Effects of Encapsulated BDNF-Producing Fibroblasts on Dorsal Root Ganglia Neurite Growth

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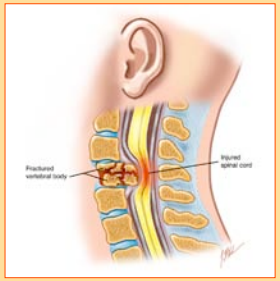
ABSTRACT

Fibroblasts genetically engineered to produce Brain-Derived Neurotrophic Factor (FB/BDNF) have been shown to promote axonal regeneration in the injured spinal cord of an immune-suppressed rat. To avoid immune suppression and protect the cells from the host immune response, FB/BDNF were encapsulated in various alginate gels. BDNF secreted by the fibroblasts was able to diffuse out of the alginate and remain bioactive, stimulating significantly more neurite growth than alginate without encapsulated fibroblasts. A BDNF concentration gradient was also shown to guide DRG neurite growth towards the source. These positive neurotrophic effects show promise as a repair strategy for spinal cord injury.

BACKGROUND

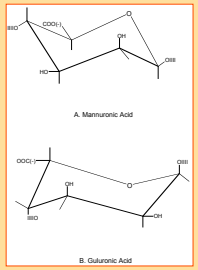
Spinal cord injury (SCI):
- Affects nearly 250,000 people in the U.S.
- Approximately 11,000 new cases every year
- Can lead to loss of sensation and motor function; chronic pain; spasticity; and bowel, bladder, and sexual dysfunction
- Effects caused by death of neurons that cannot be replaced, poor regenerative capacity of surviving neurons, damaged CNS environment is inhospitable for growth
- *Ex vivo* gene therapy (genetically engineering cells to express a transgene) is a promising strategy for SCI repair, but immune suppression is needed
- Method needed to provide benefits of *ex vivo* gene therapy without suppressing immune system

Alginate:
- Linear polysaccharide derived from seaweed
- Consists of 1,4-linked _-L-guluronic acid and _-D-mannuronic acid monomers
- FDA 'Generally Recognized as Safe' Status
- Nontoxic, biocompatible, easy to process into various shapes, capable of immobilizing biomolecules (such as BDNF-producing fibroblasts)
- Coating with polypeptides will control porosity, stability, and create an isolation barrier from the immune system



Schematic of spinal cord injury

http://www.backpain-guide.com/Chapter_Fig_folders/Ch10_Recover_Folder/Ch10_Images/10-4_Spn_Crd_Dmg.jpg



Alginate monomer structure

OBJECTIVE

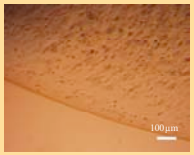
To develop a graft suitable for implantation into the injured spinal cord that will:
- Provide a favorable substrate for neuronal adhesion and growth
- Facilitate the use of *ex vivo* gene therapy without host immune suppression
- Promote the growth and directional guidance of neurons with a concentration gradient of growth factors

MATERIALS AND METHODS

Discs:
- BDNF-producing fibroblasts (FB/BDNF) resuspended in alginate
- Alginate mixed with $CaCO_3$-GDL solutions or $CaSO_4$ slurry
- Mixture shaken and poured into culture plates
- Discs coated with poly-L-ornithine (PLO) and laminin

Fibers:
- FB/BDNF resuspended in alginate
- Alginate injected into $CaCl_2$ solution
- Fibers coated with PLO and laminin

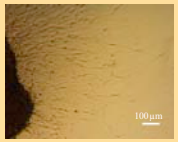
- Dorsal root ganglia (DRGs) removed from 10-day old chick embryos
- DRGs cultured on alginate and observed for up to 72 hours



FB/BDNF encapsulated in alginate (100x)

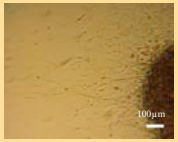
DRG NEURITE GROWTH

Culture medium

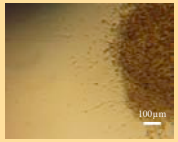


Culture medium + recombinant BDNF

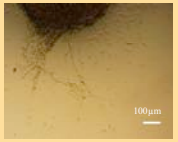
All photos: 100x



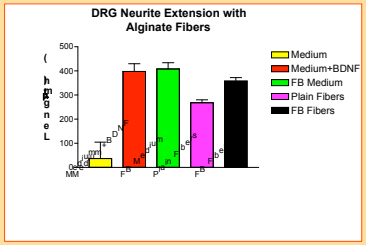
Medium from FB/BDNF in culture



Plain alginate fibers



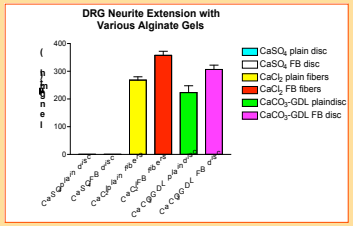
FB/BDNF alginate fibers



- Alginate with FB/BDNF promoted significantly more neurite growth than plain alginate fibers (not containing FB/BDNF) ($p<0.05$)

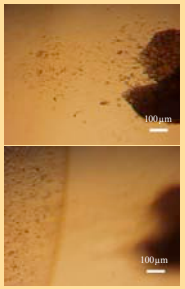
- Statistically similar neurite lengths with supplemental recombinant BDNF (positive control #1), medium from FB/BDNF in culture (positive control #2), and alginate encapsulated FB/BDNF ($p>0.05$)

ALGINATE COMPARISON



- $CaCl_2$ fibers and $CaCO_3$-GDL discs promoted significantly more neurite growth than $CaSO_4$ discs ($p<0.001$)
- $CaCl_2$ and $CaCO_3$-GDL alginates are suitable for use as a DRG growth substrate
- Choosing between the two depends on needs: gelation speed, uniformity, desired shape

BDNF CONCENTRATION GRADIENT



Growth of DRG neurites towards source of BDNF (100x)

- Concentration gradient formed by covering DRGs and FB/BDNF alginate fibers with 1% agarose
- Agarose slowed the diffusion rate of BDNF and more closely resembled an *in vivo* environment
- DRGs sprouted neurites under agarose
- Neurites grew and turned towards alginate fibers
- FB/BDNF alginate fibers promoted greater directional guidance of neurites than plain alginate fibers ($p<0.05$)

CONCLUSIONS

- BDNF secreted by fibroblasts able to diffuse out of alginate and remain bioactive, stimulating neurite growth
- Alginate-encapsulated FB/BDNF (mean neurite length = 306.4 ± 60.72 _m) promoted significantly more DRG neurite growth than alginate without FB/BDNF (mean neurite length = 223.0 ± 65.46 _m)
- Alginate not containing FB/BDNF has neurotrophic properties
- No neurite growth after 24 h suggests an inhibitory interaction between $CaSO_4$ alginate, encapsulated FB/BDNF, and DRGs
- $CaCl_2$ alginate and $CaCO_3$-GDL alginate stimulated significantly more neurite outgrowth than $CaSO_4$ alginate
- BDNF concentration gradient guided growth of DRG neurite towards the source

FUTURE WORK

- Evaluate effects of FB/NT-3 (neurotrophin-3) on DRG neurite growth
- Evaluate synergy of FB/BDNF and FB/NT-3 on DRG neurite growth
- Vary concentration gradients in alginate to find optimum concentration for FB survival and DRG growth
- Quantify release of BDNF and compare to release from YIGSR-coated alginate
- *In vivo* testing of alginate graft