MICROFLUIDIC CULTURE CHAMBER FOR THE LONG-TERM PERFUSION AND PRECISE CHEMICAL STIMULATION OF ORGANOTYPIC BRAIN TISSUE SLICES
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ABSTRACT
We have developed a microfluidic perfusion-based culture system to study long-term in-vitro responses of organotypic brain slices exposed to localized neurochemical stimulation. Using this microperfusion chamber we show that hippocampal organotypic brain slices cultures grown on nitrocellulose membranes can be stimulated for up to 24 hours in our experimental setup while preserving tissue viability. By using fluorescent probe we show that a specific area of the hippocampal slice can be precisely targeted and stimulated. The device allows a virtual pixelisation of slice, precise control of the in-vitro micro environment, long-term culture of viable brain slices, and delivery of fluids to selected brain regions in a multiplexed and spatially defined manner.

KEYWORDS: Microfluidics, Organotypic Brain slice, Long-term Perfusion, Localized Neurochemical Stimulation.

INTRODUCTION
Organotypic brain slices are extensively used in neuroscience and neuroengineering research as ex vivo systems that permit direct treatment with pharmacological agents. While the brain slice preparation has provided access to details of cellular and circuit level brain function, the relevance to the intact brain is always in question. Several methods have been developed to maintain brain slices in long-term culture; among them, the static culture of slices on micro porous membrane filters where the tissue is kept at an air-liquid interface [1] has demonstrated to be the most efficient way to preserve healthy slices for a long period of time. The principle of this membrane interface culture method is to preserve brain slices at interface between medium and a humidified atmosphere allowing sufficient oxygen flow to the cells while providing nutrition to the tissue through the membrane via capillary action.

Microfluidics have proved to be useful for neurosciences studies and some devices have been reported either allowing localized stimulation of brain slices for acute experiments [2] or showing the long-term culture of organotypic slices without a precise control of the microenvironment [3]. Most recently, Queval et al [4] presented a microfluidic system for the culture of organotypic slices; despite its convenience, their custom-made system was extremely challenging to fabricate and assemble, precluding highthroughput experimentation. Our approach, by using simple microfabrication steps, is a novel microfluidic device that allows both precise control of the micro-environment and long term stimulation of previously grown organotypic hippocampal slices.

EXPERIMENTAL
Standard rapid prototyping techniques were used in the device fabrication. Figure 1 depicts a schematic view of our experimental platform that is composed of a two PDMS layers on a glass substrate and a standard perfusion chamber. As shown in Figure 1a, the first layer consists of a network of discrete 600 µm width PDMS microfluidic channels bonded to a coverglass substrate. Each microfluidic channel has a single via hole. The second layer involves an array of 1 mm diameter by 500 µm height pillars precisely distributed and aligned atop the first layer, “stimulating” pillars containing 150 µm diameter hollow microcylinders were chosen to be positioned above the via holes of the microchannels on the first layer, that way the fluid that flows in the microchannels is directed towards the perfusion bath through the pillars.

Eight inlet ports were drilled into the standard perfusion chamber to ensure alignment of the ports when the microfluidic device and the chamber were bonded together. The PDMS microfluidic device is docked to the off the shelf perfusion chamber, as it is shown in Figure 1b. Characterization of fluid flow through this type of devices has been reported earlier by Passeraub et al [5] and Caicedo et al [6].
A post-natal 4-6 days hippocampal organotypic slice previously cultured (two days) on a nitrocellulose membrane filter was transferred in the perfusion chamber atop the pillar network then, after placing our experimental set up inside an incubator, the slice was locally stimulated by Hoechst stain (HOE) that was delivered through one stimulating pillar in the microfluidic device for 24 hours, as is shown in Figure 2.

![Figure 1](image1.png)

**Figure 1:** (A) Schematic representation of the first and second layers of the microfluidic device showing how they are aligned. (B) Perfusion chamber docked to the microfluidic device.

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The hippocampal slice and nitrocellulose membrane were transferred onto a new membrane filter. After three days *in-vitro*, we use fluorescence microscopy to show that a specific area of the hippocampal slice had been precisely stimulated and labeled while the slice was still viable, as is shown in Figure 3.

![Figure 2](image2.png)

**Figure 2:** Micrograph showing the microfluidic system inside an incubator. Close-up image is showing a slice being locally stimulated by a micropillar of the device. Scale bar measures 500 μm.
RESULTS AND DISCUSSION

From the fluorescence microscopy data it is clear that a particular region of the organotypic brain slice can be targeted and labeled over a long period of time while preserving tissue viability. As shown in Figure 3, a long-term stimulated specific region of a hippocampal slice, after three days in-vitro, shows evidence of cellular activity.

This microfluidic platform could be used to evaluate the response of inter-neuronal function across brain regions after exposure to localized neurochemical trauma. Moreover, with development of disease-relevant slice models that reproduce important characteristics of in vivo neurodegenerative pathologies, our system could be used with these ex vivo models (organotypic brain slices) for the screening of therapeutic molecules or novel genes.

CONCLUSION

A simple and modular microfluidic culture chamber for the long-term perfusion and precise chemical stimulation of organotypic brain tissue slices has been fabricated and quantified through fluorescent microscopy. Our experimental setup combines organotypic culture techniques with microtechnology thus allowing a virtual pixelisation of the slice, precise control of the in-vitro micro environment, long-term culture of viable brain slices, and delivery of fluids to selected brain regions in a multiplexed and spatially defined manner.

ACKNOWLEDGEMENTS

The first author would like to thank ILACHE (Illinois Latino Council on Higher Education) for providing a travel award, Dr. David Eddington for allowing access to his lab and a special acknowledgement to Dr. Jean Michel Peyrin and Dr. Bernard Brugg at the Universite Pierre et Marie Curie in Paris, France for their financial, scientific and professional support.

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