Microfluidic Device for Single Cell Studies of Spinal Cord Injury

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Outline

• Spinal Cord Anatomy and Spinal Cord Injury
• In Vitro Methods
• Our Devices
• Optimize Conditions
• Results for Microchannel Devices
• Results for Nanochannel Devices
• Future Studies
• Conclusion
Spinal Cord

A long, thin, tubular bundle of nervous tissue that transmits signals between the brain and the rest of the body

Photo credit: http://www.medicalook.com/Neurological_disorders/Spinal_cord_injuries.html
Spinal Cord Injury (SCI)

Any injury to spinal cord that results from trauma, not disease

Photo credit: http://swchildrens.org/health-library/library-detail?projectId=117&pid=2&gid=19619
Spinal Cord Injury (SCI)

According to University of Alabama National Spinal Cord Injury Statistical Center, 2002

• 250,000 Americans have SCI

• Common causes:
  
  Vehicular accident 37%
  Violence 28%
  Falls 21%
  Sports-related 6%
  Other 8%
Anatomy of Single Neuron

Photo credit: http://www.cs.uaf.edu/2007/fall/cs441/proj1notes/schamel/
In Vitro Methods

Difficult to maintain stable cell culture
Difficult to study specific parts of neuron cell
Difficult to induce controlled damage

Campenot chamber

Microfluidic culture system
Our Microfluidic Devices

Two parallel channels connected by either smaller microchannels (10µm width) or nanochannels
Device Characteristics

• Confinement and linear orientation of single neurons
• Spatial separation of axons and cell bodies
• Controlled application of compression and strain by home-made stretcher
Objectives

Overall objective:
Study single neuronal responses to mechanical forces via static pressure by microchannel confinement

Summer project objectives:
• Develop device design
• Stabilize cell growth
• Characterize neuron growth in devices
• Observe neuron responses to compression and strain
Materials and Methods

• Fabricate device mold by photolithography
• Make PDMS devices and treat with extracellular matrix coating
• Dissect primary dorsal root ganglia neurons from chicken embryos and seed neurons in devices
• Apply compression by home-made stretchers
Optimizing Extracellular Matrix Coatings

• SHSY5Y neuroblast cells

• Different conditions
  – poly-lysine (PK) overnight (100 µg/ml, 200 µg/ml)
  – laminin for 4 h (25 µg/ml, 50 µg/ml),
  – combinations of poly-lysine overnight (100 µg/ml, 200 µg/ml) and laminin for 4 h (25 µg/ml, 50 µg/ml, 100 µg/ml)

Combining poly-lysine and laminin produced the best cell attachments
Combinations of poly-lysine and laminin showed comparable results.

\[ \text{Laminin} \quad \text{25} \, \mu g/ml \]
\[ \text{Laminin} \quad \text{50} \, \mu g/ml \]
\[ \text{Laminin} \quad \text{100} \, \mu g/ml \]

\text{Lowest effective concentration:}

\[ \text{Poly-lysine} \quad (100 \, \mu g/ml) \quad \text{and} \quad \text{Laminin} \quad (25 \, \mu g/ml) \]
Optimizing Cell Seeding Concentration

SHSY5Y neuroblast cells in PDMS device reservoirs

$10^5$ cells/ml produced the best cell attachment for easiest visualization
Cell Growth Differs in Device Regions

Reservoir

Edge

Channel

Reservoir  Edge  Channel
Cell Growth After Channel Height Increased from 50 µm to 80 µm

Possible Explanations:

• More media volume, more nutrient availability
• Easier washing of extracellular matrix
• Slower flow speed for cell seeding and attachment
Cell Growth Timing in Microchannel Devices

- Neurons ready for compression/strain experiments on days 2-4
- Neurons survive 1 week in devices
10% Compression of Microchannel Devices
Discussion for Microchannel Compression

• 10% compression damaged many axons
• 10% compression did not provide narrow enough microchannels
• >10% compression causes bulging of the device above horizontal plane

Current microchannel dimensions not suitable
Non-stretched Nanochannel and Microchannel Devices are Comparable
Nanochannel Devices with 10% Strain
Better Cell Growth After Strain Removal

Day 3: Before strain removed

Day 4: After strain removed
Cell Growth At Various Strain

Control 3% Strain 8% Strain

Magnified:
Discussion of Nanochannel Devices

• Good cell growth in reservoir and channel for non-stretched devices

• No cell growth in channel of stretched devices
  – Stretching causes the device to lengthen and decreases the height of channels

• Cell growth in reservoir is related to % strain
  – No glia cells in stretched devices
  – Difference in topography of the stretched PDMS
Future Studies

Microchannel devices:
• Smaller microchannel widths
  – less compression, less cell damage
  – better spatial separation of cell bodies and axons

Nanochannel devices:
• Verify that increasing strain causes progressively worse cell growth
• Verify that removing strain promotes a recovery of cell growth
• Study glia/neuron ratio for various % strain
Conclusions

• Spinal Cord Injury is caused by physical trauma
• Microfluidics systems can be used in study models for Spinal Cord Injury
• Microchannel and nanochannel devices are easily compressed to apply controlled mechanical forces on single neurons
• Studying single neuronal response to mechanical forces contributes to our understanding of Spinal Cord Injury
Thank you

Questions?
Nanochannel device stretching
Optimal cell seeding concentration: $10^5$ cells/ml