Development of Nanoparticle and Nanowire Technologies to Treat Spinal Cord Injury

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Introduction

The zebrafish model, which has been used for neural degeneration/regeneration research [1] is of potentially great value because of its relative simplicity, transparency and regenerative capabilities. The transparency of the zebrafish CNS and the identifiable brainstem and spinal neurons make this an ideal preparation to evaluate retrograde delivery **(Figures 1 & 2)** of nano therapeutics and subsequent enhancement of substantive axonal regeneration using gene therapy. Such evaluations are deemed critical to the development of new therapeutic approaches for treating spinal cord injury and other neurological disorders.

Proposed Methodology

1. Rely on bulk uptake of particles by patent openings of severed axons [4]
2. Evaluate uptake of gold nanoparticles (NP) and utility of stabilizing agents
3. Evaluate particle agglomeration, size optimization and plasmid transport
4. Use fluorescent proteins to visualize efficacy of gene delivery and expression to neuronal cell bodies of injured axons

Goal:
Delivery and expression of therapeutic genes specifically to damaged descending neurons.

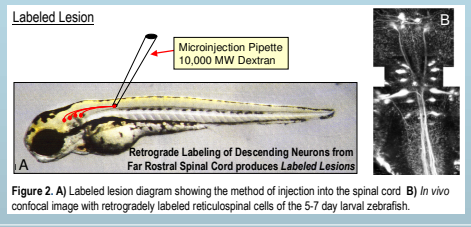


Figure 2. A) Labeled lesion diagram showing the method of injection into the spinal cord **B)** *In vivo* confocal image with retrogradely labeled reticulospinal cells of the 5-7 day larval zebrafish.

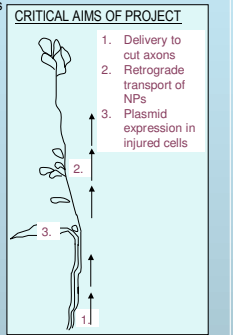


Figure 1. Diagram of the critical aims for effective gene delivery to damaged nerve cells.

Introduction

Our goal in this project is to develop a 'neurochip' which will consist of an array of conducting nanowires integrated with electrodes at one end and directly interfaced with cultured neuronal cells at the other allowing us to stimulate and detect electrical activity at the nanoscale level from simultaneous locations in neurons, as illustrated in **Fig. 1**. These nanowire arrays are intended to be used to investigate the operations of living neural networks in precise detail, tracking small changes in cell structure and electrical activity in a minimally invasive fashion and at the same time with excellent spatial and temporal resolution. Such studies will significantly improve our understanding of brain functions.

The nano-bio device under development, consists of a vertical array of conducting gold nanowires, which are prepared by means of electrodeposition **(Fig 2A, B & Fig 3A, B)** inside nanoporous alumina templates and then integrated with microscale electrodes at one end. These nanowire arrays will then be directly interfaced with cultured primary neuronal cells. Future applications will also include implants of nanowire arrays which will be used for pilot studies in the transparent CNS of zebrafish with the goal of visualizing array operations in concert with measuring neural circuit activities.

Goal: To reveal network level flows of information that underlie neural computations including sensory information processing, memory storage and motor programming.

Applications of Nanowire-Arrays to Neural Networks Entails:

- Vertically arranged nanoelectrode array
- High spatial & temporal resolution
- Capability to stimulate and record from several different sites on a neuron
- Study of live neuronal activity at the subcellular level in single neurons & neural networks

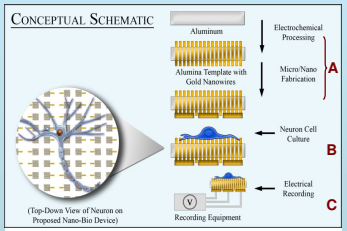


Figure 1. Diagram of the development and proposed neuronal studies with the nano-bio device.

Nanoporous Alumina Templates for Nanowire Deposition

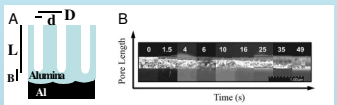


Figure 2. A) Schematic of nanoporous alumina template structure [6, 7].
B, d: Determined by Anodization Voltage and Acid
L: Determined by Anodization Time
B ~ 10-20 nm ***L*** ~ several mm ***d*** ~ 10-150 nm
B) Changing pore length as a variable of time during anodization.

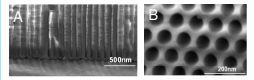
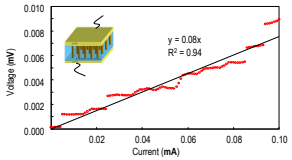


Figure 3. SEM Images of Nanoporous AluminaTemplate Structure
A) SEM cross-section image showing vertically arranged pores
B) Top view image of a template showing pore pattern.

Results & Conclusion

Resistivity of Gold Nanowires



Linear Relationship: Increase in current results in an increase in voltage.
Net Resistance calculated from the slope of the curve is approx. 80 Ω

Growth of Primary Hippocampal Cultures on Nanowire Arrays

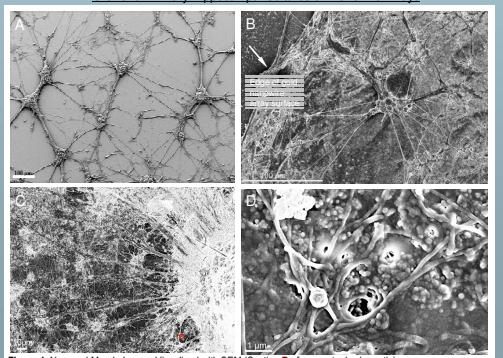


Figure 4. Neuronal Morphology as Visualized with SEM (Section B of conceptual schematic):
A) Primary culture morphology of E18 hippocampal neurons at 5 days on glass coverslips.
B) 18 day old cultures of hippocampal cells near the edge of the gold nanowire array. **C)** Morphology of attached cells on the gold nanowire array surface. **D)** Intertwined axons on array surface of hippocampal neurons from C.

Development of Nanowire Devices should help us to:

- Understand the information processing capabilities of both single nerve cells and systems of nerve cells
- Advance our understanding of how specific patterns of neural activity associated with learning and memory are created
- Comprehend the mechanisms of plasticity within neuronal networks

Calcium Imaging to Measure Neuronal Activity

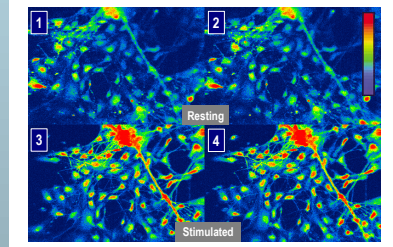


Figure 5. Neuronal Responses to KCl Stimulation
Frames 1 & 2 - resting population of neurons
Frames 3 & 4 – post-KCl calcium increase
Population imaging will allow us to calibrate array efficacy in stimulation and recording

Synaptic Connectivity of Neuronal Cells

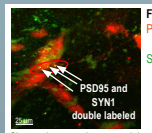


Figure 6. Plasticity of Neuronal Population
PSD 95 – a post-synaptic density protein (visualizes postsynaptic sites)
SYN1 – synapsin 1 found on presynaptic vesicles (visualizes presynaptic sites)

Change in synaptic connectivity subsequent to electrical stimulation will initially be visualized using immunocytochemistry, to confirm the synaptic nature of the observed contacts. But this can be extended to monitoring axonal and dendritic morphologies in living zebrafish to assess the efficacy of different stimulation protocols.

Results & Conclusion

Injection of Tracer/NP Mixture into Spinal Cord

We evaluated whether either gold NPs would be feasible in regards to:
1) visualization inside the spinal cord of intact larvae using confocal microscopy
2) neuronal delivery via bulk injections into the spinal cord of larval zebrafish
3) retrograde transport from the spinal injury site to neuronal cell bodies in brainstem

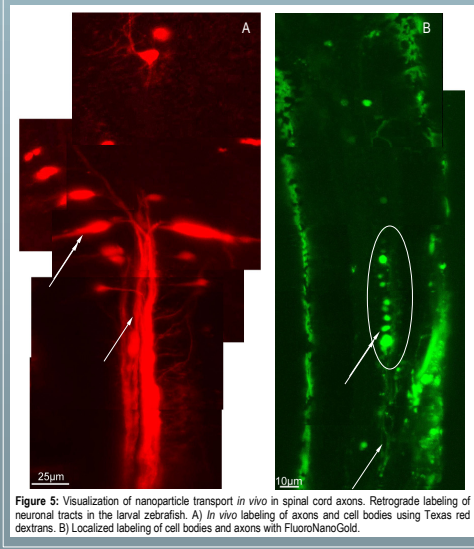


Figure 5: Visualization of nanoparticle transport *in vivo* in spinal cord axons. Retrograde labeling of neuronal tracts in the larval zebrafish. A) *In vivo* labeling of axons and cell bodies using Texas red dextrans. B) Localized labeling of cell bodies and axons with FluoroNanoGold.

Schematic of Gold Nanoparticles and Transport Characterization

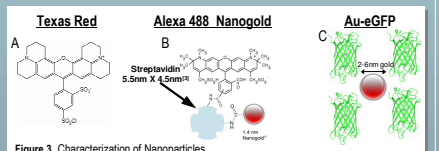


Figure 3. Characterization of Nanoparticles
A) FluoroNanogold (Nanoprobes, Inc.) conjugated to streptavidin reduces quenching of the fluorophore
B) Direct conjugation gold nanoparticles of the GFP [5] plasmid yields a larger particle size; (eGFP= enhanced GFP: [2nm (W) X 1.1 – 1.2nm (H)][2])

Improvement of NPs for efficient transport and delivery can be facilitated by:

–Optimizing particle size
–Improving dispersion of NPs in solution

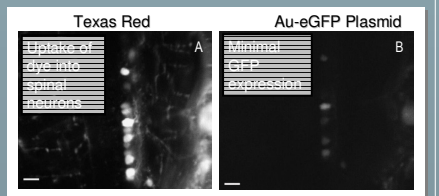


Figure 4. Efficacy of Rostral Injections of NPs into larval zebrafish
A) Injection of Texas Red dye
B) Injection EGFP plasmid conjugated to gold NPs suggests limited uptake of gold conjugated GFP plasmid given the limited expression of GFP.

Literature Cited

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