Novel Biodegradable Nerve Conduits for Functional Recovery after Spinal Cord Injury

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Spinal Cord Injury: Causes and Mechanism

- Estimated 900 Canadians suffer from Spinal Cord Injury each year *
- Causes of SCI can be vehicle accidents, falls, medical, sports, diving and industrial
- Occurs when human spinal cord receives a physical impact that damages cells such as neurons, astrocytes, oligodendrocytes, existing within it
- Formation of glial scar (Secondary Process)
  - Reactive astrocytes, microglia, transmembrane molecular inhibitors
  - Lack of pathway for regenerating axons
  - Inhibitory environment
- SCI leads to paralysis, depending on level of injury on Spinal Cord
  - Paraplegia
  - Quadriplegia

* Canadian Paraplegic Association, 2009

Nerve Conduits

- Desirable properties of an implanted nerve conduit after spinal cord injury:
  - Aid in regeneration of axons
  - Prevent glial scar tissue infiltration
  - Adequate mechanical strength, structural stability
  - Porosity and morphology for exchange of nutrients and waste materials
  - Biodegradable, biocompatible, non-toxic, non-carcinogenic

Implantation Strategy

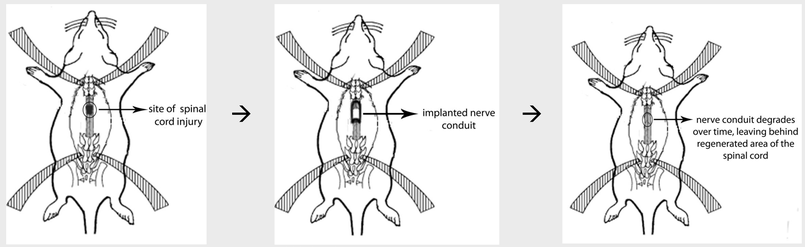

Figure 1. Implantation strategy

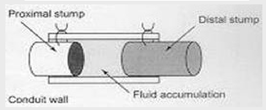

Figure 2. close-up of implanted nerve conduit

Figure 5: Microsphere-fused nerve conduits

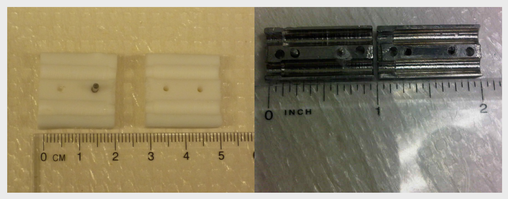

Figure 7: Teflon mold pictured left; stainless steel mold pictured right

Step 6: Placing loaded molds into the supercritical CO_2 chamber for foaming of nerve conduits

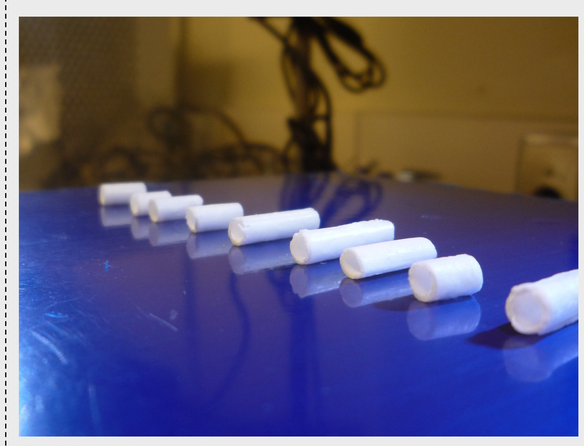

Figure 9: Microsphere-fused nerve conduits

Fabrication Techniques of Nerve Conduits: Dip-coating Method

Step 1: Preparation of 20% (m/v) poly(lactide-co-glycolic acid) (PLGA) with growth factors EGF and FGFb in chloroform

Step 2: Glass rods are immersed in the PLGA solution

Step 3: PLGA-coated rod is dried by rotating it using a mechanical stirrer for 2 hours. Steps 2 and 3 are repeated 4 more times for a total of 5 dips.

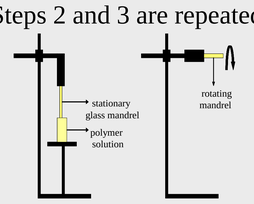

Figure 3. Schematic of nerve conduit fabrication by dip-coating

Step 4: The coated rods are dried in a vacuum drier for 48 hours
Purpose of step: removal of chloroform residues

Step 5: The dried rods are placed in a supercritical CO_2 chamber (under 850 psi) for 6 hours.
Purpose of step: the PLGA material foams and expands to take on desired properties

- Advantages of $scCO_2$
  - Inexpensive
  - Sterilization of samples
  - Extraction of solvent

Figure 4. Supercritical Carbon Dioxide Apparatus

Fabrication Techniques of Nerve Conduits: Microsphere Fusion Method

Step 1: Preparation of 20% (w/v) PLGA with growth factors in chloroform. Preparation 1% (w/v) and 0.1% (w/v) polyvinyl alcohol (PVA) solutions

Step 2: Emulsification of all three solution for 3 hours
Purpose of step: Formation of PLGA microspheres encapsulating growth factors (EGF and FGFb)

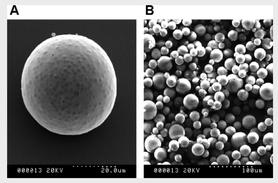

Figure 6. SEM of single microsphere on shown on left; cluster of microspheres shown on right

Step 3: Centrifugation of the microsphere-containing solution with double distilled water
Purpose of step: Separation of chloroform from desired microspheres

Step 4: Centrifuged microspheres are freeze-dried for 48 hours
Purpose of step: removal of water from microspheres

Figure 7. Microspheres being freeze-dried

Results and Conclusions

- Microsphere fused nerve conduits ensure an equal distribution of growth factor and proteins, while the distribution in dip-coated nerve guides is uneven and unpredictable
- Microsphere fused nerve guides provide a more sustained release of growth factors than the dip-coated nerve guides
- In dip coated nerve guides, a large proportion of contained growth factor was immediately exposed to the surroundings
- Thus, microsphere-fused nerve guides are more promising than dip-coated nerve guides for use in treating for spinal cord injury

Future Work

- In vivo study involving implantation of growth factor-encapsulated microsphere-fused nerve conduits to determine level of spinal cord regeneration

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