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MICROFLUIDIC HANGING-DROP PLATFORM FOR PARALLEL CLOSED-LOOP MULTI-TISSUE EXPERIMENTS

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ABSTRACT

We present a new on-chip pumping approach for microfluidic hanging-drop networks that are used for experiments with 3D microtissue spheroids. The pump includes a pneumatic chamber located directly above one of the hanging drops and uses the liquid-air-interface for flow-control. With this approach several independent hanging drop networks (HDN) can be operated in parallel with only one single pneumatic actuation line. The pump concept enables closed-loop medium circulation between different organ models for body-on-a-chip applications and allows for multiple simultaneous assays in parallel.

INTRODUCTION

The design of multi-organ devices is the next step towards more biomimetic in-vitro models. Configurable hanging-drop networks have been used to combine microtissue spheroids, representing 3D organ models, with microfluidic technology in order to realize continuous tissue-tissue interaction and study complex multi-organs systems [1]. Up to date, medium flow in such systems has been controlled by using external pumps and tubing. Tubing increases medium volume, bears the risk of increased compound adsorption at surfaces and compromises ease-of-use and the potential for highly parallelized experiments. Several on-chip micropump designs have been reported for different applications [2-3]. However, these micropumps were designed for driving liquid flow in closed microfluidic systems and are, therefore, of limited use in open microfluidic systems, such as the hanging-drop networks. Here, we present a novel integrated pump system for microfluidic hanging drop networks that helps to overcome these limitations.

DESIGN AND OPERATION PRINCIPLE

Figure 1a shows the design of one microfluidic hanging-drop loop. The loop consists of 10 hanging drops, each with 3.5 mm base diameter (circular areas); three of them form the pneumatically driven hanging-drop pump, and seven can be used to host the respective microtissues under investigation. The hanging drops are connected through 200-µm-wide bottom-open microchannels.

The pump is operated by pressurizing a pneumatic chamber located above the center pump-drop through a pressure control line (indicated in red in Figure 1a). The pressure increase deflects the membrane between pneumatic chamber and drop ceiling (500 µm thick) and closes, at the same time, an integrated valve at the inlet channel of the drop (Figure 1b). The valve is used to prevent back-flow and to obtain unidirectional flow.

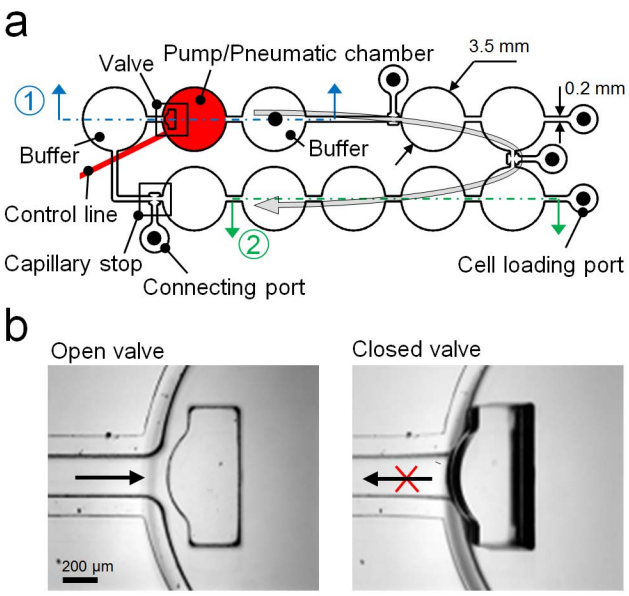

Figure 1: (a) Architecture and concept of the integrated micropump in a microfluidic loop of hanging drops. Two buffer drops are located before and after the pump-drop. A pneumatic chamber is integrated above the pump-drop (indicated in red, cross-section 1 in Figure 2). (b) Open and closed state of the valve. During pump actuation, the valve blocks the channel inlet and prevents reverse flow.

The membrane deflection virtually increases the volume of the drop. Based on Young-Laplace's equation, the decrease of the radius of the air-liquid-interface results in a pressure increase (P1>P2, Figure 2-1A) inducing a directional flow from the center pump-drop to the right neighboring drop (Figure 2-1B). When the pump membrane returns to resting state, liquid from both neighboring drops flows into the center drop thereby restoring equilibrium conditions (Figure 2-1C). This sequence is repeated with each pump stroke, and a circular and uniform flow through the hanging-drop loop can be achieved by applying an optimized actuation protocol. The two drops up- and downstream of the center pump-drop act as low-pass filters to reduce flow pulsation.

The other seven drops can be used to host spherical microtissues and have been arranged in groups of 2 and 5 drops that can be loaded with microtissues derived from different cell types (Figure 2-2). After cell medium has been filled into the microfluidic network, all three sub-networks (pump-unit, 2-drop-unit, 5-drop-unit) are fluidically interconnected upon opening of the capillary stop valves by supplying a small amount of liquid through the connecting ports. (Please refer to Ref. 1 for more details).

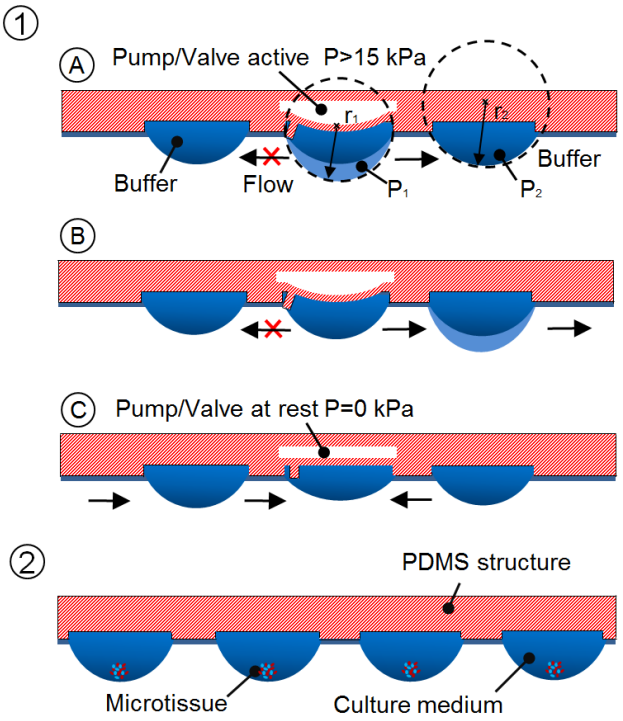

Figure 2: Cross-sections (1) and (2) as indicated in Figure 1, showing the schematic of pump actuation in a sequence. Upon pressurizing the pneumatic chamber above the pump-drop (indicated in red in Figure 1), the membrane deforms and closes the valve. At the same time, the volume change in the hanging drop below induces a pressure difference between pump drop and neighboring drop to the right and generates a directional fluid flow. The pump drop is then refilled after releasing the pressure from both neighboring drops. The two neighboring drops up- and downstream act as buffers to reduce pulsation. (2) Seven drops can be used to host different tissue types (e.g., 2 and 5 of a certain type) for tissue-tissue interaction studies.

MATERIALS AND METHODS

The microfluidic device with 8 independent loops was fabricated by using multilayer soft lithography. For both layers, pump and hanging-drop network (HDN), SU-8 100 negative photoresist (MicroChem, USA) was spin coated on thoroughly cleaned 4-inch silicon wafers. A rotation speed of 1500 rpm for 30 sec was selected to achieve a photoresist layer of 200 µm thickness. Two soft-bake steps at 65 °C for 30 min and at 95 °C for 90 min were applied. The wafer was then exposed to ultraviolet light to initiate crosslinking followed by two successive post-exposure baking steps at 65 °C for 5 min and at 95 °C for 30 min. The aforementioned steps were repeated for a second layer to reach final patterns of 450 µm height. The wafer was subsequently developed for 30 min in SU-8 developer (mrDev 600), rinsed with isopropyl alcohol, de-ionized water and dried with a nitrogen gun.

Two different transparency masks were used for UV exposure of the SU-8 layers of the HDN, whereas only one was used for the pump layer. Before replica molding, wafers were coated with trichlorosilane (Sigma-Aldrich, Switzerland) to reduce adhesion between SU-8 and the PDMS elastomer. Microfluidic devices were casted from polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning Corp., USA). PDMS base and curing agent were mixed in a ratio of 10:1 and degassed before pouring onto the SU-8 mold. Next, the PDMS was cured at 80 °C for 1 hour. The micropatterned replicas were cut and peeled off from the SU-8 mold. An inlet hole for the control line was punched into the pump layer. After rinsing with acetone and isopropyl alcohol, air drying and oxygen plasma activation, the pump layer was precisely aligned onto the HDN layer. In order to improve irreversible oxygen plasma bonding and prevent compressed air leakage, both layers were manually pressed against each other and kept in an oven at 80 °C for 1 hour. Then, inlet ports were punched through the bonded layers to be able to supply medium and/or cell suspensions (Figure 3). A pump layer of 6 mm thickness was used in order to provide the required mechanical stability for the device. The micropatterned side of device was covered with Scotch tape after a cleaning step for storage. Prior to device usage, the surface of the HDN layer was covered with a PDMS layer with openings in the drop areas and then activated with oxygen plasma in order to increase wettability of the PDMS. In this way, only the surfaces that come in contact with liquid were rendered hydrophilic, whereas rim structures were kept hydrophobic and prevented liquid overflow. Particularly, the wetting of the valve surface turned out to significantly affect pumping performance, as it changed the pump drop shape and caused additional fluid flow over the activated surface region.

For characterization experiments each individual hanging drop loop was filled with 80 µl suspension of micro-beads (5 µm diameter) in de-ionized water through the inlet holes by using a multichannel pipette.

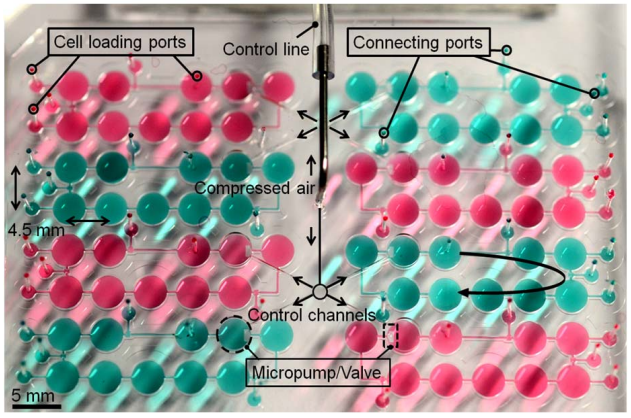

Figure 3: Photograph of the microfluidic hanging drop platform consisting of 8 identical hanging drop loops (4 loops on the left side are mirrored with respect to Fig. 1). All micropumps are connected to a single control line for parallel actuation. Alternating colored liquid show the 10 drops in each of the 8 independent loops. The drops are arranged in a 384-well plate grid (i.e. 4.5-mm pitch) for automated read out.

Characterization tests were performed on an inverted microscope (Olympus IX81) using a 5X objective in bright field mode. To minimize evaporation of the liquid from the hanging drops during experiments, microfluidic devices were placed into a closed humidified chamber. A single tube connected the pneumatic control line over a 21-gauge 90-degree-bent needle to all pneumatic chambers. The control line was connected to a 3/2-way miniature solenoid valve (Festo, Germany) controlled via a data acquisition (DAQ) device (National Instruments) operated by LabVIEW programming software. A custom-designed printed-circuit board was used to connect a DAQ card to the solenoid switch. A pressure controller was used to regulate the applied positive pressure. Compressed air (typically 30 kPa) was routed to the 8 pneumatic chambers through identical and symmetric control channels. This layout assures identical actuation frequency and membrane deflection heights and, consequently, identical flow-rates for all eight loops. The device was designed to be compatible with 384-well plate formats and multichannel pipettes (4.5 mm pitch between inlet ports of adjacent loops) to facilitate medium and cell loading as well as automated imaging.

Particles were traced along the length of the channel after the pump drops by using a digital camera with an image acquisition rate of 10 frames per second. Average particles speeds were measured manually by using ImageJ image analysis software so that the respective flow-rates could then be calculated.

The pump actuation protocol (shown as a square wave in Figure 5) consists of two states maintained for two different durations. T-on refers to the time when the pressure is applied to the pneumatic chamber. In this state the membrane is deflected, and the valve is closed. T-off stands for the time at resting state, when no pressure is applied, the membrane is at its initial flat position, and the valve is open. The rather large thickness of the PDMS membrane ensures short transition times.

RESULTS

The performance of the pumping system was investigated for different pump protocols. Figure 4 shows time-lapse images of ink circulating in the hanging-drop loop, which indicates that the integrated pumping system was able to successfully generate gradual unidirectional fluid flow. Before the pump was activated (t = 0 min), 4 µl of blue ink were added to one of the hanging drops. Some of the liquid penetrated into both neighboring drops. Upon actuation of the pump, the fluid flows clockwise, and the blue link is transported along the drops and channels through the system. Complete circulation and mixture of ink and water was observed after about 15 min of continuous pumping.

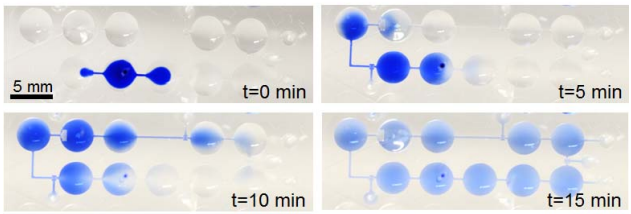

Figure 4: Time series of images showing the circulation of a small volume of blue ink within one loop of drops as a result of the integrated pumping. (Protocol: t-off = 0.025 s, t-on = 0.050 s).

Figure 5 presents experimental results of the achieved flow-rates in dependence of the different pump actuation protocols.

First, the valve was closed by applying a positive pressure in excess of 15 kPa. It has been observed that increasing the applied pressure generates higher flow-rates. Here, 30 kPa relative pressure were applied, which deformed the membrane by approximately 400 µm in the center. During each stroke, the membrane expansion adds a virtual volume of 2 µl to the hanging drop.

The t-off time was set to 0.025 s, whereas the t-on time was varied between 0.025 and 0.3 s. A constant flow-rate was achieved after 20 pump strokes. As can be seen in Figure 5, the flow-rate decreased with increasing the t-on time. Further, the pump operation generated pulsatile fluid flow, while pulsation frequency and amplitude were strongly depending on the pump actuation protocol. Flow-pulsations were larger at lower actuation frequencies (114% relative flow variation with respect to the average flow-rate for t-on = 0.2 s versus 49% relative flow variation with respect to the average flow-rate for t-on = 0.025 s).

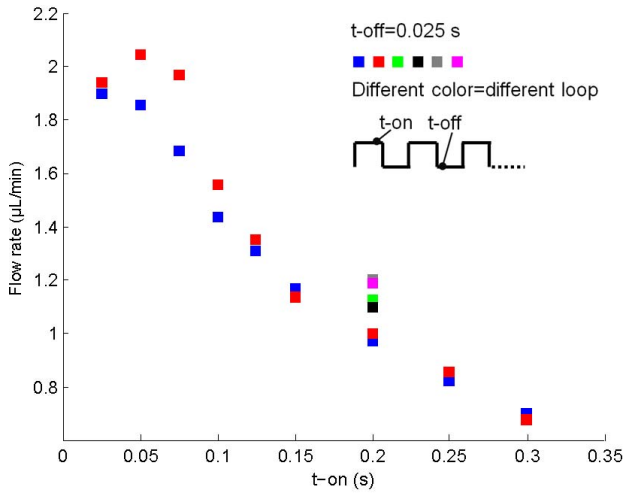

Figure 5: Measured flow-rates in dependence of the pump actuation protocol (indicated as a square wave, t-on stands for the time when the membrane is deflected, t-off when it is in the resting position). The different colors of the dots are measurements in different hanging-drop loops and demonstrate the low variability.

For t-on = 0.2 s and t-off = 0.025 s the flow-rate variation between the different loops on the same platform has been measured. The relative variation was below 15%. These results demonstrate that the generated flow-rates can be precisely modulated by adjusting pump actuation protocol and operation conditions.

CONCLUSIONS

The design, fabrication, and characterization of a novel micropump, integrated into completely open microfluidic systems, have been presented in this paper. Unidirectional simultaneous fluid flow for 8 independent hanging-drop networks has been generated via an on-chip pumping system actuated through one single pressurized control line. The developed technique offers unique opportunities for parallel medium circulation in hanging drop networks, and is of great importance for multi-tissue experiments.

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REFERENCES

[1] O. Frey, P. M. Misun, D. A. Fluri, J. G. Hengstler, A. Hierlemann, "Reconfigurable microfluidic hanging drop network for multi-tissue interaction and analysis", *Nat. Commun.*, 5:4250, 2014.

[2] Brian D. Iverson, Suresh V. Garimella, "Recent advances in microscale pumping technologies: a review and evaluation", *Microfluid and Nanofluid*, 5:145-174, 2008.

[3] C.K. Byun, K. Abi-Samra, Y-K Cho, S. Takayama, "Pumps for Microfluidic Cell Culture", *Electrophoresis*, 35:245-257, 2014.

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