EFFECTS OF NICOTINE ON HUMAN MESENCHYMAL STEM CELLS

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Purpose

To determine whether nicotine causes any effects on human Mesenchymal Stem Cell (hMSC) proliferation or migration
Problem

If nicotine adversely affects MSC proliferation or migration, treatments involving the stem cells may fail and smokers will be at higher risk of long-term injury.
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
hMSC

- Human Mesenchymal Stem Cells
- Fibroblast-like cells
- Reside in bone marrow, adipose tissue, skin, and periosteum
- Useful in regeneration of muscle/bone (artificial treatments and/or natural process)
- Effects of mechanical stimuli on MSCs are still poorly understood
Tobacco

- Known health risk
- Approximately 42 million smokers in the US alone
- Many forms (cigars, cigarettes, pipe, etc)
- Negatively affects embryonic stem cells
Cigarettes

- Contain over 4,000 chemicals, many of which have been shown to be detrimental to human health
- Emit CO$_2$
- Contains about 1 gram of tobacco and 8.5 mg of nicotine
- Responsible for 6 million deaths annually
- Contain mutagenic and carcinogenic substances
- Combustion products yield many harmful substances
Nicotine

- Derived from the tobacco plant
- Considered a stimulant
- Affects many areas in the body including the central nervous system and the endocrine (hormone) system
- Some positive effects (at low concentrations)
Hypothesis

It is hypothesized at concentrations of nicotine similar to those found in a smoker’s blood, MSC will be adversely affected, significantly altering the proliferation and migration potential of the MSC.
Previous studies

- University of Miami performed similar tests – indicate that MSC will be negatively affected
- Show that smokers are at a higher risk for long-term injury and why MSC treatments for smokers would fail
Cells were plated at 10,000 cells/cm² in high-glucose Dulbecco’s modified Eagle’s medium (DMEM; Invitrogen).

- Supplemented with 10% fetal bovine serum (Invitrogen), 10 µg/mL and 10 µg/mL penicillin/ streptomycin, respectively (Invitrogen), and 0.25 µg/mL amphotericin B on 35 mm × 10 mm tissue culture dishes.

- Cells left in incubator at 37°C and 5% CO₂ for 24 hours to allow for cell attachment.

- Nonadherent cells were washed off during the first medium change (24 hours after initial plating).

- The desired confluence of 40%–50% was reached after approximately 48 hours.

- Plates were washed with phosphate buffered saline solution twice and placed in serum-deprived high-glucose DMEM supplemented with 1% penicillin / streptomycin.

- The cells were placed in the incubator for an additional 72 hours to allow the cells to synchronize to the G0 phase of the cell cycle.

- Unsynchronized control cells while testing synchronization efficacy were left in regular culture medium for the 72-hour period.
Materials/Procedure – Nicotine

- Plates were separated into groups
- Control was synchronized as described in previous slide; following synchronization period (72 hr), starvation medium was replaced with high-glucose DMEM (10% fetal bovine serum, 1% penicillin / streptomycin, 0.1% amphotericin B)
- Other groups were also synchronized, but starvation medium was replaced with regular medium with a 1.0 μM concentration of nicotine (99%)
- Cells were left in incubator for 48 hours with the nicotine medium
- MTT assay was used to determine proliferation (approximate cell count)
- Migration assay was used to determine migration potential
Results – MTT Assay (Absorbance – Cell Count)

Absorbance (nanometers)

Day 1  Day 2  Day 3  Day 4  Day 5  Day 6  Day 7

Control
Nicotine

Chart Title
### Data Analysis – Proliferation

Anova: Single Factor

<table>
<thead>
<tr>
<th>Groups</th>
<th>Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
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<td>0.087024</td>
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<td>0.49</td>
<td>0.07</td>
<td>0.004</td>
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ANOVA

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<th>Source of Variation</th>
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<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
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</thead>
<tbody>
<tr>
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<td>0.260579</td>
<td>5.725504</td>
<td>0.033961</td>
<td>4.747225</td>
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<tr>
<td>Within Groups</td>
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<td>12</td>
<td>0.045512</td>
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<tr>
<td>Total</td>
<td>0.806721</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Results – Migration

Distance (µm)
Conclusions

- Control group had significantly more cells, and did not reach a plateau
- Control traveled significantly farther than the nicotine group
- Seems to indicate that nicotine is harmful to MSC proliferation and migration
- Smokers could be at a much higher risk of long-term injury and any treatment using MSC would likely fail
Limitations/Extensions

- **Limitations**
  - Cell health not considered – only survivorship based on absorbance
  - Plating not perfectly synchronized
  - Only one concentration of nicotine used

- **Extensions**
  - Test differentiation potential
  - Determine average speed of each group in addition to distance traveled
  - Use AFM Assay to measure elasticity
Sources

- http://scholarlyrepository.miami.edu/cgi/viewcontent.cgi?article=1362&context=oa_these