LONG-TERM STEADY STATE PERFUSION CULTURE OF MAMMALIAN CELLS USING A ROBUST MICROFLUIDIC CELL RETENTION DEVICE

T. Kwon[1], N. Madziva[2], J. D. Oliveira[2], S. K. Chandramohan[2], L. Yin[3], H. Prentice[4], M. E. Warkiani[5], J.-F. P. Hamel[2], and J. Han[1,3,6*]

[1]Department of Electrical Engineering and Computer Science, Massachusetts Institute of Technology, USA

[2]Department of Chemical Engineering, Massachusetts Institute of Technology, USA

[3]BioSystems and Micromechanics (BioSyM) IRG, Singapore-MIT Alliance for Research and Technology (SMART) Centre, Singapore

[4]H Prentice Consulting LLC, USA

[5]School of Mechanical and Manufacturing Engineering, University of New South Wales, Australia, and

[6]Department of Biological Engineering, Massachusetts Institute of Technology, USA

ABSTRACT

Cell retention devices are used to retain cells in containers during perfusion culture. However, the conventional membrane-based filtration devices have challenges such as membrane fouling/clogging and increased contamination risk due to frequent filter replacements. To solve these challenges, we introduce a new microfluidic cell retention device based on inertial cell focusing. We demonstrated a long-term steady state perfusion culture of suspended CHO cells, where high density of cells (> 3×10^6 cells/mL) and viability (> 90%) were maintained for more than a week. Our membrane-less and clog-free cell retention device has unique advantage over the conventional filtration devices for perfusion culture.

KEYWORDS: Perfusion culture, Inertial microfluidics, Filtration, Mammalian cells

INTRODUCTION

In pharmaceutical industry, continuous perfusion culture of CHO and other cells (e.g. yeast) is preferred and widely used for a large-scale production of drug molecules. In perfusion culture, nutrients are supplied and by-products are removed to facilitate cell growth. This requires cell separation devices to retain cells in culture containers. While technologies such as membrane filtration and centrifugation are available for a large, production scale perfusion culture currently used in pharmaceutical industry [1], achieving a reliable lab scale (0.1-1 L) perfusion culture is highly desirable for optimizing culture conditions and other parameters [2]. However, conventional filtration membranes are not capable of enabling truly steady-state perfusion culture, due to eventual, unavoidable fouling and clogging of filter membranes [1]. Moreover, frequent filter replacements increase the risk of bacteria or fungi contamination.

At the MicroTAS 2014 meeting, our group introduced a new filtration microfluidic device based on inertial focusing [3]. By using stacks of PDMS spiral microchannels, we demonstrated high-throughput filtration for cell culture. In this paper, we present long-term (> 200 h), continuous (steady state) perfusion culture of suspended mammalian (CHO) cells, using a robust microfluidic cell separation device utilizing inertial microfluidics principles. Our clog-free separation device has unique advantage over the conventional filtration devices for establishing lab-scale perfusion culture.

EXPERIMENTAL

The microfluidic cell retention devices were fabricated from polydimethylsiloxane (PDMS) using the standard soft lithography [4]. For PDMS casting, an aluminum mold was manufactured by micro-milling (Whits Technologies, Singapore). A drilled glass slide with holes was placed on top of the PDMS-glass

chip and they were clamped together by binder clips. The device was sterilized with bleach (10%) and ethanol (70%), and it was connected to the spinner flask containing CHO cells. These cells were incubated in a conventional humidified incubator and a peristaltic pump (Cole-Parmer, USA) flowed cell solution to the spiral retention device. Cell density and viability were monitored by a cell culture analyzer (FLEX, Nova Biomedical Corporation, USA), and glucose/lactate concentrations were measured by a biochemistry analyzer (YSI 2700, YSI, USA).

RESULTS AND DISCUSSION

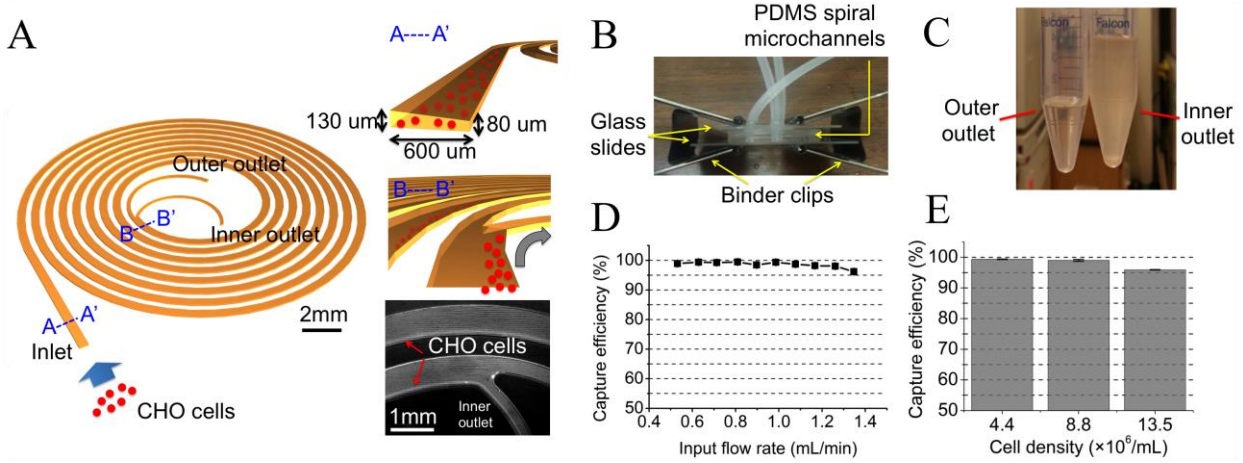

Figure 1: (A) The schematic of the spiral microfluidic channel. Most cells were focused into the inner wall in the spiral channel whereas cell-free solution was collected in the outer outlet. (B) The glass-PDMS-glass sandwich spiral microchip for robust operation. (C) Sample solution from spiral channel outlets. (D) Capture efficiencies according to different input flow rates. Two devices were used. (E) Capture efficiency dependence on input cell density.

Figure 1A describes the schematic of the spiral microchannel. Cells flowing in a spiral microchannel experience inertial lift and Dean drag forces [4]. Combination of these forces makes cells occupy their equilibrium positions based on their sizes in the channel [4]. CHO cells injected into the channel were focused near the inner wall. They were guided to the inner outlet whereas cell-free solution was collected in the outer outlet. The microscope image shows the focusing behavior of Acridine Orange-stained CHO cells at the input flow rate of 1 mL/min. The glass-PDMS-glass sandwich devices were used to minimize PDMS channel deformation and thus enable robust cell retention as described in Figure 1B. The retention device maintained high capture efficiency (> 98%) in the range of 0.6-1.2 mL/min.

Figure 2A describes the schematic of continuous perfusion culture using a microfluidic separation device. CHO cells were grown in a 500 mL spinner flask with working volume of 250 mL. Culture medium was continuously supplied to the flask using a peristaltic pump. Through the microfluidic retention device, cells were retained in the flask and the cell-free waste/by-products were harvested in a separate bottle. The perfusion rate was set to 0.69 VVD (volume of fresh medium/working volume of reactor/day) and flow rate of 0.7 mL/min was used to retain cells in the inner outlet with high capture efficiency (> 95%). Figure 2B and 2C show the results for the perfusion culture, where all the critical parameters get stabilized after ~100 h, and maintained there for more than 200 h without any apparent clogging and other issues. The cell viability and viable cell density were kept ~ 90% and 3 million cells/mL, respectively. Moreover, glucose and lactate concentrations were also saturated during the perfusion culture. In contrast, a typical perfusion culture utilizing membrane (ceramic) filter had a significant clogging problem (Figure 2F). Currently we are carrying out a detailed comparison between the two, at the same perfusion rate.

The saturated viable density of ~ 3 million cells/mL around 72 hours is low compared to the value (> 10 million cells/mL) of conventional perfusion cultures. One possible reason is insufficient oxygen level for further cell growth during exponential growth phase because oxygen of the atmosphere was supplied only through the slightly open cap of the spinner flask without control. Since there was sufficient glucose

available for cell growth and the lactate concentration was less than inhibitory level during exponential growth phase, more oxygen supply is expected to increase the steady state cell density. Currently, implementation of better oxygenation is in progress by supplying oxygen directly to the spinner flask with higher stirring RPMs and fabricating microfluidic PDMS oxygenators.

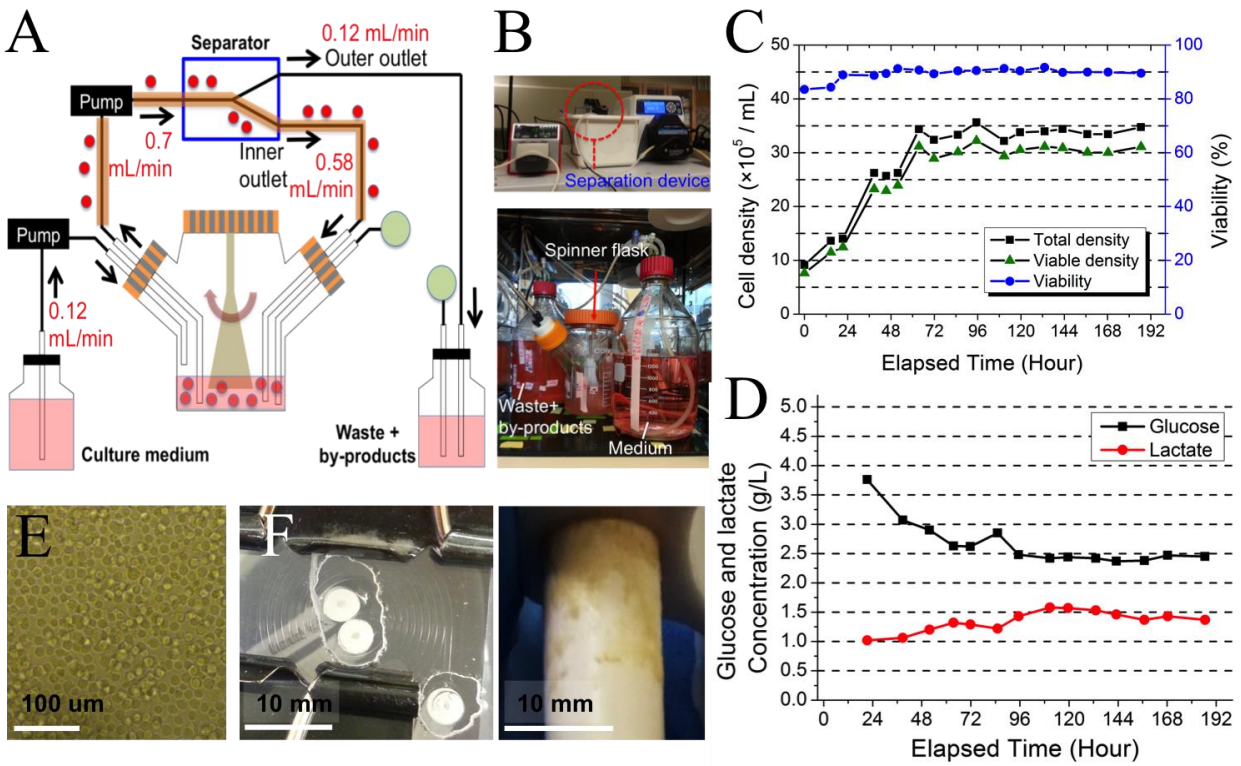

Figure 2: (A) The schematic of the continuous perfusion culture. (B) Actual perfusion culture setup. (C) The cell density and viability profiles. (D) The nutrients and metabolites profiles. (E) Sampled cells at day 7. (F) Microfluidic cell retention device, after 200 h continuous operation. No visible sign of clogging was found (left). Typical filtration membrane for cell retention in continuous culture (after 169 h operation) showing significant clogging (right; ceramic rod membrane after perfusion culture).

CONCLUSION

We demonstrated a new perfusion culture system using a microfluidic cell retention device. It maintained high density of cells and viability for more than a week in a stable and steady state condition where viable cell density, nutrient consumption, and lactate consumption were maintained stably. Our microfluidic cell retention device is membrane-less and clog-free. It has unique advantage over the conventional filtration devices for perfusion culture.

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CONTACT

* Jongyoon Han; phone: +1-617-253-2290; jyhan@mit.edu