VEIN-ON-A-CHIP: MICROFLUIDIC PLATFORM FOR FUNCTIONAL ASSESSMENT AND STAINING OF INTACT VEINS

Z. Abdi Dezfooli*, S-S. Bolz, A. Günther

1Institute of Biomaterials and Biomedical Engineering; 2Department of Physiology; 3Department of Mechanical and Industrial Engineering, University of Toronto, CANADA

ABSTRACT
We present a microfluidic approach for the combined functional assessment and immunofluorescence staining of mouse mesenteric vein segments. The blood vessel segments were isolated and manually loaded onto the microfluidic platform. Functional assessments of the endothelium and the smooth muscle cells showed 70% and 35% constriction and dilation of the veins, respectively. Pressurized veins were stained on-chip for von Willebrand Factor and CD31 markers confirming the capacity of our platform for in-situ immunofluorescence staining, without the need for tissue processing and sectioning. The selected approach is automatable and may significantly reduce the experimental throughput.

KEYWORDS: organ-on-a-chip, microphysiological systems, immunofluorescence, blood vessels

INTRODUCTION
During inflammation small veins are the key sites for leukocyte extravasation. Scalable experimental approaches that allow the structure and function of intact veins to be routinely assessed are an important requirement for an improved understanding of infectious diseases and, ultimately, for the development of new anti-inflammatory compounds. Such studies are currently limited by the lack of appropriate experimental platforms, e.g., poor optical access and limited microenvironmental control for in vivo studies1,2 and a partial representation of blood vessel structure and function in the case of microphysiological approaches3,4 Here, we present to our knowledge the first microfluidic platform that maintains an intact small vein under near-physiological conditions in order to perform a combination of functional assessment and immunofluorescence staining. Furthermore, the long duration and manual nature of conventional techniques, e.g. pressure myography for the functional assessment of small blood vessels, as well as fixation, paraffin embedding, sectioning and staining for immunohistochemistry, are barriers for scalability. The ultimate goal of our approach is to overcome these obstacles by automation of all required steps and operating multiple platforms in parallel.

EXPERIMENTAL
The microfluidic device consists of three micro-channel networks (indicated in Fig. 1a in blue, red, and green color). Mesenteric vein segments (diameter ~275 µm, length ~2 mm) were isolated from C57BL/6 mice and loaded onto the platform by putting the segments in the loading well and pulling into the perfusion channel by applying negative pressure to the other side of the channel. Vein segments were reversibly fixated (blue channels), subjected to physiological temperature (37°C) and transmural pressure (4 mmHg), perfused with a controlled flow rate (4-16 1/min) through the luminal side (green channel) and subjected to a defined convective flow on the abluminal side (red channels). Vessel function was assessed by abluminal exposure to different concentrations of phenylephrine (PE: 0-10 M) and acetylcholine (Ach: 0-1 M) through the superfusion channels (red color, Fig. 1a) by adjusting the flow rate of the drug and 3- (N-morpholino) propanesulfonic acid (MOPS).

In a second set of experiments, six reservoirs were connected to the perfusion channel, which opened to the perfusion channel one at a time, for the purpose of absolving fixing and immunofluorescence staining protocols on chip-hosted vein segments (Fig.1a). Immunofluorescence for von Willebrand Factor (vWF) and CD31 (PECAM-1) were carried out using Vein segments were stained for vWF and CD31 (PECAM-1). Vein segments were fixed by perfusion of 4% PFA for 30 minutes, permeabilized with 0.5% TritonX (Sigma Aldrich) for 45 minutes (only in the case of vWF and nuclei staining), blocked with 5%
goat serum for 1 hour and incubated with 1:50 vWF (C-20) goat polyclonal antibody (Santa Cruz Biotechnology) or 1:100 rat anti-mouse CD31 antibody (BD Biosciences) over night. All the steps were performed at 4°C with a flow rate of 4μl/min, except for incubation with primary antibodies. Then the veins were washed with PBS for 30 minutes and incubated with 1:75 mouse anti-goat IgG –TR or 1:200 goat anti-rat TRITC respectively for one hour at room temperature. Finally the veins were individually imaged on a Nikon Ti Eclipse at 20×, 10× and 40× (ELWD NA0.6) with a Qimaging Exi Blue fluorescent camera.

RESULTS AND DISCUSSION

After loading the vein segments onto the microfluidic device, the pressurized blood vessels were abluminally exposed to different concentration of PE starting with a concentration of 2.5μM in the superfusion channel, and the concentration was increased to 10μM by changing the ratio of superfusing solutions (Fig2a). Figure 2b shows the constrictor responses as a function of concentration of PE. Following that, superfusing drug was switched to Ach, starting with a concentration of 0.25μM which gradually increased to 1μM resulting in amplified dilation of the blood vessel (Fig 2c). The results show ~70% constriction and ~35% dilation of chip hosted small mouse mesenteric veins which confirms the smooth muscle cells and endothelial cells’ functionality, respectively; this is comparable with reported literature data.

After assessing the functional response of the blood vessels, the segments were washed with MOPS and immunofluorescence staining for vWF and CD31 antibodies was performed using the second
experimental setup (Fig 3a). Figure 3b indicates relative increase of vWF expression on the endothelium over control sample. Moreover, the nuclei of endothelial cells were stained with DAPI (Fig 3c). Finally, the pressurized vein segments were stained for CD31 (PECAM-1) on endothelium as shown in figure 3d where the orientation of endothelial cells was visualized.

Figure 3: Immunofluorescence for vWF, CD31 and nuclei stain (a) Schematic of experimental setup employed for vein segment immunofluorescence staining. (b) Histogram of relative increase of endothelium vWF expression over control. Error bars represent standard error. n=3. (c) Fluorescence images of nuclei stained endothelial cells of the pressurized vein (d) Pressurized vein segment stained for CD31. Scale bars: 100 µm

The presented platform is a significant advancement over the artery-on-a-chip platforms previously introduced by our group5,6. The design of the vein chip overcomes the challenges of handling fragile vein segments. Moreover, it is capable of performing combination of functional assessment and luminal immunofluorescence staining on intact veins.

SUMMARY
We have reported a microfluidic approach for the on-chip investigation of intact mouse mesenteric veins while allowing for functional assessment and immunofluorescence staining of endothelial cells. In future work, we plan to employ the vein-on-a-chip platform for the automated assessment of the activation of chip-hosted vein segments.

ACKNOWLEDGEMENT
We acknowledge financial support from Eli Lilly & Company, the NSERC CREATE Program in Microfluidic Applications and Training in Cardiovascular Health (MATCH) as well as NSERC Discovery and DAS Awards. We thank Drs. Myron I. Cybulsky and Dan Dumont as well as Firhan Malik (Bolz Lab), Ali Oskooei (Guenther Lab) and Lily Morikawa (Toronto Centre for Phenogenomics) for suggestions and helpful discussions.

REFERENCES

CONTACT
Axel Guenther, phone: 416-978-1282, axel.guenther@utoronto.ca