Lab on a Chip

Supplementary Information

Supplementary Methods

Testing PDMS material properties

The tensile material properties of the PDMS were measured using a Bose/*EnduraTEC ELF 3200* mechanical testing machine (Enduratec, Eden Prairie, MN, USA). These tests were done according to ASTM D 412 standards. The size of the test specimens were scaled down to one fourth of those specified within the standard.[1] A "dumbell" shaped die was cast using rapid prototyping (Viper, 3D Systems, Overview Drive, SC, USA) and used to cut out samples of PDMS for testing (8.25mm-length by 2.5mm-width). These samples were securely placed in the jaws of the mechanical testing machine and tensile strained at a rate of 3.75mm/s. The force and displacement readings were recorded at a rate of 80HZ. Markings were applied to the PDMS around the jaws to assess for slippage during loading.

Slice viability

The viability of slices was determined using Propidium Iodide (PI, Invitrogen, Carlsbad, CA, USA). PI is used to assess for cell death since it is membrane impermeant and thus excluded from viable cells. When cells are injured or dead, PI enters the cell and binds to nuclear DNA which enhances its fluorescence (Cy5). Cultures were incubated at 37ºC for 30mins with 5µg/ml of PI in serum free media, then washed twice for 10mins with serum free media and imaged using an Olympus IX-81 DSU microscope. The extent of cell death was assessed by completely killing control cultures (at the respective time point) by incubating in artificial cerebrospinal fluid (120 mM NaCl, 3.3 mM KCl, 1.25 mM NaH_2PO_4, 1.3 mM $CaCl_2$, 0.9 mM $MgCl_2$, 10 mM HEPES, and 10 mM glucose, pH 7.4) for 48hrs at 4ºC and then PI staining.[2] The relevant fluorescence intensity was then normalized according to the fluorescence intensity of the completely dead hippocampus slice.

Minimizing microchannel cell migration

Cell migration down the microchannels was minimized by creating a physical barrier that inhibited cell entry. This is important since some Glial cells migrate out from the organotypic slice, enter a microchannel and can affect how axons experience strain injury. This was done by fabricating microchannels that have a height in the range of cell body dimensions. Various microchannel heights were fabricated and their effect on Glial cell migration down the length of the microchannel was observed. Cell migration was visualized and evaluated using the nuclear DNA stain DAPI (2µg/ml) as a cell marker.

Supplementary Figures

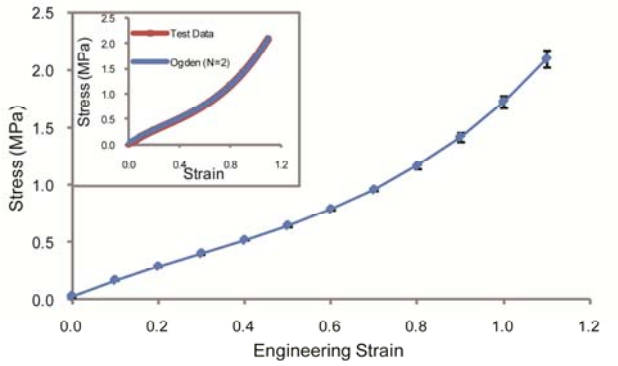

Figure S1. PDMS material properties. Tensile test results – stress vs. engineering strain curve (1.0 strain = 100% elongation). Inset: Curve fit using 2nd order Ogden hyperelastic model.

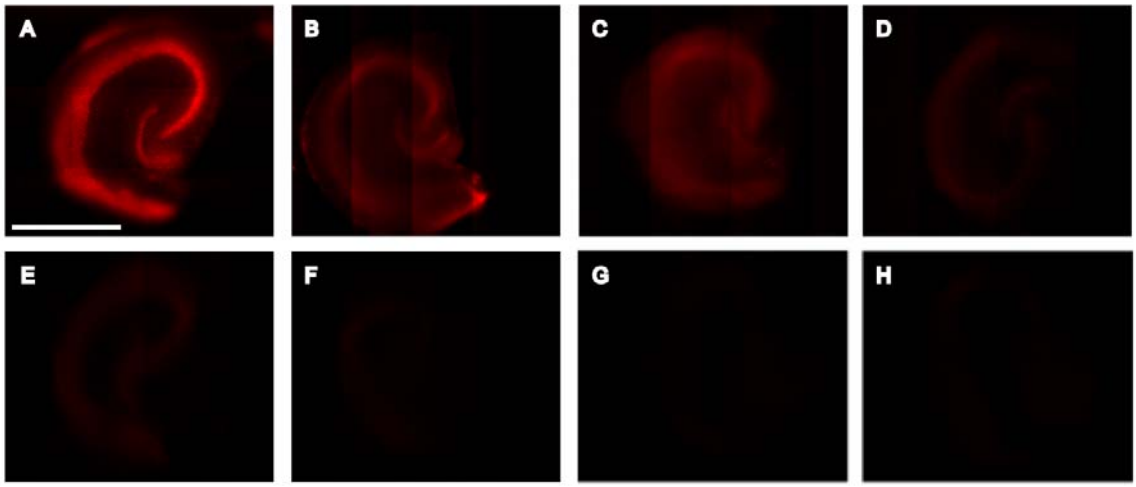

Figure S2. Assessing cell death in organotypic slices on injury device over time. Hippocampus slices on uniaxial strain injury devices stained with Propidium Iodide over time. A) Day 0, B) Day 1, C) Day 4, D) Day 7, E) Day 11, F) Day 14, G) Day 18 and H) Day 22. Scale, 1mm.

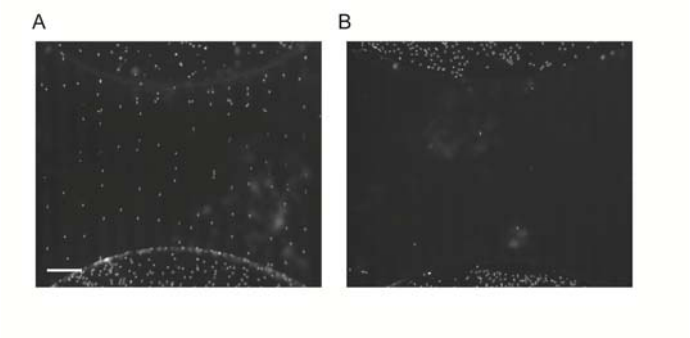

Figure S3. Minimizing microchannel cell migration. The nuclear DNA stain DAPI was used to visualize cell bodies down the microchannels. A) Representative image of Glial cell migration down microchannels with a height above 6µm, and B) Glial cell migration down microchannel with a height of 6µm or less. Scale, 200µm.

Supplementary References

1. A. Mata, A. J. Fleischman and S. Roy, *Biomed Microdevices*, 2005, **7**, 281-293.

2. M. V. Frantseva, L. Kokarovtseva and J. L. Perez Velazquez, *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*, 2002, **22**, 453-462.