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A microfluidic brain slice perfusion chamber for multisite recording using penetrating electrodes

Alexander J. Blake[a], Frank C. Rodgers[b], Anna Bassuener[b], Joseph A. Hippensteel[a], Thomas M. Pearce[a,1], Timothy R. Pearce[a], Ewa D. Zarnowska[b], Robert A. Pearce[b], Justin C. Williams[a,*]

[a] Department of Biomedical Engineering, University of Wisconsin, Madison, WI 53705, USA
[b] Department of Anesthesiology, University of Wisconsin, Madison, WI 53711, USA

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ABSTRACT

To analyze the spatiotemporal dynamics of network activity in a brain tissue slice, it is useful to record simultaneously from multiple locations. When obtained from laminar structures such as the hippocampus or neocortex, multisite recordings also yield information about subcellular current distributions via current source density analysis. Multisite probes developed for *in vivo* recordings could serve these purposes *in vitro*, allowing recordings to be obtained from brain slices at sites deeper within the tissue than currently available surface recording methods permit. However, existing recording chambers do not allow for the insertion of lamina-spanning probes that enter through the edges of brain slices. Here, we present a novel brain slice recording chamber design that accomplishes this goal. The device provides a stable microfluidic perfusion environment in which tissue health is optimized by superfusing both surfaces of the slice. Multichannel electrodes can be inserted parallel to the surface of the slice, at any depth relative to the surface. Access is also provided from above for the insertion of additional recording or stimulating electrodes. We illustrate the utility of this recording configuration by measuring current sources and sinks during theta burst stimuli that lead to the induction of long-term potentiation in hippocampal slices.

1. Introduction

The brain is composed of ensembles of small- and large-scale neuronal networks that, spontaneously or upon stimulation, generate coordinated activity patterns. In order to acquire information about the underlying spatiotemporal dynamics it is useful to record activity simultaneously at multiple locations (Csicsvari et al., 2003b). In laminated neural structures, additional information can be obtained by using current source density (CSD) analysis to derive the distribution of synchronous membrane events that give rise to the measured voltage fluctuations (Freeman and Nicholson, 1975; Mitzdorf, 1985; Buzsáki et al., 1986; Taube and Schwartzkroin, 1988; Vida et al., 1995). Penetrating multisite linear recording arrays have been developed that can be used for these purposes *in vivo*. The electrode sites are configured for specific electrophysiological applications by roughly matching them with the tissue cytoarchitecture (Buzsáki, 2004).

Brain slice preparations offer an alternative recording environment. For investigations of network patterns of activity, they provide a number of advantages over *in vivo* preparations, including the ability to record from discrete brain regions that maintain cytoarchitecturally preserved units of neuronal networks removed from the influence of afferent pathways (Sarvey et al., 1989; Traub et al., 1989). More importantly, *in vitro* brain slice recording methods offer a substantially greater control over certain experimental parameters (e.g. temperature, pH, pharmacological modulation), greatly facilitating investigations into the cellular mechanisms that underlie network activity.

A number of *in vitro* recording devices have been developed that incorporate multiple recording sites, including planar and three-dimensional microelectrode arrays (MEAs), which are capable of recording and stimulating at the sub-millimeter scale (Boppart et al., 1992; Csicsvari et al., 2003a; Gholmieh et al., 2006; Berdichevsky et al., 2009). Unfortunately, signals that originate from intact networks within the interior of slices are degraded at the injured tissue surfaces (Aitken et al., 1995), and this adversely affects the

* Corresponding author at: University of Wisconsin, Department of Biomedical Engineering, #2150 Engineering Centers Building, 1550 Engineering Drive, Madison, WI 53706, USA. Tel.: +1 608 265 3952; fax: +1 608 265 9239.
E-mail address: jwilliams@engr.wisc.edu (J.C. Williams).
[1] Current address: Department of Anatomy and Neurobiology, Washington University in St. Louis School of Medicine, St. Louis, MO 63130, USA.

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signal-to-noise ratio in recordings obtained from planar MEA recordings. This problem can be partially overcome using three-dimensional MEAs that penetrate the injured surface (Heuschkel et al., 2002; Nam et al., 2006), but this approach itself suffers from a combination of a limited recording depth and the need to obtain recordings near the non-perfused surface of the brain slice. Penetrating multisite linear electrodes potentially offer the ability to record from deep within tissue where neural networks are intact and active, while maintaining some of the same recording advantages as MEAs (Buzsáki, 2004). However, existing *in vitro* recording chambers lack access features that permit the realization of this recording configuration (Passeraub et al., 2003; Mohammed et al., 2008).

We sought to design an *in vitro* brain slice perfusion chamber that would enable extracellular recordings of network activity using a penetrating multisite electrode inserted parallel to the surface of the tissue slice, at any tissue depth, and that would incorporate laminar solution flow across both surfaces of the slice to enhance tissue viability (Passeraub et al., 2003; Rambani et al., 2009; Hájos et al., 2009). The perfusion chamber that we designed, built, and tested utilizes arrays of microposts that immobilize the tissue slice while providing fluid flow to both surfaces for delivery of oxygen and nutrients. In addition, it incorporates a lateral opening that permits the insertion of a penetrating multisite linear-array recording electrode into the edge of the slice, and an opening in the top for insertion of stimulating or conventional recording electrodes. We illustrate the utility of this recording configuration by measuring evoked activity in the CA1 area of a hippocampal brain slice undergoing long-term potentiation of excitatory synaptic strength by theta burst stimulation, and apply current source density analysis to reveal patterns of current flow during the potentiating stimulus.

2. Materials and methods

2.1. Chamber design

The microfluidic perfusion device used in this study is fabricated from poly-dimethyl-siloxane (PDMS, Sylgard 184, Dow Corning, Midland, MI). The process involves pouring a liquid pre-polymer onto a microfabricated mold, curing the polymer, and then releasing the resulting solidified material from the mold (Duffy et al., 1998). The final step requires that structural features of the mold do not mechanically impede the removal of the cured PDMS. Given this limitation, forming an enclosed 3-dimensional structure such as a microfluidic channel requires bonding the cured PDMS to another surface (Jo et al., 2000).

Perfusing both surfaces of a brain slice preparation enhances tissue viability for *in vitro* electrophysiology experiments (Rambani et al., 2009; Hájos et al., 2009). To accomplish this, we use a multi-stage process in which three layers of PDMS are individually molded, then bonded together via an oxygen-plasma surface treatment (Fig. 1). The first layer consists of a micropost array within a microfluidic channel. The brain slice is supported by the micropost array while the bottom surface of the tissue is perfused via the fluid channel. The second PDMS layer encloses the lower channel except for an opening located above the micropost array, which houses the slice. The shape of this opening closely fits the size and shape of the slice, and the thickness of the PDMS is matched to that of the tissue. The third layer of the chamber closely resembles the first, with a micropost array to hold the slice in place and a microfluidic channel to perfuse the upper surface.

An important, and novel, feature of our chamber is the inclusion of electrode access ports into the otherwise enclosed microfluidic environment surrounding the brain slice. Each port is an opening in the channel wall that connects the interior of the device with the exterior. The hydrophobic properties of the PDMS, combined with the small dimensions of the electrode access ports, create surface tension "virtual walls" at these openings. Through these virtual wall access ports, electrodes can be inserted into the slice and the perfusion bath without causing fluid to spill out of the microfluidic channels, maintaining laminar flow over both surfaces of the slice. It is important to note that this principle is not limited to the top surface of the chamber – in our device, an opening in the lateral wall of the second PDMS layer allows a multisite linear-array recording electrode to horizontally penetrate the edge of the slice, parallel to the surface. This reproduces the electrode placement used in *in vivo* experiments (Buzsáki, 2004; Ward et al., 2009), and would be difficult to accomplish using conventional perfusion chambers.

2.2. Fabrication procedure

The process for forming each PDMS layer is based on standard photolithography and microfabrication techniques utilizing silicon master molds (Jo et al., 2000). For this device, we use a monomer:crosslinker ratio of 10:1. The monomer and crosslinker are mixed by hand until the mixture becomes opaque with bubbles, and then degassed in a vacuum chamber to remove bubbles before being pouring onto the mold. The PDMS is cured at 95 °C for 2 h and slowly cooled to 40 °C before removing the solidified polymer from the mold.

Master molds for each layer are photo-patterned with ultraviolet light. First, a silicon wafer is spin-coated with a photoresist (SU8 2100, MicroChem Corp., Newton, MA) according to the manufacturer's specifications, and heated to 65 °C for 7 min and 95 °C for 1 h in a pre-exposure baking step. Next, the wafer with photoresist is exposed to ultraviolet light ($\lambda = 365$ nm) through a printed mask containing the design for the layer. Typically, a silicon master is a negative of the desired structure, and the final PDMS features are molded directly from this master. The middle PDMS layer is formed from this type of conventional negative master mold. In contrast, the top and bottom layers make use of a double casting process that serves to increase the fabrication yield of the high-aspect-ratio micropost arrays (Blake et al., 2007; Sniadecki and Chen, 2007). The features of the bottom and middle layers are of a uniform thickness, so a single coating of photoresist is sufficient. For the top layer, a second photoresist spin and UV exposure is used to create the electrode access ports on the top surface of the device (Fig. 2D). Following UV exposure, the wafers are post-exposure baked at 65 °C for 5 min and 95 °C for 20 min. Finally, the unexposed photoresist is removed with a developer solvent to form the master mold. A schematic of the procedure is shown in Fig. 2.

Next, PDMS is poured onto the silicon master molds and cured. Since the silicon wafers for the top and bottom layers were exposed to *positive* images of the desired structure, curing polymer on these masters yields a flexible *negative* PDMS master mold (Fig. 2D). A silane gas treatment of the PDMS master prevented uncured PDMS from adhering to the surface (Sniadecki and Chen, 2007). Afterwards, these masters can be used to form the final top and bottom PDMS layers (Fig. 2B). For these layers, the channel depth and micropost height are 250 μm, which is defined by the thickness of the photoresist layer on the original silicon master. The double casting process, which utilizes a flexible PDMS master, is useful because the microposts (250 μm diameter) are difficult to release from a conventional rigid silicon master mold (Sniadecki and Chen, 2007). The middle layer, which houses the brain slice, requires a thickness matched to that of the tissue (in our case 500 μm) (Wu et al., 2005), which is also defined by the depth of the photoresist spin-coated onto the silicon wafer. To create a PDMS layer of uniform depth with a fully-penetrating space for the slice, we forced