,
   6:00 PM October 7, 2015

Krotec, M. Interviewed by E. Mullen. Central Catholic High School, Phone, and Email.
    This source was contacted numerous times and acted as a mentor in my experiment.
University Thesis


Online Journals
