MICROSCALE CONTROLLED CONTINUOUS CELL CULTURE

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ABSTRACT

Continuous culture experiments provide steady state conditions where cellular activity can be measured reproducibly. Experiments are generally prohibitive due to the cost and volume of growth media for long term experiments. Microfluidic devices are well suited to reduce volume requirements for experiments, but controlling volume in elastic devices is challenging. We propose a plastic-PDMS device for integrating valves on chip while maintaining structural rigidity. The device demonstrates flow control, mixing, and volume consistency over long term operation and an example 2 week long continuous culture experiment is shown.

KEYWORDS: Chemostat, Turbidostat, Cell culture, Plastic microfluidics

INTRODUCTION

Measurements of metabolic activity through substrate and product interactions or cellular activity through fluorescent interactions are highly dependent on environmental conditions and cellular metabolic state. For such experiments to be feasible, continuous cultures [1] are utilized, but since medium must be replenished every doubling time, costs can be prohibitive. An integrated microscale bioreactor with built-in fluid metering for environmental control will enable controlled and programmed experiments capable of generating reproducible data routinely.

DESIGN AND FABRICATION

Microscale continuous culture has been explored previously [2,3], however small volumes greatly limit on-chip and off-chip measurement techniques. In Baggalalde et.al., 16nL volumes make microscopy necessary and on-chip environmental sensors difficult to integrate. In Zhang et.al., 150µL microreactors enabled on-chip sensors but had no integrated flow control. In both devices, typical 1 mL sample collection time required for off-line analysis was long (10 years and 10 hours respectively) for a 1 hour cell doubling time due to small volumetric flow rates. In addition to flow control, continuous culture devices must maintain consistent volume to ensure steady state conditions since drift in volume due to evaporation or pressure results in drift in dilution rates. To meet these requirements, devices are made out of rigid polycarbonate. Active valves and pumps are integrated using PDMS membranes bonded between polycarbonate layers as shown in Figure 1 and has been generally described in Ref. [4]. Machined polycarbonate pieces are chemically treated with NaOH to introduce surface carboxyl groups. Then the surfaces are coated with Bis-triisopropoxy-silyl-propylamine, and bonded to plasma-treated PDMS. Repeating this process on multiple layers allows for multilayer microfluidics.

The device shown in Figure 2 contains a 1mL growth chamber, peristaltic pump, and 8 fluid inputs. The mL volume enables adequate sample collection times and provides space for fluorescent sensors. A PDMS peristaltic mixer covers the growth chamber [5] and provides mixing

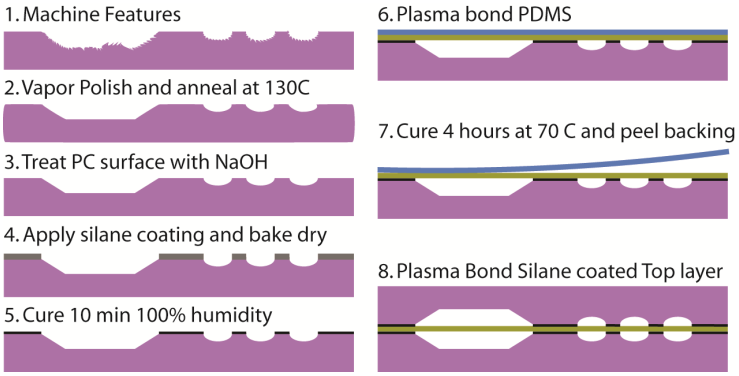

Figure 1: Fabrication process for making polycarbonate-PDMS membrane devices.

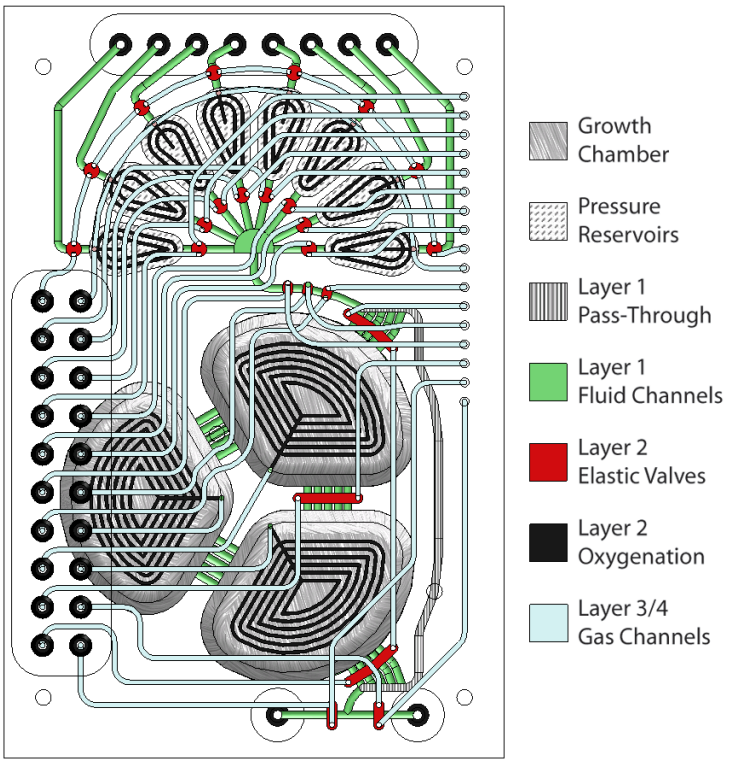

Figure 2: Schematic of the continuous culture chip.8 fluid inputs are shown at the top of the chip and 20 pneumatic connections are shown on the left. External gas and fluid lines are connected through machined hose barbs.

and oxygenation. On-chip pressure reservoirs maintain consistent backpressure and remove off-chip pressure variations. A single peristaltic pump is connected to all inputs through equal length channels for consistent fluid injection volumes. Valves between the pump and reservoirs control which input is connected to the growth chamber. To ensure equal in-flow and outflow volumes, fluid is injected into a non-deformable 26μL pass-through channel rather than directly into the growth chamber. The device operates in two modes, injection mode and mixing mode. In injection mode, the growth chamber is disconnected from the pass-through and the pass-through connects the reservoir to the waste. Any cells in the pass-through are sent to waste or sample collection as new media is pumped through. In mixing mode, the reservoir and waste are disconnected from the pass-through, and the pass-through is reconnected to the growth chamber where the newly injected media is mixed with the existing cells. In addition, evaporation is eliminated through an additional mode which replenishes lost fluid volume as shown in Figure 3. During evaporation compensation, the growth chamber is reset to the initial inoculation condition and water is injected from the pressurized water reservoir until equilibrium is reached. This ensures that the volume remains consistent. The device operates by having two of the three growth chamber sections full at any given time. By deflating individual sections in sequence, the liquid is simultaneously mixed and oxygenated by the PDMS membrane from above.

RESULTS

To demonstrate the ability to control the input concentration, ratios of blue dye and water are mixed in the chip using the peristaltic pump. Consistent volume injections are shown in Figure 4 with blue dye and DI water inputs. Measurements of the dye concentration are performed by a transmission measurement through the chip using a 585 nm

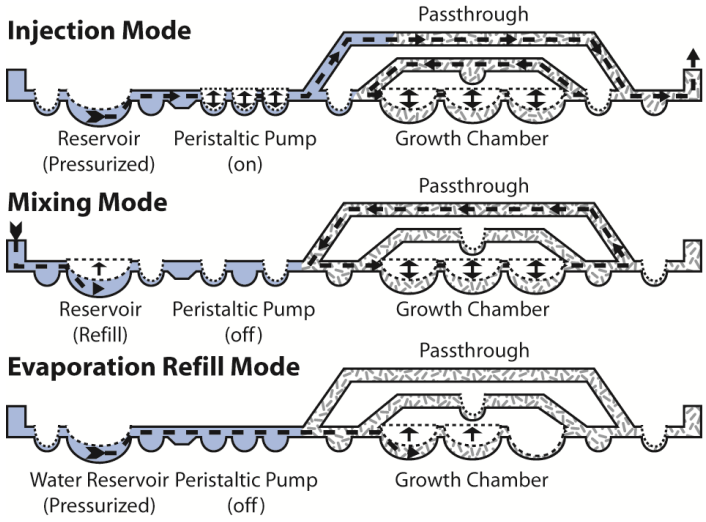

Figure 3: Diagrams of the three operating modes for the continuous culture device. In injection mode, the growth chamber is disconnected from the inputs and fluid is injected into the pass-through. In mixing mode, the pass-through and growth chamber are connected to mix newly injected contents into the chamber. In evaporation refill mode, the growth chamber is connected directly to the water reservoir to replace any lost fluid.

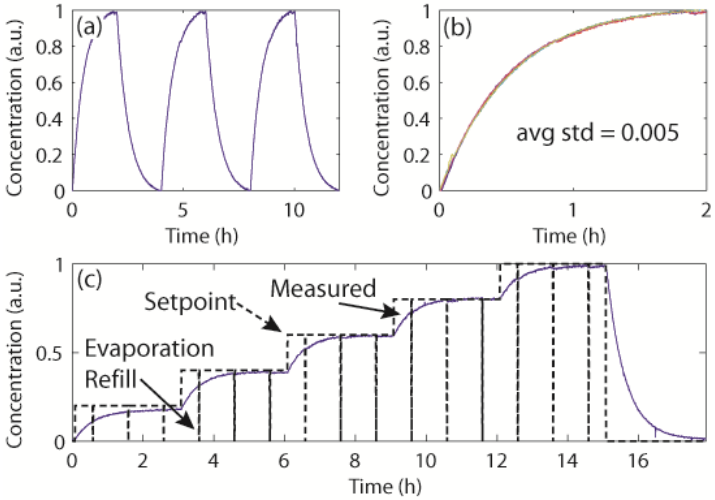

Figure 4: (a) Plot of the step response between an input with dye and an input with water. (b) The 3 dye and 3 water step responses are overlaid to show consistency. (c) Control over injected inputs is demonstrated by varying the concentration of dye in the growth chamber.

LED. To demonstrate injection volume consistency between different inputs, multiple step responses between DI water and blue dye are shown in Figure 4a. Comparing the dynamics of the step response in Figure 4b shows only a 0.5% variation between different steps. Arbitrary mix ratios are also shown in Figure 4c where the dye concentration is varied from 0 to 100 percent in 20 percent increments.

Culture experiments are shown in Figure 5 using E. Coli ATCC31883 in 5g/L glucose and defined medium with 100µg/ml ampicillin. Cell density is measured with a forward scattering optical sensor through the 800 µm deep pass-through channel at 585nm. The system is initially run in batch mode to demonstrate cell viability within the reactor, then run in various continuous modes. A steady state without oxygen control is first demonstrated and the lower pH reflects a slightly anaerobic environment. After oxygen control is turned on, both the cell density and the pH increases indicating more aerobic growth conditions. To demonstrate control over fluid flow as well as the ability to measure cell density on-line, the cell density is then maintained at OD 2 by adjusting the input flow rate. Turbidostatic conditions are demonstrated since the flow rate reaches steady state at the maximum growth rate of 0.79 h^{-1}. Finally, the buffering capacity is changed during a media change and chemostatic conditions are re-established. The reduced pH reflects the reduced buffering capacity.

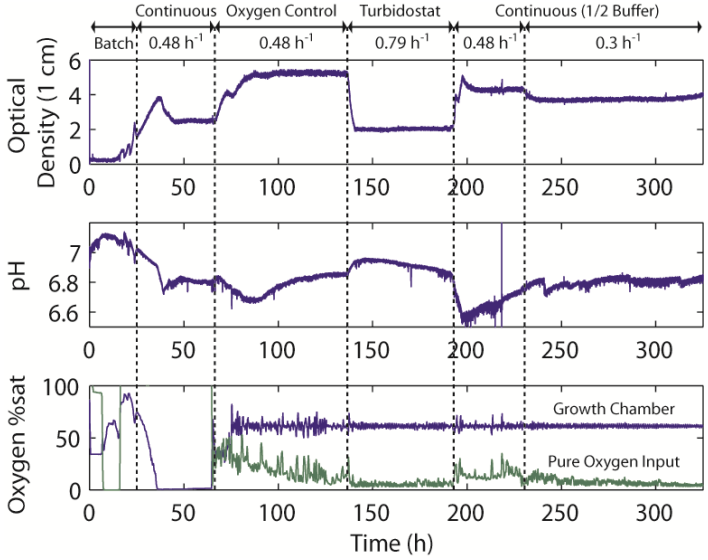

Figure 5: Demonstration of a 2 week continuous culture in the microreactor. After initial batch growth, the device is switched into a chemostat mode with a dilution rate of 0.48 h^{-1} without oxygen control. At 60h, oxygen control is turned on. At 140h turbidostat mode is enabled, where the cell density is controlled at OD=2 by varying the injection rate. At 195h growth media is switched to half buffer capacity and run in chemostat mode at the original dilution. At 230h the dilution rate is reduced to 0.3 h^{-1}.

CONCLUSION

In summary, we present a plastic-PDMS microfluidic device capable of sustaining continuous culture conditions for over 2 weeks. Flow control is demonstrated through dye measurements and continuous culture is verified through a 2 week long growth of E. coli ATCC31883. With an operating volume of 1 mL, the output stream can also be used for off-line chemical analysis.

ACKNOWLEDGEMENTS

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