STANDARDIZED, MODULAR MICROFLUIDIC BUILDING BLOCKS FOR AUTOMATED CELL CULTURING SYSTEMS

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ABSTRACT

We report an emerging toolkit of modular and standardized MicroFluidic Building Blocks (MFBB) to ultimately form a versatile and automated system on a Fluidic Circuit Board (FCB) for high-throughput cell culturing and screening assays. The toolkit is composed of four different MFBBs to meet a total of four different purposes: (1) a metering and mixing MFBB for upstream sample preparation, (2) a gut-on-a-chip MFBB for increased biological complexity, (3) a 64-chamber MFBB for multiplexed cell culturing, and (4) a cell-in-droplet encapsulation MFBB for downstream analysis preparation.

KEYWORDS: Modular, Standardization, Automation, Microfluidic Building Blocks

INTRODUCTION

We report an emerging toolkit of modular and standardized MFBBs to form a versatile, automated and multi-functional system on an FCB. The development of such a system addresses the yet unfulfilled microfluidic potential in many research labs by aiming to bring together off-the-shelf solutions and unique applications. Previously, modular systems have been presented as reviewed in [1], however, they lack standardization and automation. At µTAS 2018 we reported an automated system in which an FCB could parallelize three MFBBs with 64 independently addressable cell culturing chambers each [2]. Here we expand upon this system by adding three new MFBBs to meet a total of four different purposes: (1) upstream sample preparation, (2) increased biological complexity, (3) highly multiplexed cell culturing, and (4) downstream analysis preparation. These MFBB prototypes demonstrate the widespread applicability of a modular system with standardized interfacing.

EXPERIMENTAL

Our toolkit contains the following four MFBBs which are formatted to fit the ISO Workshop Agreement 23:2016 standards [1].

(1) Preliminary metering and mixing MFBB for upstream sample preparation: The design is based on multiple arrays of parallel dosing units (fig. 1). This allows for programmable concentration profiles with high dynamic range. This MFBB is a 3 layer device fabricated from micro-milled poly(methyl methacrylate) (PMMA).

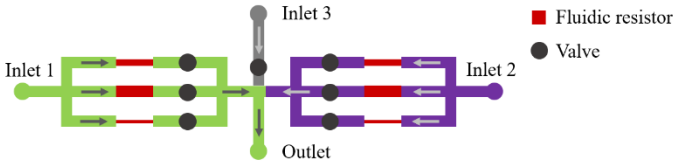

Figure 1: Schematic diagram of mixing MFBB with two dosing arrays.

(2) 3-organ-on-chip MFBB for increased biological complexity: This MFBB aims to simulate a more complex 3D cellular microenvironment, including a tissue-tissue interface (fig. 2). The fabricated MFBB contains three individually addressable organ-on-chips, all consisting of two microfluidic channels on top of each other, separated by a porous, in-house made, ±27 µm thick, (polydimethylsiloxane) PDMS membrane.

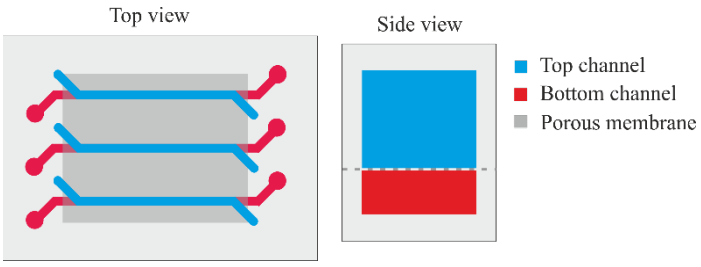

Figure 2: Top and side view of the 3-organ-on-chip MFBB.

(3) 64-chamber cell culturing MFBB for efficient multiplexing: This previously presented [2] MFBB consists of two PDMS layers. The top layer contains the flow channels and 64 microchambers and the bottom layer contains integrated "push-up" valves for multiplexed, automated flow control (fig. 3).

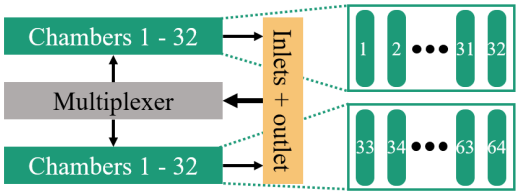

Figure 3: Schematic diagram of 64 chamber MFBB.

(4) Preliminary droplet encapsulation MFBB for downstream analysis preparation: One way to remove cell content from an MFBB for off-chip analysis is by packaging the content in droplets. This two-layer PDMS MFBB has four independently addressable chambers that connect to a droplet generator (fig. 4).

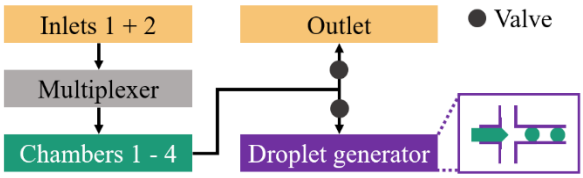

Figure 4: Schematic diagram of encapsulation MFBB.

RESULTS AND DISCUSSION

The proof-of-principle is demonstrated for each MFBB as follows: **(1) Mixing MFBB**: Using fluorescein and water as input solutions, mixtures in a dynamic range of 1:5 were achieved for each liquid within 7 seconds (fig. 5a). **(2) 3-organ-on-chip MFBB:** In the top channel a mixture of Caco2 and HTMX-29 cells (75%-25%) was seeded and cultured under static conditions for 7 days. ZO-1 staining (fig. 5c) shows that after 7 days, a confluent cell layer with both larger Caco2 cells and clusters of the smaller HTMX-29 cells was observed. **(3) 64-chamber MFBB:** For the first time in this chip, we now demonstrate uniform cell culturing across all chambers for up to 72 hours (fig. 5b). **(4) Droplet encapsulation MFBB**: Human umbilical vein endothelial cells (HUVECs) were cultured in the four chambers for 24 h and subsequently detached and encapsulated in a droplet (fig. 5d).

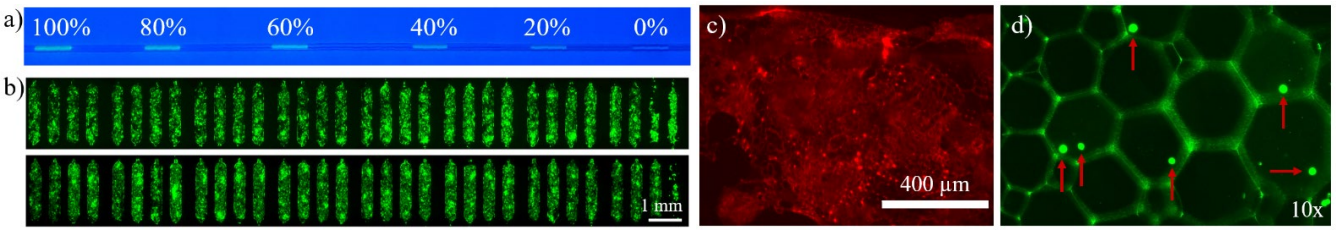

Figure 5: a) Automatically generated concentrations of fluorescein in water. b) 64 chambers filled with green fluorescent protein (GFP) expressing HUVECs cultured for 72 hours. c) Caco2 – HTMX-29 (75%-25%) coculture on chip at day 7, ZO-I staining. d) GFP-HUVECs encapsulated in droplets after having been cultured in the four chambers overnight.

CONCLUSION

In summary, we present this toolkit as the first step towards an open system where new application-specific MFBBs can be combined with generic commercializable MFBBs. Moreover, this hybrid approach ultimately presents the opportunity for industry and academia to work together to enable complex microfluidic applications in any lab.

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