INTRODUCTION

Traumatic Brain Injuries (TBI) affect up to 1.5 million people annually within the United States, with as many as 250,000 being hospitalized and 50,000 dying [1]. TBI events occur when the brain experiences a sudden trauma such as a rapid acceleration or deceleration. These events produce high inertial forces that result in a shearing or elongation of axons (commonly known as Diffuse Axonal Injury [2]).

Currently, there are no indicated treatments for TBI, resulting in an inconsistent and non-standardized approach to care. In order to develop novel therapeutic solutions that can be used in clinical TBI care, a model that can accurately mimic certain mechanisms of injury needs to be developed. We will employ a combination of organotypic slices and a microfabricated mechanical deformation device that is able to monitor axonal response to traumatic strain injury in an *in vitro* platform while maintaining the natural architecture and neural circuitry found *in vivo* [3].

MATERIALS AND METHODS

**Fabrication of PDMS microchannels and culture wells**

PDMS microchannel networks were patterned via a modified “soft lithography” technique. A combination of two microchannel widths of 25µm or 50 µm, and two heights of 2.5µm or 5µm (with a constant channel spacing of 50µm) were fabricated. Liquid PDMS was spin-coated onto a silicon master at 1000rpm, and cross-linked at 65ºC overnight. Miniwells of 2mm diameter for organotypic slice placement, with a large channel for culture medium access was then cut from the membrane (Fig 1A&B).

A schematic of the device is shown in Fig 1C, depicting how an increase in internal cavity pressure results in axonal strain within the microchannels. The PDMS on which the slices attach and axons extend was coated with Poly-d-Lysine (PDL) (1mg/ml) and Laminin (80µg/ml). Each layer of PDMS is irreversibly bonded to the adjacent layer using oxygen plasma (100W, 30s).

**Organotypic cultures.** Hippocampal slices of 450µm thickness were dissected from postnatal day 4 and 5 rat pups (Sprague Dawley) and placed into PDMS mini-wells (Fig. 1). The device was then filled with just enough serum containing medium (1:1:2 horse serum, Hanks’ Balanced Salt Solution, and Basal Medium Eagle, supplemented with 0.5mM L-glutamine, 10mM HEPES and 30µg/ml gentamicin) to cover the bottom of the well, and incubated in a humidified 5% CO₂ incubator. Serum-free medium (Neurobasal A/B27, with 0.5mM L-glutamine, 10mM HEPES and 30µg/ml gentamicin) was substituted on the second day of organotypic culture, and was used in all subsequent medium changes (every 2 days).

**FEA Analysis.** FEA software (Abaqus) was used to design and simulate the strain device. The PDMS material tensile properties were tested according to ASTM D412 and input into the model as an incompressible first-order Ogden hyperelastic material. The three dimensional model was meshed using Abaqus standard and 8-node quadrilateral elements.
Applying Strain. The pneumatic pressure was applied to the cavity beneath the microchannels through a pressure injection system that consisted of a syringe, linear actuator, pressure sensor and controller. These were coupled to the device through pressure injection and sensing ports (Fig 1A). The applied strain is varied by changing the injected volume and the rate of strain is varied by using different linear actuator speeds.

RESULTS

Device Design. Using Abaqus FEA software, the device is designed such that a uniform strain field is applied along the area in which axons will be extending and connecting to the adjacent slice (Fig 2).

An example of varying the rate of strain applied to the device is shown in Figure 3.

Axon extension within microchannels. On PDMS that has been surface coated with PDL and Laminin, good axon extension is observed from the periphery of the organotypic hippocampus slice.

Using different channel widths and heights, the number of axons in a given channel can be varied and cell migration down the channel can be minimized. In channels that are 50µm wide by 5µm high, multiple axons are seen, whereas in channels that are 25µm wide by 2.5µm high single axons can be obtained (Fig 4). These axons were confirmed to be Tau⁺ (axon marker) and MAP2⁺ (dendrite marker) through immunocytochemical analyses.

Axon Strain. An example of axons being strained is shown in Figure 5. Immediately after the applied injury a delayed elastic effect can be seen (Fig 5B - waves along the length of the axon) and within a short period of time post injury (Fig 5C - 4hours) significant axonal beading and degeneration has occurred.

DISCUSSION

Our results indicate that our device is capable of inducing uniaxial strains to axons that are guided within microchannels. By varying the dimensions of the microchannels we are able to control the number of axons that enter a given channel, thus giving the device the capability of applying strains to either individual axons or to larger axon bundles. This device will be capable of monitoring molecular and functional changes by placing microelectrode arrays beneath the organotypic slices and observing the effects of axonal strain on resulting slice-slice communication. This device could be used to elucidate potential therapies in neurodegenerative diseases such as Alzheimer’s and Epilepsy that have been linked to axonal strain injuries [2].

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REFERENCES

