AUTOMATED MEDIUM RECIRCULATION USING MACRO VALVES FOR HIGH FLOW RATES IN AN ENDOTHELIAL CELL CULTURE CHIP

Elsbeth G.B.M. Bossink[1*✉], Anke R. Vollertsen[1,2*✉], Loes I. Segerink[1†], Andries D. van der Meer[2,3 †], and Mathieu Odijk[1 †]

[1]BIOS Lab on a Chip group, University of Twente, Enschede, the Netherlands

[2]Applied Stem Cell Technologies group, University of Twente, Enschede, the Netherlands

[3]Organ-on-Chip Center Twente, University of Twente, Enschede, the Netherlands

*,† equal contribution

ABSTRACT

The vascular endothelium serves an important function in many signaling processes and throughout all of our organs. Accurately mimicking its dynamic environment *in vitro* requires a wide range of flow control. We present a microfluidic chip fabricated from rapid prototyped molds with which we can automate medium refreshment and medium recirculation using an integrated peristaltic pump. This macro valve-based pump can reach flow rates up to ~30 µL/min and over 3 × 10^6 valve actuations. Finally, we show that endothelial cells can be cultured in the device for 96 hours under peristaltic flow and with automated medium refreshment.

KEYWORDS: Peristaltic pump, micromilling, macro valve, recirculation, endothelial cells

INTRODUCTION

Endothelial inflammation plays an important role in the cytokine release syndrome (CRS) which, in severe cases, can result in multi-organ failure. Since both shear stress and the consistent exposure to paracrine signaling factors have a major impact on the integrity of the endothelium, medium recirculation in blood vessels-on-chips is essential. However, current methods involve either off-chip pumps [1] or on-chip peristaltic pumps composed of microvalves [2]. The off-chip pumps offer limited parallelization possibilities and generally have a large culture medium to cell ratio. The microvalve peristaltic pumps rely on complex photolithography for fabrication and are limited in valve size and consequently flow rate. This limits the implementation of microvalves in typical organs-on-chips, which are usually in the order of hundreds of micrometers and for modeling arteries, as the microvalve-based pump cannot reach sufficiently high shear stresses. Previously, we reported a Quake-style 'macro valve', fabricated by direct micromilling of rounded channels in the mold used for polydimethylsiloxane (PDMS) [3].

Here, we present a microfluidic chip designed for automated medium refreshment and recirculation which contains several of these integrated macro valves. We demonstrate that our macro valve-based peristaltic pump can reach pumping rates of up to ~30 µL/min. Due to our higher stroke volumes, this is 4x higher than reported microvalve-based pumps [4], despite using an order of magnitude lower actuation frequency. Finally, we provide proof-of-concept of its application in cell culture.

EXPERIMENTAL

The chip is shown schematically in Figure 1. The valves are 1 mm wide and close off rounded channels which are 200 µm high and 1 mm wide. The molds and PDMS chip were fabricated by micromilling and multilayer soft lithography, respectively, as reported in [3]. The on-chip pump consists of three valves, which can be actuated in different patterns (3- or 6-phase, Figure 2A) to achieve pumping. The pumping rate was determined by actuation of the three valves for at least 5 minutes and collecting the outflow in an Eppendorf vial which was weighed before and after pumping.

For cell culture experiments, the chip was coated with 0.1 mg/mL collagen I and GFP (green fluorescent protein)-expressing HUVECs (Angio-proteomie, USA) were seeded at 4 × 10^6 cells/mL

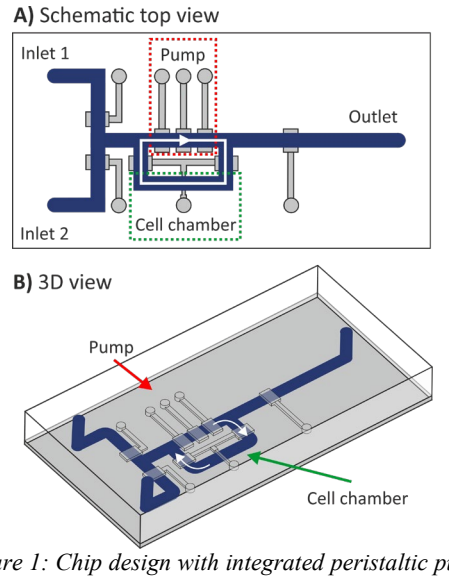

Figure 1: Chip design with integrated peristaltic pump and recirculation loop (indicated with white arrows). A) Schematic top view. B) Three dimensional (3D) view.

in the cell chamber. The peristaltic pump was programmed to run at 10 Hz with a 3-phase (011-101-110) actuation pattern. The cell culture medium in the loop was partially replaced every 2 h by automated opening of the inlet 1 and outlet valves while closing the cell chamber and pumping fresh medium in the chip for 1 min.

RESULTS AND DISCUSSION

The pumping rate was determined for two different actuation patterns, indicated as the 3-phase and 6-phase actuation, as shown in Figure 2 A. In addition, we demonstrated that our chip can be used for automated medium recirculation in cell culture experiments. To this end, the macro valves in the peristaltic pump were switched ON/OFF over 3 × 10^6 times within 96 h. No valve rupture or delamination was observed which demonstrates the robustness and suitability of this pump for (long-term) cell experiments. Figures 2B and C show the HUVECs forming a confluent layer in the channel at 96 h after seeding. The peristaltic pump at 10 Hz while using the 3-phase pattern generated a shear stress of approximately 0.01 Pa. Due to the large width of the channel (1 mm), the shear stress did not yet reach a physiologically relevant level for blood vessels (>0.1 Pa). However, this can be solved by reducing the cell chamber width to <0.5 mm in future devices and by using the 6-phase pattern. For other organ-on-chip applications, such as a gut-on-chip, this generated shear stress is already sufficient.

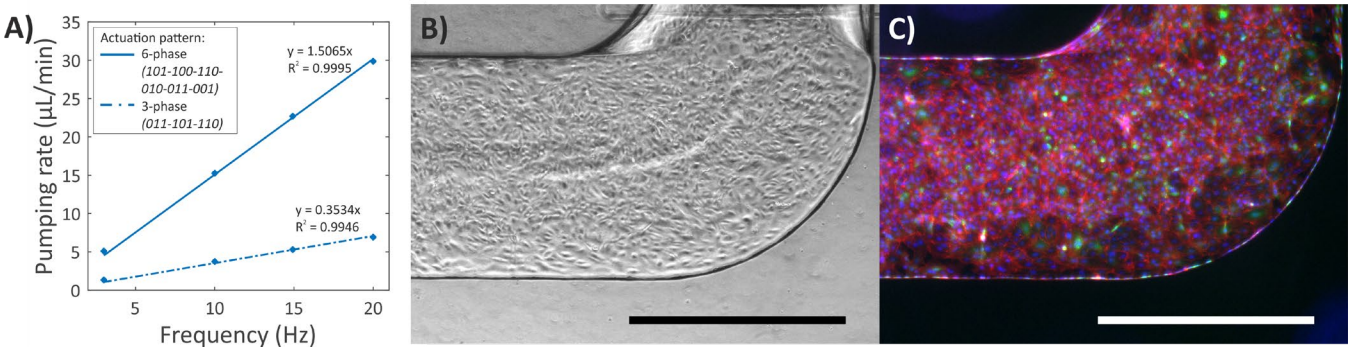

Figure 2: A) Pumping rate at different frequencies with different actuation patterns (both n=1). B) Phase contrast microscopic image of HUVECS cultured on-chip for 96h under constant peristaltic flow. C) Fluorescence image of GFP-expressing (green) HUVECS stained for cell nuclei (NucBlue) and F-actin (Red). Scale bars represent 1 mm.

CONCLUSION AND OUTLOOK

In summary, we present a parallelizable recirculation chip for automated cell culture under flow using pneumatic macro valves fabricated from a micromilled mold. The macro valves can be switched over 3 × 10^6 times without breakage or leakage and can reach pumping rates of up to 30 µL/min. This is, to our knowledge, the highest on-chip microfluidic pumping rate, taking advantage of our fabrication strategy based on micromilling. These flow rates make it possible to achieve physiologically relevant shear stresses in larger blood vessels-on-chips, either stand-alone or when integrated in more complex organs-on-chips. In the future, we will use the current chip to study endothelial inflammation and CRS by dynamic dosing with cytokines coupled with cell culture under different shear stresses.

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CONTACT: ✉ E.G.B.M. Bossink; phone: +31-53-489-6405; e.g.b.m.bossink@utwente.nl; & ✉ A.R. Vollertsen; phone: +31-53-489-6436; a.r.vollertsen@utwente.nl