NEXT-GENERATION MICROFILTER: LARGE SCALE, CONTINUOUS MAMMALIAN CELL RETENTION FOR PERFUSION BIOREACTORS

Majid Ebrahimi Warkiani[1], Andy Kah Ping Tay[1], Guofeng Guan[1], and Jongyoon Han[1,2,3*]

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

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

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

ABSTRACT

In this study, we report on the development of the first membrane-less, clog-free microfiltration platform for ultra-high throughput (up to 1000 mL/min) cell separation, using a massively-multiplexed array of inertial microfluidic cell sorting channels. Our developed system consists of multiple layer (10~20) of PDMS sheets with embossed microchannels (i.e., ~ 200 individual spirals) bonded together for continuous size-based cell sorting from a large volume of biological fluid. Subsequent perfusion culture experiments using the cell retention system show the potential to significantly enhance overall efficiency of perfusion cell culture.

KEYWORDS: Inertial microfluidics, Mammalian cells, Perfusion bioreactor, Antibody, Filtration

INTRODUCTION

Mammalian cell cultures are widely used in manufacturing large and complex chemicals in biotechnology [1]. Over the past decade, mammalian cells have been employed for large-scale production of various diagnostic and therapeutic products such as monoclonal antibodies [2, 3], recombinant proteins [1] (e.g., Glycoproteins) and viral vaccines [4] against polio, hepatitis B and measles. The growing demand for these products resulted in unrelenting push for the 'upstream' (bioreactor operation) improvements. Perfusion bioreactors have been used extensively for this purpose as they can sustain high cell number with continuous feeding of nutrients and removal of waste, as well as better control of pH and other conditions. A major challenge for continuous perfusion bioreactor design and operation is the cost and reliability of the cell retention device. A variety of techniques (e.g., microfilters and centrifugation) have been employed for cell retention or recycle, however none of these are without shortcomings [4]. In this study, we demonstrate that massive multiplexing of inertial microfluidics can process macroscopic scale sample volumes in a unique, continuous and clog-free particle separation process. The technique utilizes the hydrodynamic forces present in curvilinear microchannels for cell focusing and sorting [5]. Cells in our system are separated solely due to fluidic interactions driven by externally-driven flow, thus the system is inherently clog-free and can run continuously without the need for membrane filter replacement or external force fields.

THEORY

When neutrally buoyant particles flowing through a spiral microchannel, suspended particles experience inertial lift forces and Dean induced viscous drag at the same time. The combination of these forces will balance the particles at certain equilibrium positions of the channel cross-section in a continuous flow [5]. By selecting the appropriate channel dimensions and also optimum flow rates, suspended particles can be focused near outer wall and get trapped (i.e., due to presence of strong vortex) and eventually exist the device from the outer outlet (see Fig. 1A).

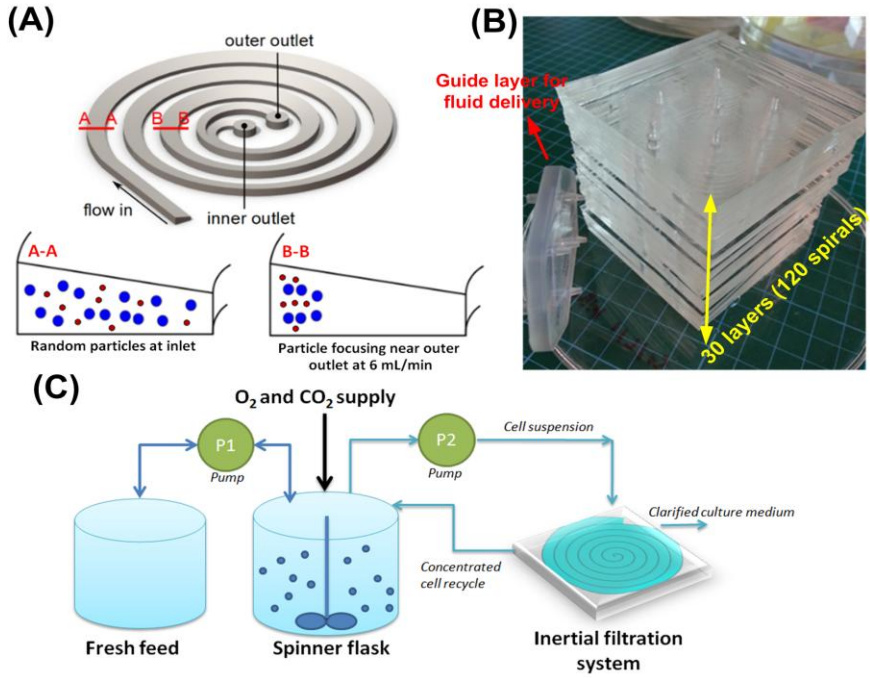

Figure 1. (A) Schematic representation of the configuration and operational mechanism of a single spiral microfluidic chip for filtration of mammalian cells with one inlet and two outlets. (B) Optical image of a high-throughput system consists of multiple layers of PDMS sheets with embossed microchannels (i.e., 120 spiral microchannels) bonded together for continues cell retention from large sample volumes. (C) Sample processing workflow showing process of cell enrichment using the high throughput filtration system from spinner flasks imitating condition of a perfusion bioreactor.

EXPERIMENTAL

The high-throughput microfiltration system was fabricated using soft-lithography techniques in poly-dimethylsiloxane (PDMS) [5]. Our molds were fabricated from aluminum using conventional micromilling described in our previous publication [5]. The device was fabricated by stacking of individual layers (embossed with spiral microchannels) together using manual alignment and oxygen plasma bonding. The fluidic inlets and outlets were punched inside the PDMS layers (4.5 mm holes) after bonding two of them together, and the final device obtained by attaching the whole assembly to a thick PDMS layer.

RESULTS AND DISCUSSION

To characterize our system while mimicking condition of a perfusion bioreactor, cell cultures were carries out using the 250 mL disposable spinner flasks inside a humidified incubator for three different cell lines. Microfiltration tests were performed daily by separating the products from cells using our inertial filtration system inside a sterilize environment while fresh media was added to each flask after each experiment along with enriched cells. Cell densities, viability, glucose, antibody titers and pH were monitored in each sample separately. Microfiltration tests using different cell concentrations revealed usefulness of our system for continuous cell separation from bioreactores with over 95% cell separation efficiency (Fig. 2), with the ability to process up to 10^8 cells/mL. The viability of the sorted cells was similar to that of the unsorted (control), with more than 90% of the cells excluding the dye suggesting minimum physical damage due to the separation. Cell productivity was also assessed by measuring activity of the secreted IgG protein using an enzymatic assay. Our results suggest sustainable growth of the cells and antibody production for a period of 10 days indicating the value of this new technology for separation of animal cells from the culture medium.

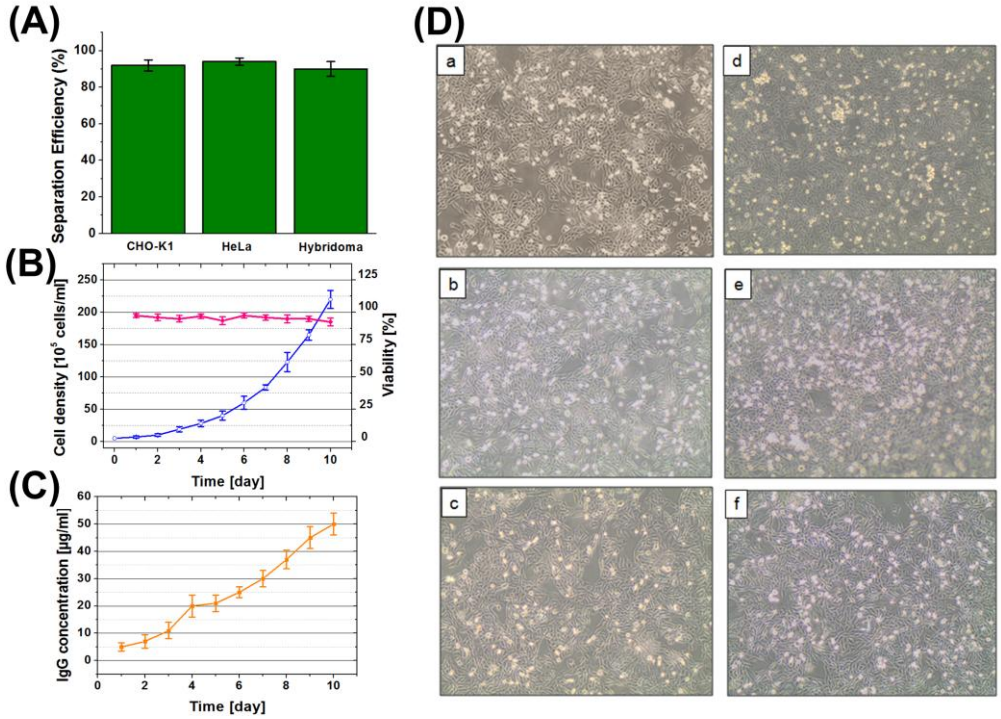

Figure 2. Characterization of the high-throughput microfiltration system for cell retention from a perfusion bioreactor. (A) Recovery efficiency of different cell lines. (B) Viability and cell densities over operation time. (C) Rate of IgG production by the Hybridoma cells over operation time. (D) Phase contrast micrographs of cultures of control (unsorted) CHO cells (a-c) and sorted cells (d-f) by inertial microfiltration system. The images indicate no significant differences between the morphology and proliferation rate of the cells suggesting high viability and sterility.

CONCLUSION

The novel microfiltration system presented here can be produced with extremely low-cost using conventional micro-milling and PDMS casting. In contrast to membrane filters, this system doesn't suffer from progressive protein and cellular fouling of the filters and can be operated non-stop for a long period without any flux decline. This platform has the desirable combinations of high throughput, low cost, scalability and small foot-print, making it inherently suited for various microfiltration applications.

ACKNOWLEDGEMENTS

Financial support by the Singapore-MIT Alliance for Research and Technology (SMART) Centre (BioSyM IRG) is gratefully acknowledged.

REFERENCES

[1] D. Low, et al., "Future of antibody purification", *Journal of Chromatography B*, 848, 48-63, 2007.
[2] G.G. Banik, C.A. Heath, "Partial and total cell retention in a filtration-based homogeneous perfusion reactor", *Biotechnology progress*, 11, 584-588,1995.
[3] Y.M. Deo, et al., "Practical Considerations in Operation and Scale-up of Spin-Filter Based Bioreactors for Monoclonal Antibody Production", *Biotechnology progress*, 12, 57-64, 1996.
[4] L.R. Castilho, R.A. Medronho, "Cell retention devices for suspended-cell perfusion cultures", in: Tools and *Applications of Biochemical Engineering Science*, Springer, 129-169, 2002.
[5] M.E. Warkiani, et al., "Slanted spiral microfluidics for the ultra-fast, label-free isolation of circulating tumor cells", *Lab on a Chip*, 14, 128-137, 2014.

CONTACT

*Jongyoon Han: +1 617 253 2290, jyhan@mit.edu