A MICROFLUIDIC PLATFORM FOR PROBING MULTIPLE INTACT BLOOD VESSELS

Byeong-Ui Moon[1], Steffen Sebastian-Bolz[2] and Axel Günther[1,3]
[1]Mechanical and Industrial Engineering, [2]Department of Physiology,
[3]Institute for Biomaterials and Biomedical Engineering, University of Toronto, Canada

ABSTRACT

We present here further development of an artery-on-a-chip (AOC) technology in which multiple small blood vessel segments are co-hosted on a single microfluidic platform. We previously introduced an organ-based microfluidic platform to investigate small blood vessels for probing structure and function [1]. In this contribution we present a further development of the platform that accommodates three mouse mesentery artery segments and allows their function to be assessed in parallel.

KEYWORDS

Small blood vessel, Artery-on-a-chip, Organ-on-a-chip

INTRODUCTION

Resistance arteries (diameter range from 30 µm to 300 µm) play an important role to regulate blood pressure homeostasis by maintaining constant blood flow through different tissues. The maintenance of blood flow is modulated by dynamically changing the artery inner diameter (tone) and thereby the peripheral vascular resistance. The regulation of artery tone plays a key role in many cardiovascular diseases, particularly in hypertension, heart failure and diabetes [2].

The conventional method of probing a single small blood vessel *in vitro* is pressure myography where a blood vessel segment is manually cannulated, i.e., it is sutured on both ends onto glass micropipette tips [3]. Pressure myography requires highly trained personnel and is very labor consuming. Despite its ability of functionally assessing an intact cardiovascular organ, the technique's lack of scalability has so far limited its use to microvascular research laboratories and prevented its introduction in the biopharmaceutical drug development process.

The long-term goal of this project is to harness the scalability of a microfluidic approach that allows investigating the structure, function, and interaction of intact small blood vessels. Recently, our group developed an artery-on-a-chip (AOC) platform to perform dose response experiments on a single resistance artery [1]. Here, we present further development of the device that accommodates multiple small blood vessels. Figure 1 schematically compares characteristics of the present approach with the manual pressure myography technique.

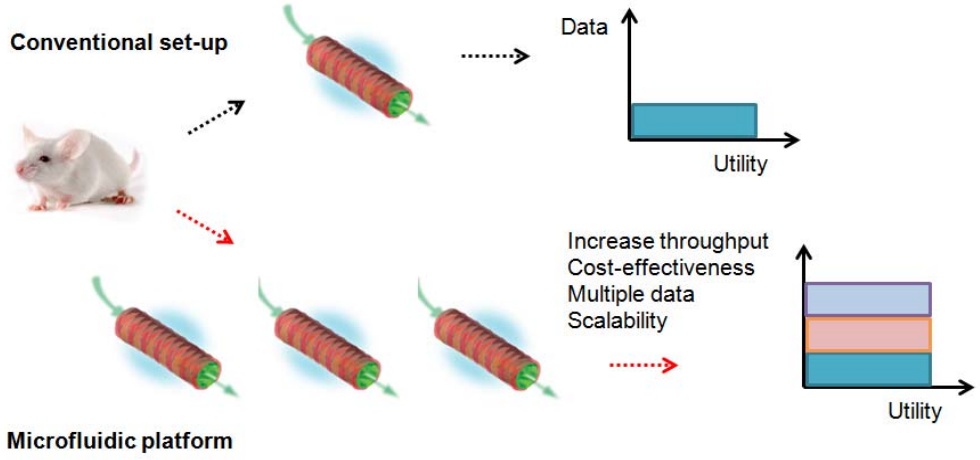

Figure 1. Schematic comparison between organ-based approaches for probing intact small blood vessels. The conventional pressure myography technique (top) allows probing a single vessel, whereas the present microfluidic platform (bottom) allows hosting and simultaneously assessing multiple vessels. Its advantages are scalability, cost effectiveness and an increased experimental throughput.

EXPERIMENTAL

A microfluidic device was designed to host three small blood vessels and to perform a functional bioassay. The current chip design is composed of individual three loading wells, three sets of fixation channels, three perfusion channels and one superfusion channel of artery segments (Figure 2a, b). One source superfusion fluidic stream distributes six superfusion channels to deliver drug to the side wall of the vessels. Figure 2c shows a fabricated device of in the regions of interest. The device was fabricated by using standard single-layer soft lithography in a

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poly(dimethylsiloxane) (PDMS) substrate bonded to a glass slide.

Mesenteric artery segments were obtained from 10-14 week old wild type CD1 mice (Charles River, Montreal, Canada). A small resistance artery was isolated and carefully dissected from the 2nd of 3rd order of mesenteries. Once connective tissue was removed from the artery by using ultra-fine forceps and scissors (Fine Science Tools, Vancouver), an approximately 1.5 mm long vessel segment was obtained. The prepared vessel segments were then opened on both ends to allow for the luminal perfusion and to maintain physiological levels of the transmural pressure. All mesenteric artery preparation was done under a dissecting microscope.

Artery loading process and dose-response measurement were described in detail by Günther *et al.*[1] Briefly, the first artery segment was inserted into the first loading well and a syringe pump that was operated in the withdraw mode allowed positioning the segment in the first inspection area. Once the artery segment reached its dedicated position, the vessel was then immobilized by applying a subatmospheric hydrostatic pressure at the fixation channels located at both vessel ends. Subsequently, the second and third artery segments were loaded as the same manner. Prior to testing the viability of the chip-hosted vessels, a transmural pressure of 60 mmHg was applied on all vessels and the temperature was maintained to physiological levels (37.0 °C) using a metal-deposited on-chip heater.

A functional bioassay was performed by administrating phenylephrine, a vasconstrictor. Two individually controllable syringe pumps delivered a physiological buffer solution and a drug solution that were diffusively mixed before being subjected to the vessel abluminal side. The vessel tone was measured from bright-field images in real time with a high resolution CCD camera (Q Imaging, 1280×1024 pixels) and recorded by a custom LabVIEW program (National Instruments).

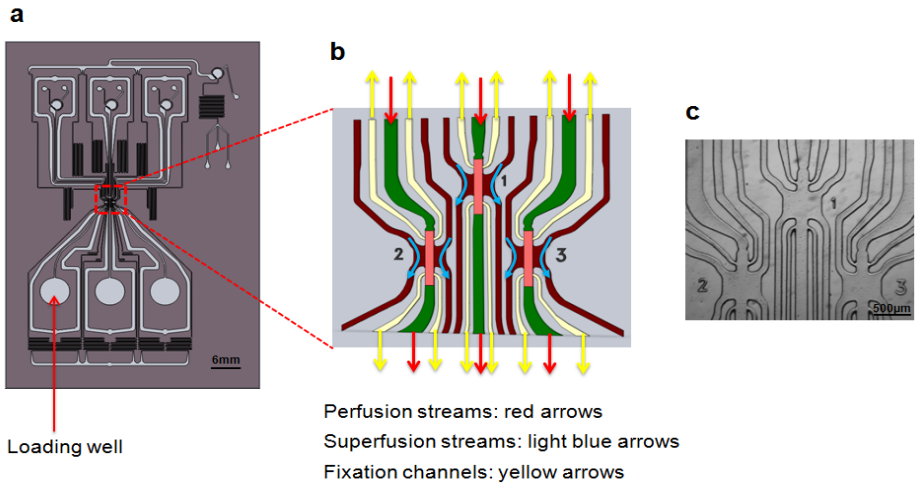

Figure 2. Design and working principle of artery-on-a-chip platform hosting three small blood vessel segments (a) Overall lay-out of a chip design with a size of 50 mm (w) × 75 mm (h), (b) Enlarged view of regions of interest, red arrows indicate perfusion channel; light blue arrows for superfusion channel and yellow arrows for fixation channel. Arrows indicate the direction of flow. (c) Microscopic image of the region of interest from a fabricated device (without blood vessel segments loaded).

RESULTS AND DISCUSSION

The assessment of the microfluidic platform was evaluated by positioning three mouse mesenteric artery segments in their appropriate locations and performing functional bioassays to determine viability. Figure 3a shows bright field micrographs of the three vessels loaded and fixed within the region of interest. The ability to consistently load the second and third vessel segment required the unwanted interference caused by crosstalk between the different inspection areas to be reduced by increasing flow resistance. Individual fixation channels that were operated at a subatmospheric pressure of 45 mmHg allowed to individually and subsequently fixate artery segments. The result of phenylephrine-induced dose response shows the three vessels are intact in three different organ baths introduced at 3 µM phenylephrine concentration (Figure 3b). Figure 4 shows the result of dose-dependent response as a function of phenylephrine concentration. The phenylephrine was delivered to vessels by upstream diffusive mixing of a physiological buffer solution and phenylephrine at a total flow rate of 2 mL/h. Different concentrations of phenylephrine (0.9 µM, 1.5 µM, 2.1 µM and 3 µM) were obtained by changing the relative flow rates of the two streams while the total flow rate remained constant. Comparable dose-response relationships were obtained for the three vessel segments. We conclude that the smooth muscle cells in our engineered microenvironment are intact and will be feasible to further assess endothelial cell viability as well as vessel-to-vessel interaction using modified chip designs.

The present artery-on-a-chip platform allows multiple vessels to be loaded and enables functional bioassays to be performed on intact small blood vessels. Compared to pressure myography, our platform significantly increase the throughput by hosting multi-vessels for the investigation of intact small resistance artery and may provide one step towards the adaptation of the artery-on-a-chip platform in the context of cardiovascular research and drug development.

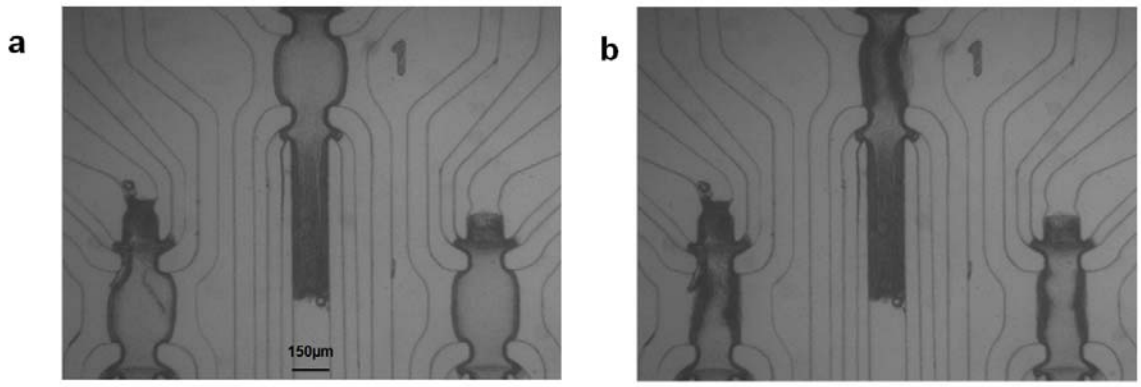

Figure 3. (a) Bright-field micrograph showing three loaded mouse mesenteric arteries, (b) Image showing the constriction of three vessel segments after drug administration. The region of interest is 2.1 mm (w) × 1.7 mm (h), at a resolution of approximately 1.7 µm/pixel. A 4× magnification was used and the organ bath had a size of 500 µm (w) × 300 µm (h). All three vessels were kept in identical microenvironments regarding their temperature, transmural pressure and drug concentration.

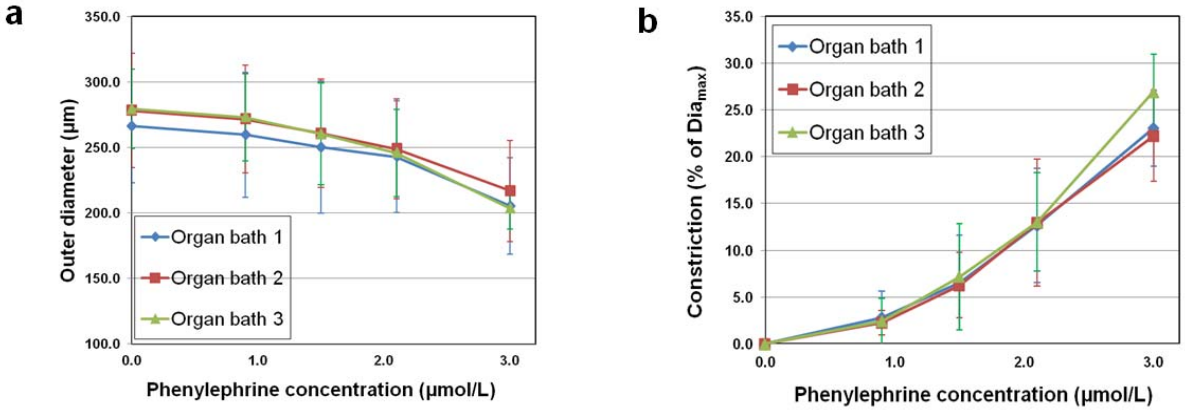

Figure 4. Measured phenylephrine dose-dependent response (a) Change of artery outer diameters and (b) Percentage of constriction, (Dmax-D)/Dmax (n=4). Organ baths 1, 2 and 3 indicate in Figure 3 the center, left and right locations, respectively.

CONCLUSION

We have successfully demonstrated the development of an artery-on-a-chip platform for the simultaneous functional assessment of three intact small blood vessels. Three mouse mesenteric arteries were co-hosted in three individual organ baths. The functional response to the constrictor phenylephrine was similar for the three vessels. We anticipate an increased utility of the increased experimental throughput of our platform in the context of cardiovascular drug development on intact and perhaps in the future even tissue-engineered small blood vessels.

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CONTACT

Axel Guenther, tel:+1-416-978-1282; axel.guenther@utoronto.ca