3D PRINTED MICROFLUIDIC DEVICES FOR OXYGEN CONTROL IN CELL CULTURE

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ABSTRACT

Recently 3D printing has emerged as a method for directly printing complete microfluidic chips, although printing materials have been limited to non-oxygen permeable materials. We demonstrate the addition of gas permeable PDMS (Polydimethylsiloxane) membranes to 3D printed microfluidic devices as a means to enable oxygen control cell culture studies. Two devices are presented and characterized.

KEYWORDS: 3D printing, Oxygen Control, Cell Culture

INTRODUCTION

3D printing of microfluidic devices enables rapid, one-step fabrication of complex designs infeasible to make with planar lithography and replica molding techniques. In addition, planar lithography is time consuming, requires specialized equipment and facilities, and has a high failure rate. On the other hand, 3D CAD printing allows for unambiguous specifications and nearly eliminates time and effort spent on fabrication which is outsourced to a 3D printing company for around $100 per device [1, 2]. Printing is currently limited in choice of materials when compared to MEMS style fabrication. As of yet there is no widely available methods or materials to facilitate direct printing of gas permeable materials. Here we report on the development of 3D printed microfluidic devices for the control of oxygen in cell culture microenvironments. Two devices are demonstrated: an open-well petri dish design and a device that nests into a 24 well culture plate.

EXPERIMENTAL

The 3-inch (75 mm) petri dish device contains a single 500 µm wide channel following a serpentine path leaving 500 µm of spacing between channels and has integrated hose barbs directly printed onto the device. The 3D part is printed with the channels embedded in the bottom of the petri dish. A 120 µm thick PDMS membrane is adhered across the channels by spin coating a thin layer of PDMS on the membrane and allowing it to cure in place. The device is designed so that a standard 3" petri dish lid may be used. This device allows cell culture in a large open well format compatible with assays requiring cell scraping. The membrane is easily peeled away allowing additional fixing/staining assays to be performed and can also be replaced with a new membrane to reuse the device.

In addition a device was designed to integrate with a multiwell format, specifically a 24 well plate. The 24 well plate insert is designed to control gas in 6 wells of a 24 well culture plate from one input, borrowing the working principle of previous work [3] and also incorporates integrated hose barbs to simplify device operation. The pillars extend into each well leaving a ~500 µm gap for media between the diffusion membrane and the culture surface at the bottom of the well. Diffusion occurs rapidly across this gap allowing control of the dissolved gas environment around the cells. The 6 well design is a prototype intended to be expanded to control all 24 wells. A distribution network stems from the central input that equalizes the flow along each path length by varying the channel width leading to the proximal, intermediate, and distal wells.

The device also features a 'pipe within a pipe' design so that gas flow enters and leaves the diffusion area in a uniform, and symmetrical flow pattern, which would not be possible with standard lithography and demonstrates the capabilities of 3D printing (Figure 1c). Again the membranes are adhered with a thin layer of PDMS on the membrane and allowed to cure in place.

Both devices were printed by Fineline Prototyping in Watershed XC using stereolithography. 3D CAD models were designed and printed with microfluidic delivery channels, and then completed by adhering a gas permeable membrane of PDMS to enable diffusion of gas to the culture area.

RESULTS AND DISCUSSION

Oxygen was measured in the 3” petri dish device with a commercial fiber-optic probe (Figure 2). Oxygen control was demonstrated with gas flowing through the channel under the PDMS membrane.

The 24 well insert device was quantified with a platinum based (PtOEPK) planar oxygen sensor as shown in Figure 2. Oxygen control was able to be controlled in each of the 6 wells equally from one gas input demonstrating the distribution network. The device depletes the wells of oxygen for 30 minutes and can then hold the oxygen level near 0% indefinitely, while 100% nitrogen is pumped through the device.

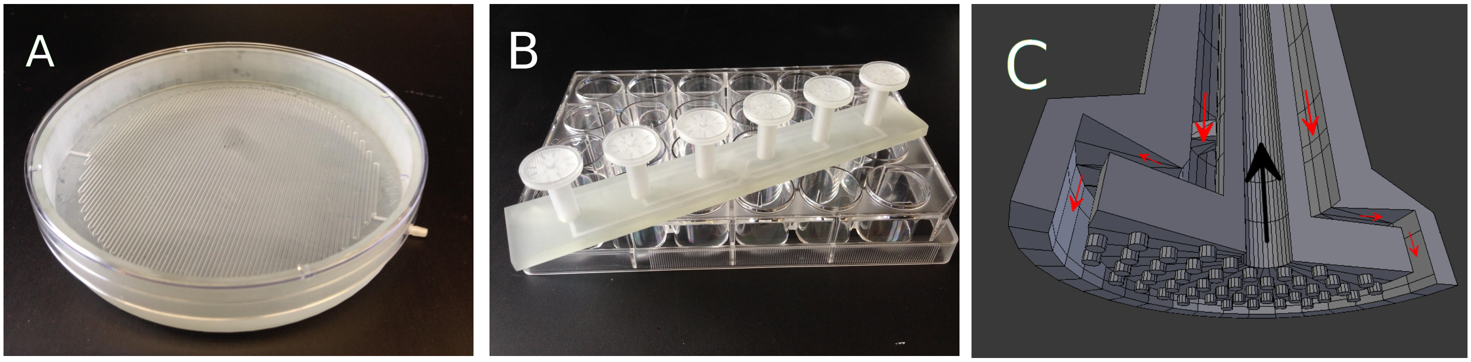

Figure 1: 3D printed devices. (A) 3” petri dish device. (B) 6 well prototype of 24 well plate insert. (C) Cross sectional CAD rendering of ‘pipe in pipe’ design. Red arrows indicate flow to the diffusion chamber and the black arrow is exhausted gas.

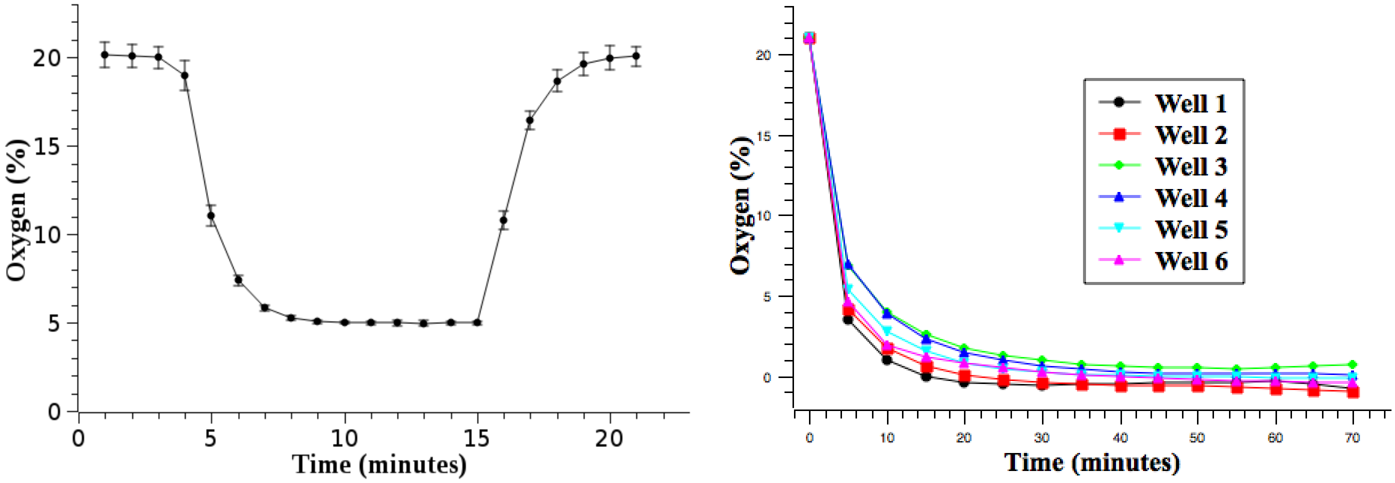

Figure 2: Preliminary oxygen characterization data. (Left) Oxygen control in the open well petri dish device measured with a commercially available fiber optic oxygen probe. Error bars are the standard deviation, N=3. (Right) Oxygen control in 6 wells of a 24 well plate from a single gas input. Intensity measurements were taken from a platinum(II) octaethylporphyrinketone planar sensor spaced 500 µm away in a standing well insert and analyzed with the Stern-Volmer relationship.

CONCLUSION

3D printing microfluidic chips have been limited to non oxygen permeable materials. We demonstrate oxygen control in 3D printed devices the addition of a gas permeable PDMS membrane. Oxygen control is demonstrated in a open well petri dish design and a 24 well plate.

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