STANDARDIZED, MODULAR PARALLELIZATION PLATFORM FOR MICROFLUIDIC LARGE-SCALE INTEGRATION CELL CULTURING CHIPS

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ABSTRACT

Standardized high-throughput devices for microfluidic cell cultures are necessary to translate discoveries made in academia to applications in pharmaceutical industry. Here we present a platform with integrated pneumatic valves for **s**tandardized **par**allelization of multichamber **c**hips (SPARC). In total, 192 chambers divided over three microfluidic building blocks (MFBBs) can be filled and purged with spatial and temporal independence. The dimensions of both the MFBB and the platform are standardized and thus compatible with common lab equipment. We characterize the valves at different pumping and gate pressures and show that the MFBBs are suitable for culturing human umbilical vein endothelial cells (HUVECs).

KEYWORDS: Parallelized, Standardization, Microfluidic Large-Scale Integration, Cell Culture

INTRODUCTION

Developing microfluidic systems which meet the high-throughput demands of the pharmaceutical industry requires parallelization, automation and standardization [1]. Previously, microfluidic large-scale integration (mLSI) chips with 96 and 128 independently addressable chambers for cell culturing have been reported [2,3]. However, further upscaling in a single chip is limited by fabrication challenges such as PDMS shrinkage and limited wafer sizes. Here we report a modular, automated and standardized platform to efficiently upscale the number of independently addressable cell culturing chambers. The SPARC platform consists of two types of parts: modular microfluidic building blocks and a fluidic circuit board (FCB). Both the MFBB and the FCB formats are in accordance with ISO Workshop Agreement 23:2016 standards [4,5]. The novelty in the presented system lies in operating multiple mLSI chips as parallelized MFBBs. This is achieved by integrating a "chip select" function based on latched multiplexing in the FCB. In this way, the number of fully independently addressable microchambers can be easily upscaled without having to fabricate large mLSI chips. Furthermore, the development of a mLSI MFBB adds a new building block to the library of standardized MFBBs [5], thereby extending the library's range of applications.

EXPERIMENTAL

The FCB and MFBB designs are shown in figure 1 A and B, respectively. The polystyrene FCB fits the 96-well plate format and is therefore compatible with common lab equipment. It has three MFBB ports (dashed lines in figure 1A) which can be independently enabled or disabled for MFBB control. Each of the ports is connected to a common fourth port for external pressure control. For each MFBB, a set of pneumatically actuated, normally closed valves forms a "chip select" in the FCB. Applying either vacuum or positive pressure will, respectively, enable or disable the corresponding MFBB (figure 1C). Standardized PMMA clamps [5] and O-rings are used to seal the MFBBs and the external pressure control block to the FCB (figure 1D).

The MFBBs are mLSI chips with 64 cell culturing chambers that are independently addressable using a combinatorial multiplexer with "push-up" valves. The chips are fabricated from two layers of polydimethylsiloxane (PDMS), whereby the fluidic (top) layer contains the chambers (each has a volume of 25 nL) and the control (bottom) layer contains the valves. In total, 13 control channels are used to control approximately 750 valves. The PDMS layers are bonded to a 30 x 60 mm glass slide which has powderblasted holes to enable pressurization of the

control channels through the FCB. The holes are located on a standardized grid as defined in [4]. Figure 1E shows the fully assembled SPARC platform with the chambers of the MFBBs filled with food coloring.

The opening and closing behavior of the FCB valves was characterized by measuring the flow from the external pressure control port to a MFBB port for different pumping and gate pressures. The measurements were taken at 200 mbar intervals and range from 0 mbar to 1400 mbar for the pumping pressure and from -200 mbar to 1600 mbar for the gate pressure. Furthermore, the flow through the fluidic layer of a MFBB was measured upon closing the MFBB valves and saving their state by subsequently disabling the MFBB. Finally, first cell experiments were performed by seeding HUVECs in the chambers of a MFBB and observing their morphology. For this, the channels leading to the chambers were coated with poly(L-lysine)-poly(ethylene glycol) (PLL-PEG) and the chambers with 0.1 mg/mL collagen I.

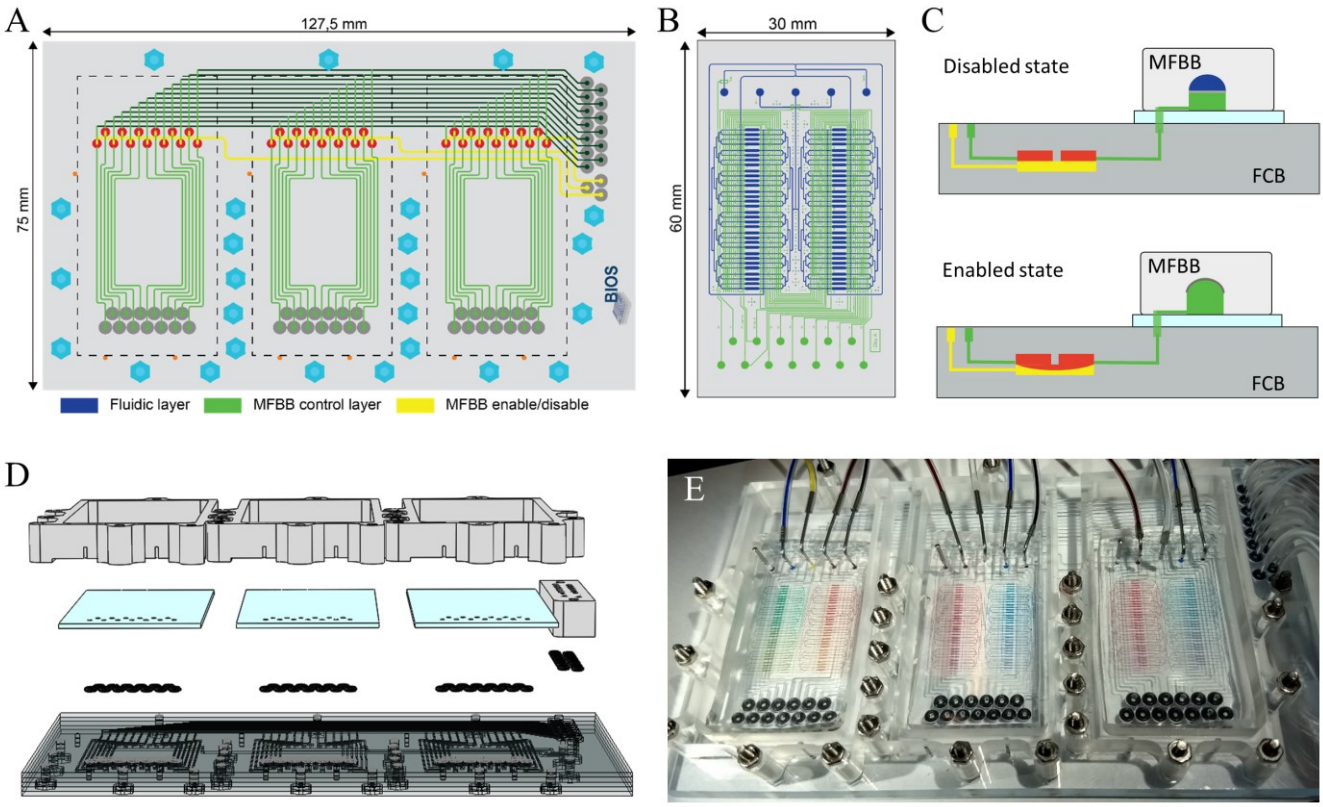

Figure 1: A) Schematic of the FCB design. The dashed lines indicate where the MFBBs are placed. B) Schematic of a MFBB. C) Working principle of SPARC. The MFBB is disabled (enabled) if the valves in the FCB are closed (open). D) Exploded view of the parts needed to assemble the SPARC platform. E) Fully assembled SPARC platform with the 192 chambers filled with food coloring.

RESULTS AND DISCUSSION

The "chip select" functionality is demonstrated in figure 2A. From left to right, the MFBBs are enabled individually and then filled with food coloring. In the first two MFBBs the chambers are filled simultaneously with all three inlets open, whereby the chip acts as a passive gradient generator. In the third MFBB each chamber is independently addressed and filled with either red or blue food coloring or a mix thereof. Figure 2B shows the decrease in measured flow rates through a FCB valve at increasing gate pressures. Even at high pumping pressure (1400 mbar) the FCB valves close, in most cases at equal or lower gate pressure (figure 2C). In addition, we show that the FCB valves can hold the closed state of MFBB valves for over 17 hours without leakage (figure 2D). Finally, figure 2E shows HUVECs in MFBB chambers 2.5 hours after seeding. The cells show substrate adherence and a healthy morphology.

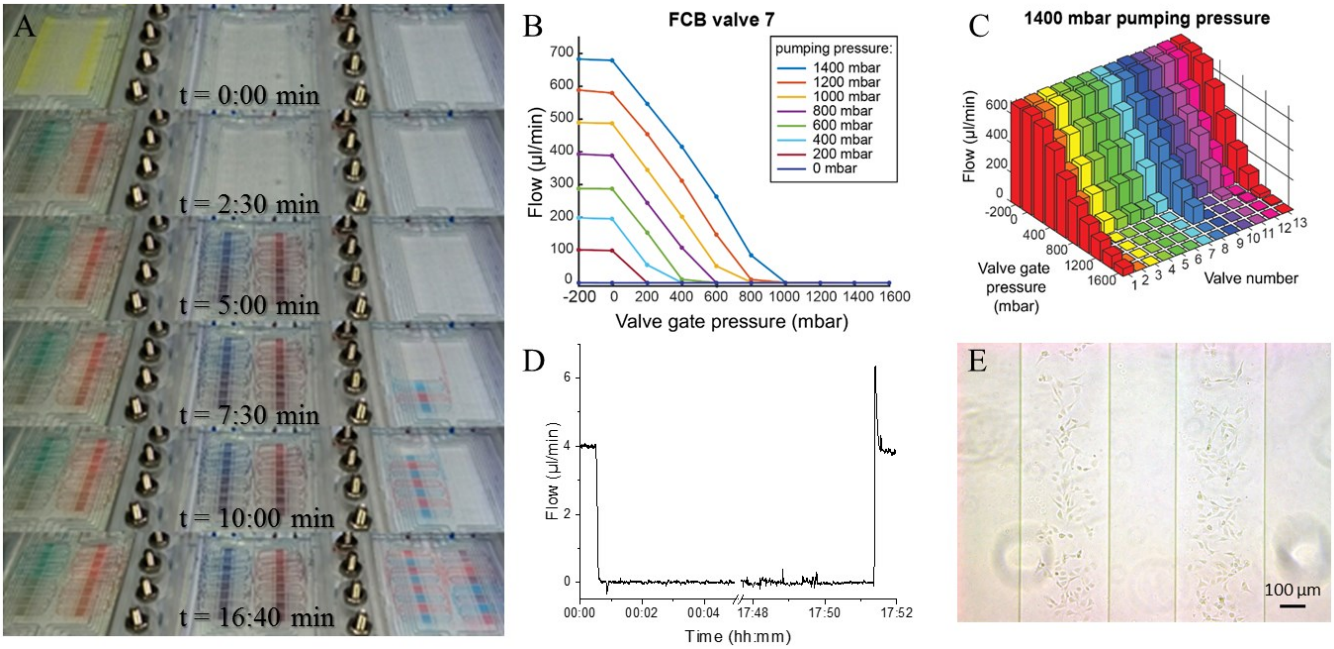

Figure 2: A) Time sequence of the three MFBBs enabled individually to fill the chambers with food coloring. B) Flow through a FCB valve at different gate und pumping pressures. C) Flow through all 13 valves of a "chip select" for different gate pressures at 1400 mbar pumping pressure. D) Flow through an MFBB after closing of the "push-up" valves and subsequent disabling of the MFBB. E) Brightfield image of HUVECs in the chambers of a MFBB 2.5 hours after seeding. The scale bar represents 100 µm.

CONCLUSION AND OUTLOOK

In conclusion, we show that we could successfully upscale the total number of individually addressable chambers by parallelizing several MFBBs, thus circumventing fabrication limitations. Moreover, the modular approach featuring a "chip select" function significantly increases versatility, as multiple MFBBs can be filled independently of each other. In combination with standardization this approach facilitates further scalability, ultimately bringing the experimental setting in academia one step closer to an industrial setting.

In the future, we hope to build upon our promising first cell culturing experiments and use the SPARC platform as an automated, high-throughput drug screening platform.

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