

MICROFLUIDIC CULTURE PLATFORM FOR STUDYING NEURONAL RESPONSE TO AXONAL STRETCH INJURY

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ABSTRACT

We have developed novel device where a localised axonal stretch injury is achieved by the integration of a pressure-flexible valve in the axon compartment of a fluidically isolated neuron culture device. Defined stretch injuries applied using this device resulted in delayed neuronal alterations typical of those observed *in vivo*. These results confirm the suitability of this system for studying the neuronal response to axonal stretch injury. Therefore, this system provides a valuable tool for studying the degenerative and regenerative neuronal responses induced by acute stretch injury and provides a suitable platform for testing potential therapeutic agents.

KEYWORDS

Axon Stretch Injury, PDMS Device for Cell Culture, Neuroscience, Neuron Culture, Traumatic Brain Injury

INTRODUCTION

Traumatic brain injury (TBI) is a leading cause of mortality and morbidity in children and young adults, making TBI a significant public health problem. TBI may be caused by rapid brain deformation, stretching, compression or shear forces occurring as a result of traumatic incidents such as motor vehicle accidents, falls and assaults [1]. A number of *in vitro* models to study the neuronal response to stretch injuries have been developed, including a dynamic stretch injury model where pressurizing a culturing chamber deflects a section of the flexible substrate containing the cultured axons, inducing tensile elongation [2-3]. However, this model do not offer the ability to expose the neurons to different neuronal microenvironments. This makes it difficult to investigate the pathological changes within distinct parts of the neuron (soma, axon and dendrites) in response to pharmacological manipulation and therapeutic treatment. This may be particularly important in the investigations of mechanisms of secondary degeneration where the role of retrograde signalling to the soma is unclear.

In the past few years, microfluidic devices have brought new direction in the development of models in neurobiology due to their ability to control both spatial and temporal dimensions, closely mimicking conditions found *in vivo*. The introduction of commercially available devices based on the research by Taylor and coworkers [4] has accelerated research in this area. They developed a microfluidic device enabling the isolation of axons from somata or dendrites allowing physical and chemical treatment on one side of the device without effecting the other side of the device by using microgrooves (10 μm wide and 3 μm high). To date no system able to study axonal stretch injury in a fluidically isolated microenvironment has been presented. In addition, the pathways to successful therapeutic intervention in TBI are still not clear and new models are required that mimic specific aspects of injury in order to examine the axonal response and trial different therapeutic agents. Therefore, a novel microfluidic device to simulate a stretch injury of axons by incorporating microfluidic valve technology[5] into our devices has been developed. The microfluidic culture chamber design developed by Taylor and coworkers [4] for fluidic isolation of the axon and soma was used, together with a poly (dimethylsiloxane) (PDMS) membrane that can be locally deflected using the valve technology. This allows axons growing on top of the membrane to be discretely stretched. This model provides an ideal platform to study stretch injuries and, in later stages, to test the effects of therapeutic agents in isolated axonal or somal compartments following stretch injury.

EXPERIMENTAL

The axonal stretch injury device consisted of two independent PDMS structures separated by thin PDMS membrane (Figure 1). Dissociated rat cortical neurons (harvested at embryonic day 18, E18) were grown in the upper PDMS microfluidic culturing device (Xona Microfluidic, CA), which has 450 μm long microgrooves connecting the soma and axon compartments. The bottom structure contained the air channel and was irreversibly sealed with the PDMS membrane using air plasma. Air pressure was applied to the air channel by using an in-house assembly valve system. In response to a pressure pulse, the air channel inflated and the PDMS membrane deflected upward, stretching the axons growing on top. The air channel microfluidic device was fabricated in PDMS by soft lithography and replica molding procedure. The template to make the PDMS air channel device was fabricated by using an office laminator as described previously [6]. A thin PDMS membrane was formed on a 1H, 1H, 2H, 2H perfluorooctyltricholasilane coated silicon wafer at rate of 1500 rpm and spinning time of 30 s. A series of increasing air pressures were applied to the air channel and after steady state was reached, the membrane deflection was determined with an optical profiler system. Primary cortical neurons were seeded inside the culturing devices and allowed to grow for 6-8 days *in vitro* (DIV) for adequate axonal extension prior to stretch injury. A pressure pulse (35 psi, 7 s) was applied to the air channel to induce an axonal stretch injury to the overlying axons. We then investigated the neuronal response to axonal stretch injury by using immunocytochemistry technique.

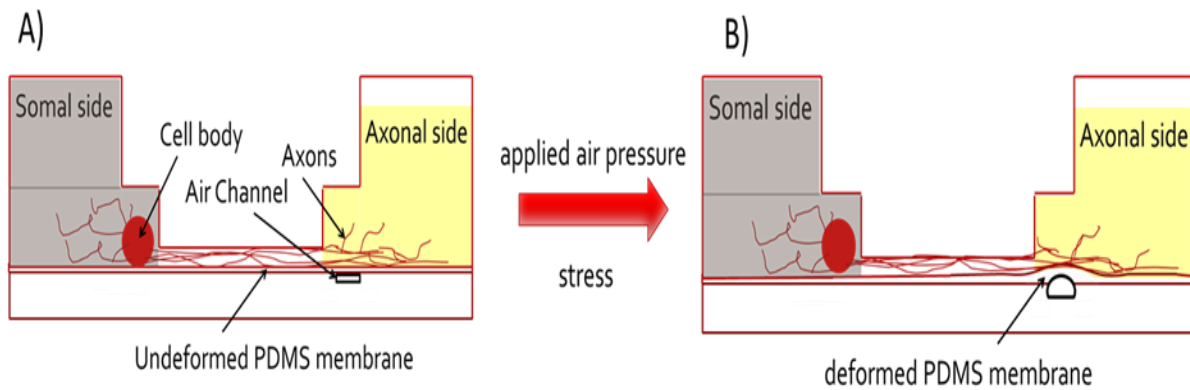


Figure 1. Schematic drawing of microfluidic device used for simulating axonal stretch injury. (A) A thin PDMS membrane separates the air channel layer (bottom) from the overlying culturing chamber (top). (B) Application of an air pulse to the air channel (positioned at 200-300 μm from microgrooves), cause upward deflection of the thin PDMS membrane, which stretches the overlying axon.

RESULTS

To determine the degree of axonal stretch, the physical extent of membrane deflection was examined. Membrane deflection, and therefore the degree of axonal stretch, depends on the thickness of the PDMS membrane and the applied pressure. In these experiments, a PDMS membrane of $\sim 60 \mu\text{m}$ thickness was used (spin speed 1,500 rpm), as determined by an optical profiler system (data not shown). A linear relationship was found between the applied pressure and the PDMS membrane deflection for the $\sim 60 \mu\text{m}$ thick PDMS membrane with applied pressures below 30 psi, which appeared to plateau when higher pressures were applied (Figure 2).

For the experiments presented here, a 35 psi pulse was applied, resulting in a 4.3 μm upward deflection. The air channel in this study was 90 μm wide, therefore a mild, 0.5% strain was obtained (estimated by using Pythagoras Theorem). Live imaging of the axonal compartment pre- and post-injury was used to determine the morphological changes occurring to axons following application of the pressure pulse.

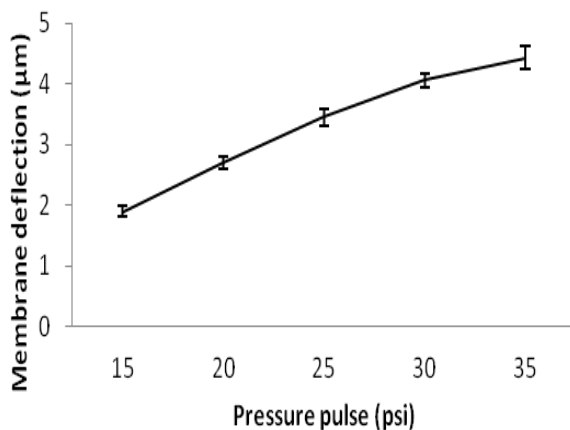


Figure 2. Relationship between applied air pressure and membrane deflection at steady state for $\sim 60 \mu\text{m}$ PDMS membranes. A constant valve opening of 7s was applied to ensure deflection measurements at steady state.

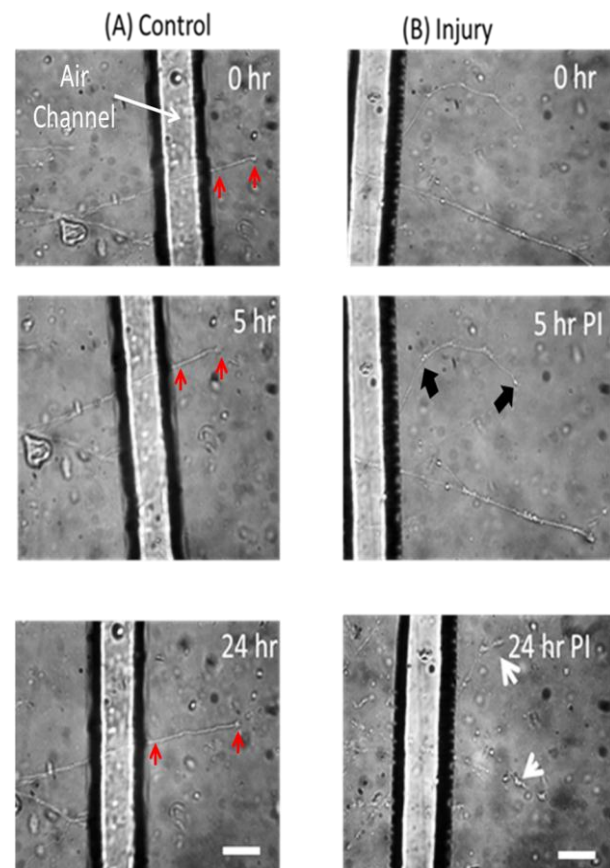


Figure 3. Live imaging of control and injured axons. (A) In the control, healthy and intact axons continue to grow at 8 DIV (indicated by red arrows). (B) Top image demonstrates that injured axons were intact directly after the stretch deflection at 8 DIV. At 5 h PI, axonal beading can be observed (indicated by black arrows). At 24 h PI, axons that had been stretched stop growing and multiple swellings were observed along the axon (indicated by white arrow heads). Scale bar = 20 μm .

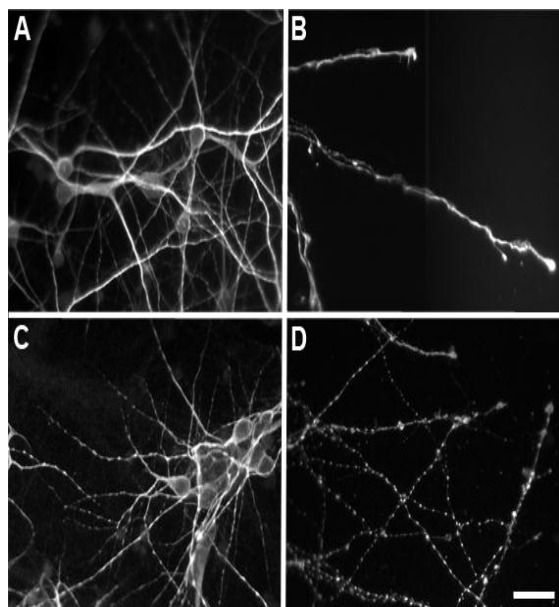


Figure 4. Immunocytochemistry images of uninjured and injured neurons 24 h following injury. Cell bodies and dendrites (A, MAP2 labelling), and axons (B, Tau and NFM labelling), in the control chambers were smooth and uniformly labelled for cytoskeletal markers. At 24 h post injury cortical cultures exposed to axonal stretch injury showed dendritic blebbing, and irregular MAP2 expression (C, MAP2 labelling). The injured axons (D, Tau and NFM labelling) underwent characteristic beading, swelling and degeneration, showing punctate accumulation of tau and NFM within the swollen portions of the axon. Scale bar=50 μm

In the control, the non-stretched axons remained intact and continued to grow (Figure 3A). Following stretch, at 5 h post injury (PI), axons were beaded but continuous (Figure 3B), confirming the axons were not cut during the stretching. At 24 h PI, the axons exhibited further signs of degeneration including multiple swellings along the axon (Figure 3B). Double fluorescent immunolabelling for the dendritic marker protein , microtubule associated protein 2 (MAP2) and the axonal proteins tau and neurofilament M (NFM) demonstrated smooth continuous expression of these proteins in control uninjured cultures (Figure 4A, B). Tau and NFM immunoreactive beading was also observed along the length of injured axons (Figure 4D). In order to examine if axonal injury resulted in retrograde signs of degeneration in the neuronal soma, we examined the somal compartment. At 24 h PI stretched neurons demonstrated dendritic beading and irregular MAP2 expression in the somal compartment (Figure 4C). These findings are consistent with previous experiments [2-3] and indicate that this new model can be used as an *in vitro* culture platform for studying the neuronal response to brain trauma and the testing of potential therapeutic agents.

CONCLUSIONS

A simple, reproducible *in vitro* model of discrete axonal stretch injury of cultured primary neurons is presented. When used for applying a very mild axonal stretch injury (4.3 μm upward deflection, 0.5 % strain) to neurons at 7-9 DIV, live imaging and immunostaining revealed several pathological alterations characteristic of TBI *in vivo* and *in vitro*. Our device allows alterations in individual axons to be investigated. Moreover, fluidic isolation of the axonal and somal compartments enables targeted exposure to potential therapeutic agents. This model provides an ideal platform to study stretch injuries and, in later stages, to test the effects of therapeutic agents in isolated axonal or somal compartments following stretch injury.

REFERENCES

- [1] D.H. Smith and D.F. Meaney, *Axonal Damage in Traumatic Brain Injury*, Neuroscientist, 6, pp. 483-495, (2000).
- [2] H. Monnerie, M.D. Tang-Schomer, A. Iwata, D.H. Smith, H.A. Kim and P.D. Le Roux, *Dendritic Alterations after Dynamic Axonal Stretch Injury In Vitro*, 224, pp. 415-423, (2010).
- [3] M.D. Tang-Schomer, V.E. Johnson, P.W. Baas, W. Steward and D.H. Smith, *Partial Interruption of Axonal Transport Due to Microtubule Breakage Accounts for the Formation of Periodic Varicosities after Traumatic Axonal Injury*, Experimental Neurology, 233, pp. 364-372, (2012).
- [4] A. M. Taylor, M. Brunton-Jones, S.W. Rhee, D.H. Cribbs, C.W. Cotman and N.L. Jeon, *A Microfluidic Culture Platform for CNS Axonal Injury, Regeneration and Transport*, Nature Methods, 2, pp. 599-605, (2005).
- [5] M.A. Unger, H.P. Chou, T. Thorsen, A. Scherer and S.R. Quake, *Monolithic Microfabricated Valves and Pumps by Multilayer Soft Lithography*, Science, 288, pp. 113-116, (2000).
- [6] A.A. Kazarian, E.F. Hilder, M.C. Breadmore, *Capillary Electrophoretic Separation of Mono- and Di-saccharides with Dynamic pH Junction and Implementation in Microchips*, Analyst, 135, pp. 1970-1978, (2010).

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