INTEGRATED PASSIVE BUBBLE TRAP FOR LONG-TERM CELL CULTURE MICROFLUIDIC SYSTEMS

Karina Ziółkowska, Iga Hofman, Artur Dybko, Zbigniew Brzózka
Department of Microbioanalytics, Institute of Biotechnology, Faculty of Chemistry,
Warsaw University of Technology, Poland

ABSTRACT

One of the major advantage of microfluidic systems is a possibility of precise control over fluid flow. However, this can be significantly disturbed by air bubbles penetrating microchannels. Many efforts are taken to improve tubing connections and tightness of experimental set-ups. However, application of gas permeable materials, such as PDMS, which are beneficial for cell cultures, is strictly connected with increased risk of air bubble occurrence. Therefore, degasing elements should be considered to be implemented in microfluidic chips made of these materials.

In this paper, we presented a simple and effective bubble trap structure. The bubble trap (b-trap) can be easily integrated with a PDMS microfluidic chip. It can be fabricated during any manufacturing process and its implementation is a low-cost solution. The b-trap's usefulness was proved during long-term cell culture.

KEYWORDS

Bubble trapping, integration, long-term cell culture, tumor spheroids

INTRODUCTION

Microfluidic systems made of PDMS have been widely used for cell culture and cell based studies recently. They present many advantages over traditional cell culture methods, *i.e.* possibilities of mimicking *in vivo* environment [1]. Control over fluid flow via microchannels leads to the ability of precise control over culture parameters. For example, stable culture conditions have already enabled four-week 3D human cell culture on-chip [2]. Long-term cell cultures are particularly useful in drug screening research, i.e. for repeated doses treatment or delayed toxicity evaluation.

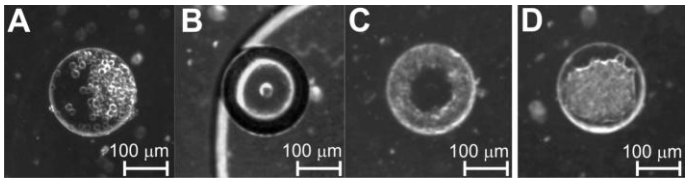

Figure 1: Effect of air bubbles on 3D cell culture: (A) cells seeded to the microwell, (B) air bubble, (C) organoid of changed morphology. (D) correct morphology of a spheroid.

PDMS is an advantageous material for cell culture applications, mostly because of its biocompatibility, transparency and gas permeability. However, application of PDMS has also some drawbacks. PDMS is permeable for air and water vapor, therefore air bubbles occur in the microfluidic systems made of PDMS. Occurrence of air bubbles often limits success of long-term on-chip cultures (Fig.1). The longer culture is, the more frequently bubbles are observed. Therefore, development of a bubble trap (b-trap) integrated with a microfluidic system is an understandable effort [3,4].

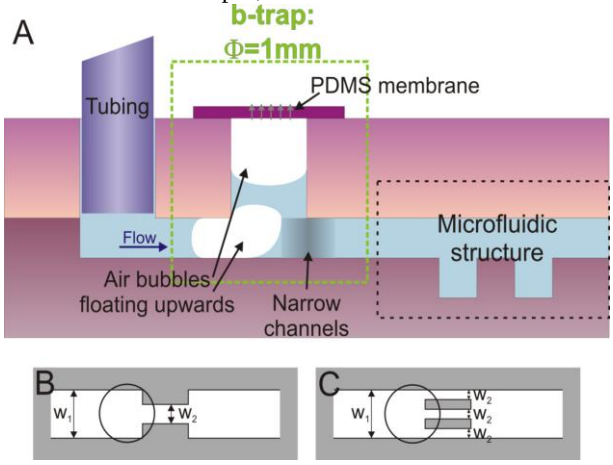

Figure 2: (A) Construction of the b-trap containing PDMS chip: bottom layer contains microfluidic structure and upper layer contains tubing connection ports and b-trap. PDMS membrane was bonded using oxygen plasma treatment. Variants of narrow channels: (B) single channel and (C) multichannel.

We present a simple passive b-trap (Fig.2), which can be easily integrated with PDMS microfluidic systems. It takes an advantage of two phenomena: (1) increasing fluidic resistance of a narrowing of a channel and (2) air bubble up-floating. Integration of our b-trap, on the contrary to already reported solutions [3,4], does not require additional fabrication steps or sophisticated machining. Moreover, the b-trap works without any external force or pumping. It can be fabricated during any manufacturing process and its implementation is a low-cost solution.

EXPERIMENT

The b-trap consists of an obstacle-containing microfluidic channel and an above, drilled-through well, sealed with a 0.5 mm thick, air-permeable PDMS membrane. Fluidic resistance can be increased by

978-0-9798064-5-2/µTAS 2012/$20©12CBMS-0001

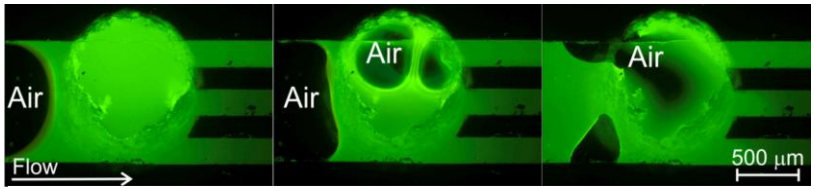

Figure 3: Sequencing frames of the experiment on bubble trapping in the multichannel b-trap.

narrowing of one channel (Fig.2:B) or the channel split into several narrow channels (Fig.2:A). A bubble pushed via the channel, when coming into the obstacles is more preferably trapped in the well than pushed via the obstacles (Fig.3). Overpressure present during medium flow provides air penetration through the membrane out of the device. It was observed, that obstacle-less b-traps were effective enough during stop-flow periods of cell-chip incubation, but were not able to catch bubbles while medium flow was applied. Different types of obstacles were considered and an increase of fluidic resistance was calculated for them (Fig.4). Experimental results demonstrated that minimum of 1.5 fold fluidic resistance increase was sufficient for bubble trapping in the well (for flow rates up to 10 µL/min). Moreover, multichannel b-traps were more effective at lower values of resistance increase than single-channel ones. A b-trap with three parallel narrow channels was chosen as optimal for cell culture device. It provided uniform cell seeding, which is a crucial factor for future applications.

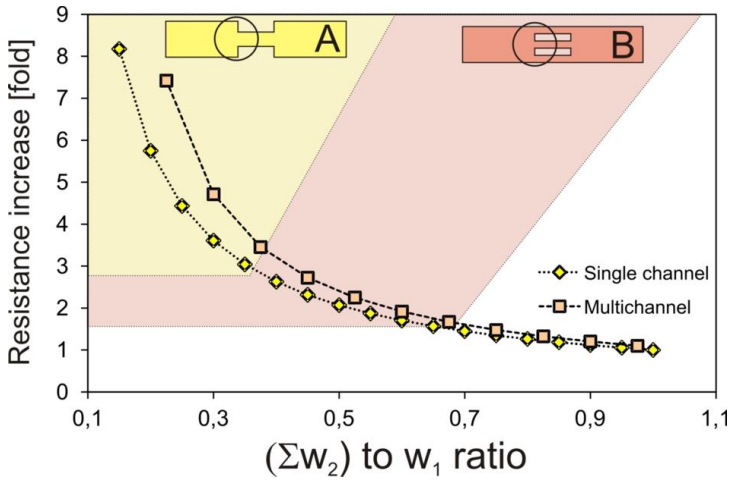

Figure 4: Results of fluidic resistance calculations for different variants of obstacles. Fluidic resistance increase suitable for effective bubble trapping according to experimental results marked for a single channel (A) and multichannel (B) b-traps.

Possible flow disturbances caused by the b-trap integration were verified using sheath-flow microsystem (Fig.5). The b-trap was placed above one of the side-stream channels and deflections of the central stream were observed. Different flow rates and different side-to-central stream ratios were examined and no significant flow disturbances were detected.

Finally, the b-trap was integrated with a microfluidic chip for long-term spheroid culture, which was presented before [2]. 3D cell culture system was fabricated using double casting in PDMS with a thermal aging step [5]. B-traps were located at the inlet microchannels (Fig.6). HT-29 cells were cultured for over three weeks in the

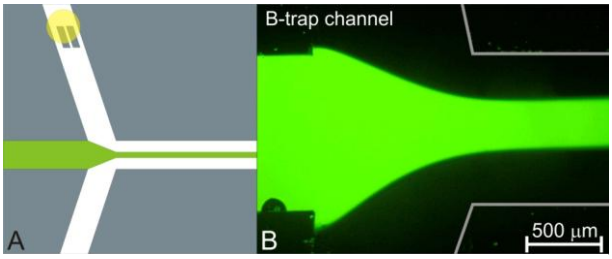

Figure 5: Study of stability of the sheath-flow zone: (A) geometry of the microsystem and (B) symmetrical sheath flow, flow rates at each inlet = 4µL/min.

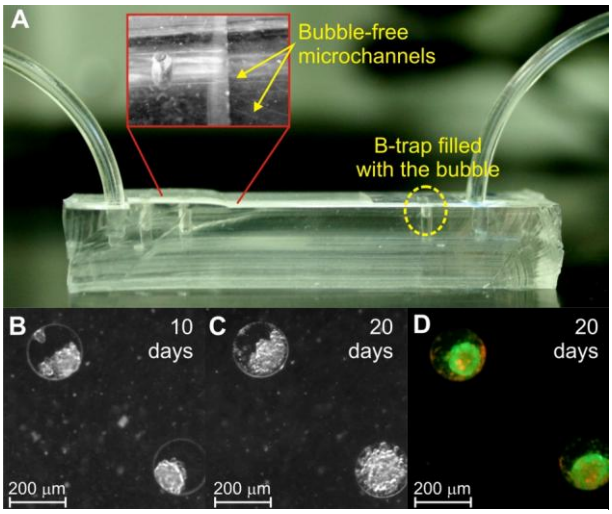

Figure 6: (A) Bubble traps integrated with the cell culture microsystem (after stop-flow 24h incubation). (B,C) Growth of correct spheroids. (D) Cell viability assay.

b-trap containing chip. Effective bubble trapping was observed within this time. Bubble-free culture of spheroids provided unimpeded growth and high viability of the cells. Experiments requiring long-term culture and utilizing cell chips integrated with the b-traps are in progress.

ACKNOWLEDGEMENTS
This work was financially supported by Polish Ministry of Science and Higher Education through "Iuventus Plus" Programme, contract No. 0643/IP1/2011/71.

REFERENCES
[1] K. Ziółkowska, R. Kwapiszewski, Z. Brzózka, *Microfluidic devices as tools mimicking in vivo environment*, New J. Chem., 35, pp. 979-990 (2011).
[2] K. Ziółkowska, A. Stelmachowska, R. Kwapiszewski, M. Chudy, A. Dybko, Z. Brzózka, *Long-term Multicellular Spheroids culture and anticancer drug activity evaluation in a microfluidic system*, Proc. MicroTAS 2011, pp. 124-126 (2011).
[3] E. Kang, D.H. Lee, C.B. Kim, S.J. Yoo, S.H. Lee, *A hemispherical microfluidic channel for the trapping and passive dissipation of microbubbles*, J. Micromech. Microeng, 20, 045009, pp. 1-9 (2010).
[4] W. Zheng, Z. Wang, W. Zhang, X. Jiang, *A simple PDMS-based microfluidic channel design that removes bubbles for long-term on-chip culture of mammalian cells*, Lab. Chip, 10, pp. 2906-2910 (2010).
[5] K. Ziółkowska, K. Żukowski, M. Chudy, A. Dybko, Z. Brzózka, *Enhancing efficiency of double casting prototyping by thermal aging of poly(dimethylsiloxane)*, Proc. MicroTAS 2011, pp. 1164-1166 (2011).

CONTACT
Karina Ziółkowska kziolkowska@ch.pw.edu.pl