STROKE ON A CHIP: SPATIAL AND TEMPORAL CONTROL OF OXYGEN FOR IN VITRO BRAIN SLICES

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ABSTRACT

A new device docked to a standard off-the-shelf perfusion chamber is used to deliver spatially defined, transient hypoxic conditions to a brain slice. When compared to traditional methods, our diffusion device allows complete spatial and temporal control over the oxygen environment with microscale precision, something that is not possible with any current method. The ability to deliver localized oxygen stimuli to an *in vitro* model will undoubtedly motivate advances in stroke research, as well as in other tissues where cyclic oxygenation can provide further insight into the behavior of the body.

KEYWORDS: Microfluidics, Oxygen, Stroke, Brain Slice, Perfusion Chamber

INTRODUCTION

Current methods used to expose brain slices to hypoxic environments use a perfusion driven oxygen delivery where oxygenated artificial cerebral spinal fluid (aCSF) is alternated with deoxygenated aCSF [1]. However, perfusion lacks spatial control and in using a strictly perfusion method, it is difficult to determine the difference between a global and local response, as in a stroke.

In order to address this problem, we designed a microfluidic add-on to a commercially available perfusion chamber that diffuses oxygen throughout a thin membrane and directly to the brain slice. A microchannel is responsible for the rapid and efficient oxygen delivery and can be modified to allow different regions of the slice to experience different oxygen stimuli.

Previously, we have developed other modified perfusion chambers to control the spatial and temporal delivery of chemical stimuli to *in vitro* brain slices [2]. In addition, we have also used microfluidic oxygenation devices for adherent mammalian cell studies [3]. This current work leverages these past successes to develop a system to control oxygen in thick tissue slices and demonstrates a new method for neuroscience investigations.

THEORY

The microfluidic add-on consists of 4 independent parts: a glass slide for support, a microfluidic channel that delivers the oxygen (250 μm thick), a thin membrane (100 μm thick), and a commercially available perfusion chamber (Warner Instruments, RC-26GPL). The thickness of the membrane allows oxygen to diffuse from the microchannel to the brain slice/aCSF in a fast and uniform way. The oxygen gas is supplied at a rate of 38ccm to the microfluidic gas channel in order to allow good diffusion throughout the membrane without distending the membrane. The perfusion chamber allows the slice to be completely submerged under the aCSF while the PDMS membrane provides a mechanically stable surface for the tissue.

EXPERIMENTAL

The microfluidic channel and the membrane were fabricated out of the elastomer polydimethylsiloxane (PDMS) using soft lithography as previously described [3]. Alignment marks were used to create holes in the PDMS membrane and in the perfusion chamber in such a way that they allowed the oxygen to flow into and out of the microfluidic channel. Once the individual parts were aligned, they were irreversibly bonded to complete the device.

A hand-held optical sensor (Neofox, Ocean optics) was used to determine the oxygen concentration inside the brain slice. The sensor was calibrated according to the manufacturer's instructions, namely, 95% N_2/ 5% CO_2 and 95% O_2/ 5% CO_2 was used to represent 0% O_2 and 95% O_2 respectively. Once calibrated, the oxygen sensor is able to obtain oxygen readings in liquid and solid environments.

The Animal Care and Use Committee at the University of Illinois Chicago approved all of the procedures outlined here. Experiments were carried out on male and female wild type C57BL/6 mice at P17-22 (late postnatal). 350μm thick hippocampal slices were cut with a tissue slicer (Vibratome Series 1000 Classic) along the horizontal plane. They were stored in chilled slicing solution for 35 minutes and then in aCSF for 25 minutes.

RESULTS AND DISCUSSION

Validation of the Device Using an Oxygen Sensor

The microfluidic add-on allowed complete temporal control over the hypoxic insult and is able to reach greater differences in oxygen concentration when compared to the perfusion method, as shown in Figure 1. During 4 minutes of hypoxia, the device is capable of creating a hypoxic environment in less than four minutes and is able to revert back to its initial settings in

the same amount of time compared to perfusion, which requires over eight minutes to equilibrate. It is also capable of achieving a level of hypoxia of 9% as compared to the perfusion method, which was only able to achieve 22%.

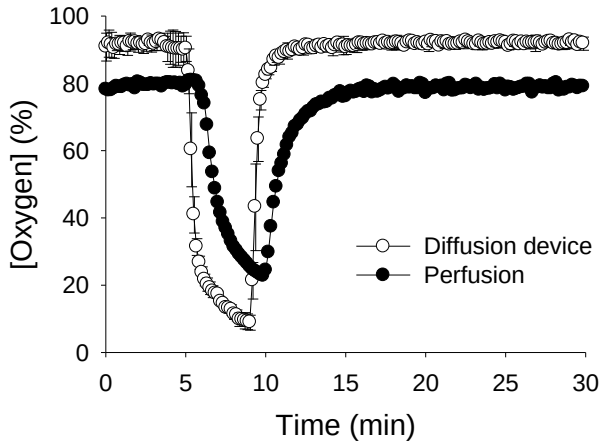

Figure 1: Differences between the diffusion device and perfusion. Oxygen concentration values in aCSF demonstrate that the device obtains a more controlled as well as a bigger change in oxygenation.

Constant Oxygen Environment

Oxygen concentration values measured inside the brain slice demonstrate that our device created a gradient as the diffusion distance increased. As shown in Figure 2A, This gradient moved in the opposite direction as the gradient produced by the perfusion method. By using the perfusion method and the device, a relatively uniform oxygen environment is created in the brain slice with the device dominating on the lower part of the slice and the perfusion dictating oxygen concentration on the top of the slice. From our results, we demonstrated that even though the device does not create a uniform high oxygen environment, it is capable of producing a uniform hypoxic environment throughout the entire slice with only an 8% difference from the bottom to the top of the slice, while perfusion creates a gradient of over 20% from top to bottom (Figure 2B). These data suggest that the device is both capable and efficient at producing hypoxic insults on the brain tissue in a well-controlled manner superior to current methods.

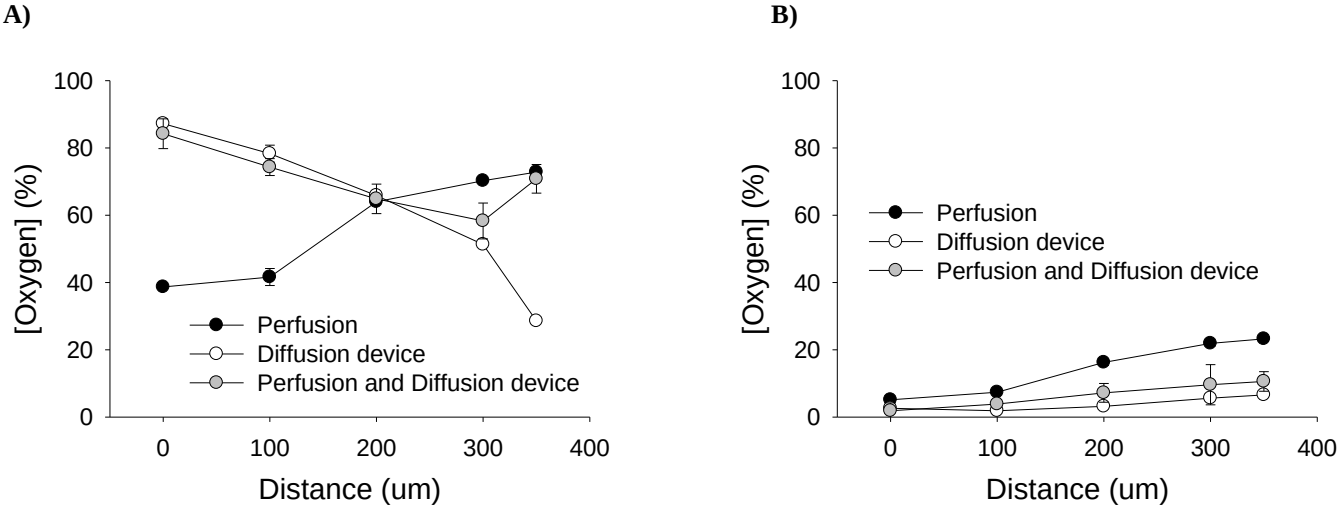

Figure 2: Oxygen concentrations at a depth of 0, 100, 200, 300, and 350μm were measured. Perfusion and device measurements are recorded. Figure 2a shows the maximum concentrations obtained at different distances. Figure 2b shows the minimum levels obtained from the different methods.

Spatial Control Over the Oxygenated Region

We determined the limits of the second design of the microfluidic add-on by measuring the oxygen concentrations in the aCSF at certain distances away from the microfluidic channel walls. While 95% oxygen gas was flowing in one channel, in

the adjacent channel 0% oxygen was flowing. As shown in Figure 3, the oxygen measurements taken show a steep change from one microfluidic channel to the adjacent one. This clearly demonstrates the ability to deliver hypoxic stimuli with microscale precision and on time scales similar to in vivo stroke events.

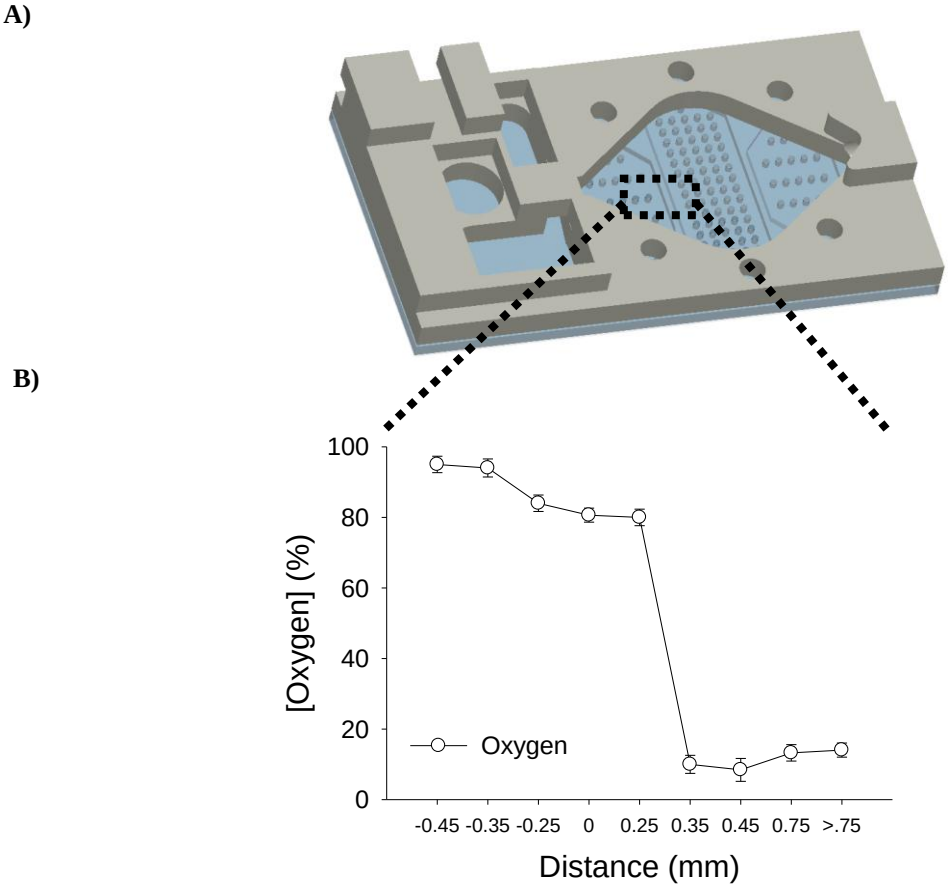

Figure 3: Schematic and validation of a microfluidic device capable of spatio-temporal oxygen control. A) Image showing the completed microfluidic device used to oxygenate different regions of the brain. B) As shown, the oxygen measurements take a steep change from one microfluidic channel to the adjacent one.

CONCLUSION

Until now, localized deoxygenation of a brain slice was not possible in a way suited for stroke research. Using our microfluidic device, we can adjust the spatial oxygenation conditions inside the brain slice within a matter of minutes. Stroke research is a prime candidate to take advantage of this technology where having the ability to control the oxygen environment of different brain regions independently of each other will lead to more insightful research into more relevant neuronal circuits.

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