

New Jersey Commission on Brain Injury Research (NJCBIR)

Final Narrative – December 2010

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2. Name of Organization/Institution
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Final Narrative Report.

We proposed to develop a new and unique traumatic brain injury (TBI) model that employs a combination of organotypic slices with microfabricated mechanical deformation and electrophysiological detection device. This model will serve in enhancing the ability of researchers to monitor axonal response to traumatic injury in an *in vitro* platform that focuses on easy on-line long-term visualization of axons and detection of the axonal ability to propagate action potentials. The following are the specific aims for the project:

Specific Aim 1: *Design, fabrication, and testing of the silicone microchannel network for application of mechanical deformation at pre-defined locations.* The network will consist of 2 layers of regularly-spaced channels placed orthogonally to each other. The top layer will contain channels for axon guidance, while the bottom channels will be permanently bound to the culture substrate and connected to an external array of syringe pumps to regulate the pressure within the channel. Application of increased pressure will result in the deformation of the thin membrane separating the two layers, at the location precisely determined by the activation of a given set of pumps. The microfluidic network will be fabricated in polydimethylsiloxane (PDMS, a transparent silicone polymer). Each layer will be separately defined with soft lithography process (a technique for micro-pattern transfer); layers will be aligned under a microscope and bonded using oxygen plasma. The resulting microfluidic network will then be reversibly bound to a glass slide (same substrate as used for multiple electrode array devices in aim 3). Fluid connections will be made using small diameter flexible tubing, devices sterilized either in autoclave or by washing with ethanol.

Specific Aim 2: *Validation of the TBI model with the culture of hippocampal and entorhinal slices.* Slices of the early postnatal mouse brain will be placed into pre-defined wells on either side of the culture substrate-bonded microchannel network. The slices will be maintained at the air-liquid interface by controlling the amount of medium in the culture dish (we have succeeded in maintaining organotypic slices of the cortex and hippocampus in this manner for several weeks in our preliminary studies). We will monitor the axon growth along the microchannels connecting the slices, and selective disruption of the connection by application of mechanical deformation to individual microchannels. We will characterize the deformation magnitude (pressure and deflection distance) and application protocol (deformation speed and duration) in terms of the disruption to the bundle of axons in a microchannel. We will also monitor post-deformation axonal degeneration and any spontaneous sprouting of new axon collaterals.

Specific Aim 3: *Integration of the microchannel network with a multiple electrode array.* We will use a planar MEA (fabricated in our laboratory) as the substrate for bonding of the microchannel network and culture of organotypic slices. We will then monitor electrophysiologically both the new functional connections made by extended axons in the microchannels (the magnitude and spatial spread of evoked post-synaptic field potential following an extra-cellular stimulation pulse) and the long-term changes in the two-slice neural circuit elicited by applied mechanical damage to interconnection between two brain regions— something that is difficult or impossible to do in the intact brain or in other *in vitro* models.

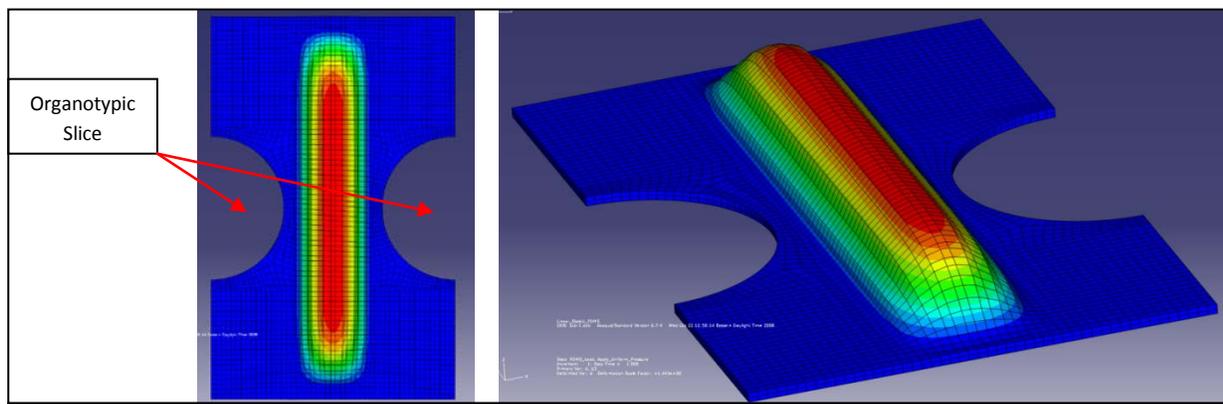
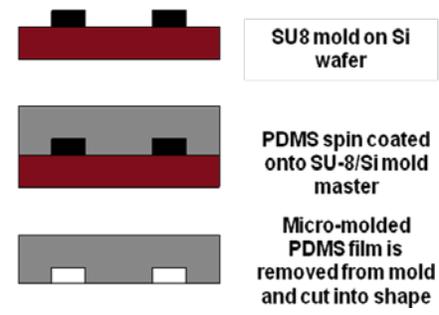
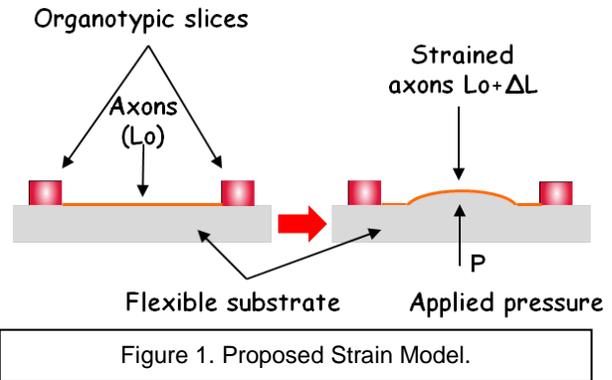
The following is the progress that has been made on the individual specific aims.

Specific Aim 1: As stated above, this aim investigates the use of a PDMS microfluidic network that guides axonal extension from the periphery of organotypic hippocampus slices through microchannels that connect two organotypic slice cultures together. A schematic of the device is shown in Figure 1.

The microfluidic network that guides these axons is manufactured using soft lithography techniques (Figure 2). This PDMS is bonded to a glass substrate. It is essential that you get very good bonding between the PDMS and the glass in order to withstand the pressure that will be applied to produce the desired strain. It took some time to optimize this bonding technique.

Using the Finite Element Analysis (FEA) software Abaqus we were able to optimize a number of design parameters that would enable us to apply uniaxial strain to axons traversing the microchannels and connecting the two organotypic slices. The PDMS thickness, PDMS mechanical properties and width to length ratio of the pressure channel were determined and an example of a strain result can be seen in a simplified FEA model in Figure 3.

The FEA software was also used in order to ensure that no compression occurs within the microchannel, since this *in vitro* model is designed only for uniaxial strain (later models could possibly have a combination of strain with compression). It was determined that the PDMS thickness above the microchannels should be equal to or less than the thickness below the microchannel in order to minimize these compression effects.



The pressure required to induce a deflection of the device has been determined both experimentally and theoretically using Abaqus. Figure 4 shows how the theoretical values correspond well to those obtained experimentally.

The rate at which the pressure is applied, using a linear actuator attached to a syringe, can be varied by changing the actuator speed. Figure 5 shows four different actuator speeds that correlate to specific strain rates. A speed of 0.1m/s relates to a strain rate of 2.3 s⁻¹ (which is a strain of 230% applied over a second), 0.5m/s relates to a strain rate of 9.7 s⁻¹, 1.0m/s relates to a strain rate of 16.1 s⁻¹ and a speed of

1.5m/s relates to a strain rate of 18.5 s^{-1} . The maximum pressure of 7.2psi relates to a strain of approximately 37%.

We have thus demonstrated that we were able to design and fabricate a microchannel network that is capable of producing a mechanical deformation (uniaxial strain) across these microchannels.

Specific Aim 2: Using a modified Gahweiler method of culturing organotypic slices on a substrate, we have demonstrated that organotypic slices remain healthy on a PDMS surface for a culture period of at least 22 days. Figure 6 shows a phase contrast image and corresponding MAP-2 staining (neuron marker). The neuron marker demonstrates that the organotypic slice architecture, i.e. the specific regions of the hippocampus such as the dentate gyrus, CA3, CA1 remain intact over the culture period. At higher magnification, we also see that the cell orientation, i.e. neuronal dendrites, are also maintained (data not shown).

Slice attachment and subsequent axon extension from the periphery of the slice required that the PDMS surface be coated with Poly-d-Lysine and Laminin.

In order to: 1) control the number of axons that enter and traverse the distance connecting the two organotypic slices and 2) to minimize cell migration into the microchannels, we investigated the use of two different microchannel widths ($25\mu\text{m}$ and $50\mu\text{m}$) and two different microchannel heights ($2.5\mu\text{m}$ and $5\mu\text{m}$). As expected, the wider the channels the more axons enter but more cell migration into the channels occurs (Figure 7A). By both narrowing the channel and reducing the height, both cell migration and the number of entering axons is reduced (Figure 7B).

The projections within the microchannels have been confirmed to be axons through double labeling of cultures with Tau (axonal marker) and MAP-2 (dendrite marker). Figure 8 shows that only axons traverse the entire length of the microchannel.

Axons within microchannels were subjected to a strain of approximately 37% (applied at a rate of approximately 18.5 s^{-1}) resulting in characteristic axonal beading. Figure 9A and B shows single axons beading after being strained, whereas C shows how axon bundles bead. We are currently preparing a manuscript describing the characterization of the device and these initial results (Specific Aims 1 and 2). Using these initial results we are planning to submit a grant application to the NSF (April of 2011) in the

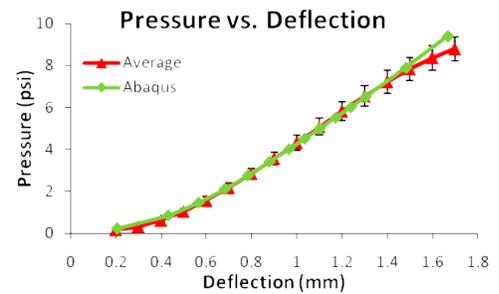


Figure 4. PDMS deflection results – FEA simulations vs. experimental.

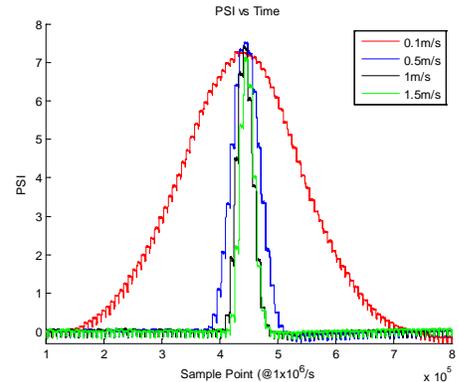


Figure 5. Change in cavity pressure with time. Red – 0.1m/s (2.3 s^{-1}), Blue – 0.5m/s (9.7s^{-1}), Black – 1.0m/s (16.1s^{-1}), Green – 1.5m/s (18.5 s^{-1}).

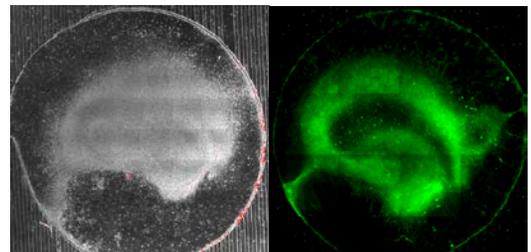


Figure 6. Phase contrast and MAP-2 staining of organotypic slices on Day 22.

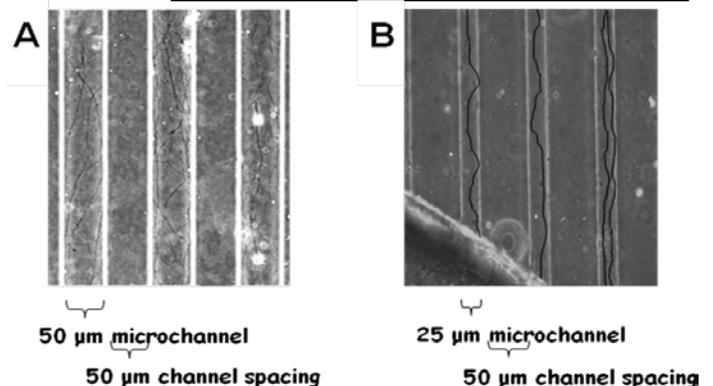
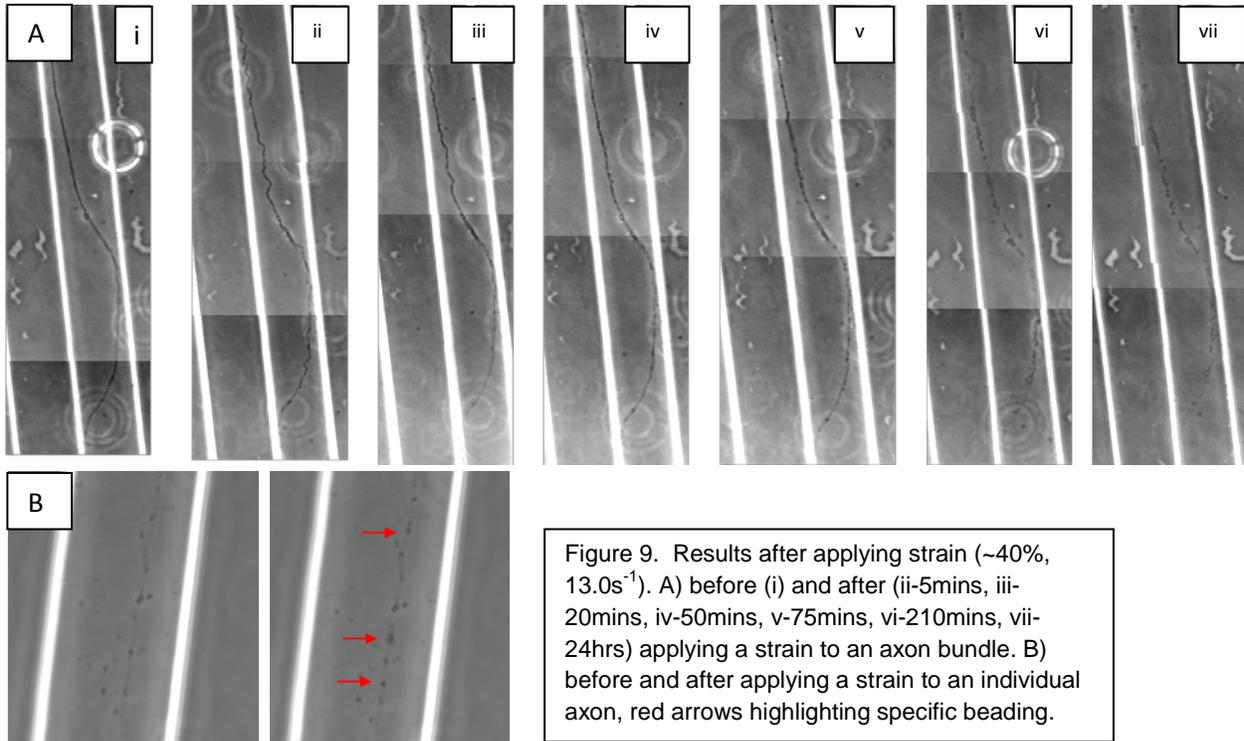
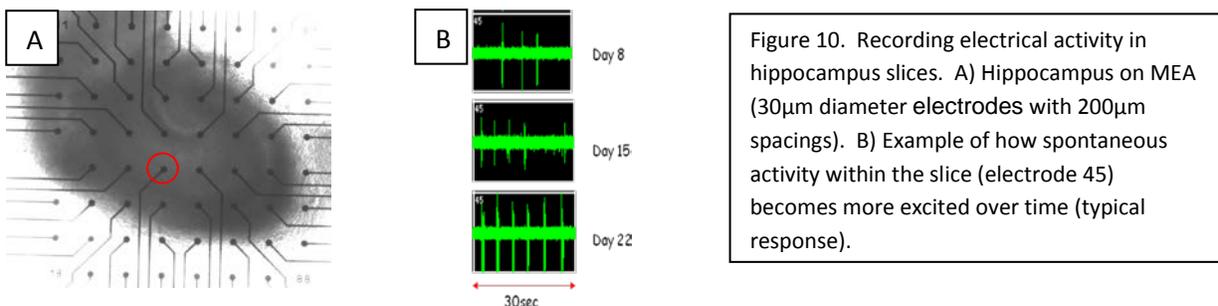


Figure 7. Axons restricted to extending in PDMS microchannels, A) multiple axons - $5\mu\text{m}$ high channels, B) single axons - $2.5\mu\text{m}$ high channels.

Biomedical Engineering section with the specific focus on cellular biomechanics. Two manuscripts are currently in preparation, one that describes and characterizes the device (“Development of a model for studying the effects of axonal strain injury on action potential propagation”, Jean-Pierre Dollé, Rene Schloss, Martin L. Yarmush) and a second that focuses on the axonal response to the strain injury (“Axonal diameter plays a role in the delayed elastic effect after strain injury”, Jean-Pierre Dollé, Oleg Milberg, Rene Schloss, Martin L. Yarmush).



Specific Aim 3: In preparing for integrating MEA's onto the strain device we first wanted to demonstrate that we are capable of being able to perform long-term action potential recordings through MEA's placed beneath hippocampal slices (MEA's purchased from Multichannel Systems). Figure 10A shows a hippocampus slice placed on an MEA (electrodes are $30\mu m$ in diameter with a $200\mu m$ spacing). Figure 10B shows characteristic spontaneous activity over time (from electrode 45 – highlighted in Figure 10A) demonstrating how the slice becomes more active as it ages (Berdichevsky et al). We are currently working on methods to incorporate electrodes onto the strain device in order to record the change in neural circuitry between two tissue slices after mechanical deformation.



References:

Berdichevsky Y, Sabolek H, Levine JB, Staley KJ, Yarmush ML. Microfluidics and multielectrode array-compatible organotypic slice culture method. *J Neurosci Methods*. 2009 Mar 30;178(1):59-64.