A microfluidic platform for probing small artery structure and function^{†,‡}

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Abstract

Although pathologic changes to the structure and function of small blood vessels are hallmarks of various cardiovascular diseases, limitations of conventional investigation methods (*i.e.* pressure myography) have prohibited a comprehensive understanding of the underlying mechanisms. We developed a microfluidic device to facilitate assessment of resistance artery structure and function under physiological conditions (37 °C, 45 mmHg transmural pressure). The platform allows for on-chip fixation, long-term culture and fully automated acquisition of up to ten dose–response sequences of intact mouse mesenteric artery segments (diameter ≈250 micrometres and length ≈1.5 mm) in a well-defined microenvironment. Even abluminal application of phenylephrine or acetylcholine (homogeneous condition) yielded dose–response relationships virtually identical to conventional myography. Unilateral application of phenylephrine (heterogeneous condition) limited constriction to the drug-exposed side, suggesting a lack of circumferential communication. The microfluidic platform allows us to address new fundamental biological questions, replaces a manually demanding procedure with a scalable approach and may enable organ-based screens to be routinely performed during drug development.

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1. Introduction

High blood pressure (hypertension), a major risk factor for many cardiovascular diseases (*e.g.* heart disease and stroke), is already at epidemic proportions and predicted to further grow in the coming decades.¹ Since changes to the structure and function of small blood vessels are important pathogenic factors that promote and sustain cardiovascular diseases, organ-based approaches that lead to an improved understanding of the underlying mechanisms will be central to develop better treatment strategies.

In cardiovascular research, microfluidic approaches have been employed to study single and co-cultured cell populations,^{2–8} tissue slices,⁹ and bioengineered tissues.^{10–12} However, to our best knowledge, scalable approaches to assess the structure and function of intact cardiovascular tissues in health and disease (*e.g.*, heart failure, diabetes, vasculopathies, tumor biology, and toxicology) have yet to be developed.

Our work focuses on probing the structure and function of small arteries, particularly resistance arteries, *in vitro*. Resistance arteries are highly specialized structures with diameters ranging from 30 μm to 300 μm located in the terminal sections of the arterial vascular tree. Their luminal side is lined with one layer of endothelial cells (ECs) that are wrapped by several layers of circumferentially orientated smooth muscle cells (SMCs, Fig. 1a). Tone in resistance arteries, defined as amount of constriction relative to the artery's maximal diameter, D_{max} , is collectively established through diffusible neurotransmitters (*e.g.* noradrenaline), which generally promote vasoconstriction, and vasodilatory factors released by ECs in response to mechanical stimuli, *e.g.*, fluid-induced shear stress, pressure and strain,^{13,14} and chemical stimuli, *e.g.* prostacyclin,¹⁵ nitric oxide¹⁶ and the endothelium-derived hyperpolarizing factor.¹⁷ Vascular tone in resistance arteries represents the net effect of these multiple inputs from a microenvironment that dynamically changes in space and time. Current pathogenic concepts for hypertension assume that even subtle microenvironmental changes can disturb this dynamic relationship and have pronounced effects on arterial tone that subsequently alters peripheral vascular resistance (*e.g.*, it can lead to a hypertensive phenotype).

Despite this clinical relevance, technologies to study microvascular structure and function have, for the most part, remained unchanged since their introduction by Mulvany and Halpern in 1976–1978.^{18,19} In essence, small arteries are either mounted on two wires (isometric approach) or cannulated and perfused with glass micropipettes (isobaric method), which more accurately reflects the *in vivo* conditions. Both procedures require manually skilled personnel and are not scalable; *i.e.*, time consumption and workload increase proportional to the throughput. This lack of scalability has confined microvascular research to a relatively small number of scientific laboratories and so far prevented its utilization for artery-based pharmacological screens.

We present an organ-based microfluidic platform that allows for routine determination of resistance artery structure and function. The platform overcomes several of the previously mentioned limitations by providing a scalable, inexpensive and high throughput ready

alternative to manual conventional myographs,²⁰ with a large potential for automation and standardization.

In addition, this new microfluidic platform offers the opportunity to assess the role of spatiotemporal heterogeneities in the regulation of small artery tone. Such aspects have not yet been appreciated using conventional myography protocols that assume a homogenous *in vivo* environment and, by virtue of their design, establish a homogenous microenvironment (*i.e.*, drug concentrations) around the arteries. This is a specific experimental limitation of pressure myography that may have profoundly impacted (and limited) our understanding of the regulatory mechanisms governing microvascular function. In fact, a complex network of regulatory factors modulates resistance artery tone *in vivo*, with one important determinant being the dynamically varying structure of the artery itself.^{21,22} In addition, resistance arteries are exposed to signals that are spatially non-homogeneous, for example: region-specific nitric oxide release at bifurcation points;²³ discrete noradrenaline gradients generated by focal release from noradrenergic synapses;^{24,25} heterogeneous electrical inputs conducted from downstream arteries;²⁶ and mosaic, non-uniform metabolic activity in the surrounding tissue. We therefore propose that a comprehensive assessment of microvascular function critically depends on the ability to engineer and control microenvironments that are capable of accurately simulating these complex influences. The versatile design of the platform presented here allows imposing dynamic changes on the microenvironment surrounding the arterial wall so that effects of heterogeneous environmental changes on microvascular structure and function can be assessed. Although engineered microenvironments have previously been used to investigate the effect of heterogeneity on cells, fungus-like organisms and pancreatic islets,^{27–31} the present study is to our knowledge the first microfluidic approach to study whole organ function.

2. Experimental

2.1 Device design, fabrication and characterization

We designed, fabricated and tested microfluidic devices that subject small blood vessels to controlled spatiotemporal perturbations in their microenvironment. The geometry of the microenvironment remains identical throughout the paper and forms the small artery inspection area on a microfluidic device. The device allows for loading, precise placement, fixation as well as controlled perfusion and superfusion of a small artery segment. Fig. 1b shows a three-dimensional representation of the micro-fluidic device, accommodating three microchannel networks for artery fixation (yellow), perfusion (green) and superfusion (red) in one layer. Standard soft-lithographic techniques^{32,33} were employed to define the microchannel networks in the elastomeric substrate poly(dimethylsiloxane) (PDMS), see ESI[†]. Prior to starting the experiments, all microchannels were primed with aqueous 1% Bovine Serum Albumin (BSA) solution that was replaced after ~1 min with a MOPS buffer.

At the beginning of the artery loading process, a resistance artery segment (typically 250 μm in diameter) was immersed in the loading well while the fixation and superfusion in/outlet lines remained closed. The loading well remained open and microchannel A was connected

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to a syringe pump that withdrew a constant flow rate of buffer, thereby transporting the artery segment towards its dedicated position in the inspection area of the chip (Fig. 1c). Upon reaching the final position, the pump was stopped and the cylindrical artery segment was reversibly fixed at both ends (Fig. 1d). This was accomplished by first applying a sub-atmospheric pressure through the top fixation channels, E_1 in Fig. 1d, to fix one end of the segment and co-axially align the elastic artery segment within the inspection area. Next, the loading well was sealed using a removable lid and microchannel A was subjected to an external pressure of 45 mmHg above atmosphere, establishing a transmural pressure and aligning the artery segment symmetrically at the center of the inspection area. A sub-atmospheric pressure was then also applied at the bottom fixation channels E_2 (Fig. 1d). The pressure in all fixation channels was kept at 45 mmHg below atmospheric pressure throughout the experiment to maintain the artery segment within the inspection area of the chip. The remaining channels (B and C in Fig. 1e) were opened and constant perfusion and superfusion flow rates were adjusted using separate syringe pumps. A pressure of approximately 45 mmHg above atmosphere was applied through microchannel D, maintained throughout the experiment and defined the physiological transmural pressure.

Fig. 2a shows a schematic of a microfluidic device with the previously described artery inspection area (Fig. 1c–e) that also allows for on-chip control of the temperature in the artery inspection area. A disk of sapphire with a thermal conductivity exceeding the one of the PDMS substrate by a factor of 200 was embedded in PDMS, uniformly distributing the heat generated by a thermoelectric heater in the horizontal direction and keeping the inspection area at physiological temperature. An aluminium lid served to compression-seal the loading well during experiments. Fig. 2b shows the numerically calculated temperature distribution at the top surface of the glass slide (see also ESI[†], Fig. S3).

During dose–response measurements a hydrostatic head was applied at microchannel A (green) and the flow through microchannel D was kept at zero flow rate in order to match the conditions previously used in pressure myographs. Two computer-controlled syringe pumps (see also ESI[†], Fig. S2) delivered a buffer stream, Q_{B1} , and a drug-containing stream, Q_{B2} , at a constant total superfusion flow rate $Q_{B1} + Q_{B2}$ of 4 mL h^{−1}. Time-dependent changes in the drug concentration of the superfusing stream Q_B were obtained by altering the ratio Q_{B1}/Q_{B2} . The length of the $w = 100\ \mu\text{m}$ wide mixing channel, 115 mm, exceeded the distance $U(w/3)2/D_{PE} = 90\ \text{mm}$ that is necessary for the concentration of phenylephrine (PE) (diffusivity $D_{PE} \approx 9.0 \times 10^{-10}\ \text{m}^2\ \text{s}^{-1}$)³⁴ to homogenize across the entire channel (U is the bulk velocity of the flow through channel B). Diffusive mixing was illustrated by labeling stream Q_{B2} with fluorescein, a molecule larger than PE, with an estimated diffusivity of $4.8 \times 10^{-10}\ \text{m}^2\ \text{s}^{-1}$ (according to the Wilke–Chang method³⁵). Three fluorescence images (Fig. 2c–e) were obtained at the location where the two streams meet, at an intermediate location, and just before the combined stream Q_B reached the outer artery wall where mixing was completed. Flow-through time (residence time) for the mixing channel was approximately 6 s.

[†]Electronic supplementary information (ESI) available: Microfluidic device design, experimental protocols, details of numerical flow simulation as well as video files showing artery perfusion, superfusion and functional response. See DOI: 10.1039/c004675b

2.2 Artery isolation and functional assessment

Resistance arteries were isolated by microdissection from 2nd or 3rd order mesenteries from wild type CD1 mice (Charles River) or CD1 mice expressing a Tie2-GFP transgene in their ECs³⁶ (Fig. 3f and g, Jackson Laboratory) and loaded onto microfluidic devices. Computer-control of the relative flow rates supplying microchannels B₁ and B₂ resulted in stepwise increasing PE concentrations while maintaining a constant superfusion flow rate and recording bright-field images of artery segments (QImaging).

3. Results

3.1 Artery perfusion and superfusion

Fig. 3a shows the velocity field and Fig. 3b the concentration field as obtained from a two-dimensional finite-element simulation that considered the geometry of the inspection area and assumed the artery segments to be straight-walled. The elasticity of the arterial wall and any resulting deformation during pressurization were therefore neglected. The model assumed a 140 μm wide artery segment, 5 μm wide gaps at the fixation locations, a flow rate of 4 mL h^{-1} through channel B (at 150 μm channel depth), a pressure of 45 mmHg for channel A, atmospheric pressure for channel C, and a zero flow rate through channel D. The exit location of the fixations channels, E, was kept at a pressure of 45 mmHg below atmosphere. The simulation supported a key requirement of the microfluidic chip, the complete separation of perfusing and superfusing streams, and predicted a total flow rate Q_E of 0.75 mL h^{-1} .

The inverted fluorescence micrograph in Fig. 3c shows the corresponding experimental results for a pressurized mouse mesenteric artery. The artery segment was first superfused with a fluorescently labeled 3-(*N*-morpholino)-propanesulfonic acid (MOPS) solution and perfused with a non-fluorescent, clear buffer. The fluorescence micrograph in Fig. 3d was obtained for a different mesenteric artery segment, where the perfusing stream was fluorescently labeled while the superfusing stream was kept non-fluorescent or transparent. In agreement with the numerical result, small fractions of the perfusing and superfusing streams are continuously removed through the fixation channels. Fig. 3e shows a fluorescent micrograph of a pressurized resistance artery segment that can be distinguished in the inspection area due to its autofluorescence. Intactness of the EC layer in the inspection section (Fig. 3f) and at the fixation points (Fig. 3g) was confirmed using confocal microscopy in arteries that expressed GFP under an endothelial Tie2 promoter (*i.e.*, GFP expression was confined to ECs).

3.2 Functional tests and long-term artery culture

Following artery segment loading, the microfluidic chip hosting the arterial segment was placed on the stage of an inverted microscope and the artery was heated to 37 °C. For functional tests, the lumen of the artery was not perfused; the superfusion flow rates Q_{B1} and Q_{B2} were dynamically controlled *via* a computer interface. Stream Q_{B1} contained the respective drug, *i.e.*, either phenylephrine (PE) or acetylcholine (ACh), in a buffer solution. The second stream, Q_{B2} , contained buffer solution only. Both fluid streams mixed by diffusion before the combined stream Q_B was split into two equal parts to abluminally

superfuse the artery (Fig. 4a). Bright-field images of the inspection section (Fig. 4b) were continuously recorded and provided the basis for determination of artery inner and outer diameter in real time. Fig. 4c illustrates contour changes to the arterial outer wall in response to a stepwise increasing PE concentration. Fig. 4d shows the temporal change in the artery outer and inner diameter while the PE concentration in the superfusing stream was stepwise increased from $0 \mu\text{mol L}^{-1}$ to $1.5 \mu\text{mol L}^{-1}$ that was evaluated from bright-field images at the center of the inspection area (indicated by the dashed box in Fig. 4b).

Results from a local cross-correlation (see ESI[†]) of the two successive images, depicted in Fig. 4b, that were recorded while increasing the PE concentration from $0.3 \mu\text{mol L}^{-1}$ to $0.9 \mu\text{mol L}^{-1}$ are shown in Fig. 4e and provide spatially resolved information on artery constriction. The resulting two-dimensional displacement map consists of vectors that represent direction and magnitude of the local artery deformation. The mean displacement $\bar{x}(x)$ averages all vectors that are located at an equal distance x from the axis of symmetry. A symmetric displacement pattern was found, with $\bar{x}$ vanishing at the axis of symmetry and increasing at both sides of the artery. Finally, Fig. 4f shows the results from a fully automated experiment in which ten dose–response curves were consecutively acquired using the same artery segment.

Smooth muscle and endothelial function of mesenteric arteries in a spatially homogeneous microenvironment—For systematic tests of resistance artery structure and function, mesenteric arteries (max. outer diameter $D_{o,\text{max}} = 272 \pm 16 \mu\text{m}$ and max. inner diameter $D_{i,\text{max}} = 220 \pm 16 \mu\text{m}$, $n = 6$) were loaded onto the chip and subjected to spatially homogeneous microenvironments in the perfusing and superfusing streams. Smooth muscle function was assessed by superfusing the non-perfused artery on both sides with PE concentrations that increased from 0.1 to $1.5 \mu\text{mol L}^{-1}$. All arteries constricted in response to PE and showed a sigmoidal dose–response relationship, with maximal constriction at $1.5 \mu\text{mol L}^{-1}$ (Fig. 5a). The different behavior of the artery inner and outer diameters that was observed at high PE concentrations was attributed to thickening of the microvascular wall at high constriction levels. A virtually identical dose–response relationship was obtained when conventional pressure myography (arteries mounted onto glass micropipettes) was utilized (Fig. 5a). Maximal constriction (at $1.5 \mu\text{mol L}^{-1}$ PE) was not different for arteries studied on the chip ($42 \pm 4\%$ of D_{max} , $n = 6$) *versus* the conventional setup ($44 \pm 3\%$, $n = 6$).

Endothelial function was assessed by superfusing arteries with the endothelium-dependent vasodilator acetylcholine (0.01 to $10 \mu\text{mol L}^{-1}$, Fig. 5b) that diffuses through the vascular wall, binds to endothelial muscarinic receptors and ultimately stimulates the synthesis of endothelial vasodilators (primarily nitric oxide in mouse mesenteric arteries³⁷). Arteries pre-constricted by $32 \pm 2\%$ of $D_{o,\text{max}}$ with $1 \mu\text{mol L}^{-1}$ PE ($n = 5$) dilated in a dose-dependent manner to ACh (Fig. 5b).

The chip-based platform can hold microarteries in place for extended time periods and thus, in principle, permits use of a previously established tissue-culture protocol³⁸ for chip-loaded mesenteric arteries. Smooth muscle and endothelial function were assessed following 24 h of culture on the chip. Cultured mesenteric arteries constricted robustly in response to 1

$\mu\text{mol L}^{-1}$ PE ($37 \pm 4\%$ of D_{max} , $n = 5$) and almost completely dilated (to $95 \pm 8\%$ of D_{max} , $n = 5$) in response to $10 \mu\text{mol L}^{-1}$ ACh (Fig. 5c).

Smooth muscle function of mesenteric arteries in a spatially heterogeneous microenvironment—The question whether smooth muscle cells in an intact resistance artery wall are circumferentially coupled remains unsolved. Thus far, functional and dye injection experiments have largely excluded longitudinal coupling between SMCs.³⁹ We engineered a microenvironment on the microfluidic chip that provided the opportunity to expose discrete regions of the artery wall to different environmental conditions, *i.e.*, spatially inhomogeneous stimulation (Fig. 6a). Complete separation of the right and left superfusion streams, as confirmed by the inverted fluorescence micrograph in Fig. 6b, was achieved by sandwiching the artery segment between the top and bottom walls of the microfluidic device (Fig. 1c–e). Increasing concentrations of PE (0.3 to $1.5 \mu\text{mol L}^{-1}$, $n = 5$) in the right but not the left superfusion stream induced a dose-dependent displacement of the drug-exposed (right) but no movement of the drug-opposed (left) wall (Fig. 6c).

Local cross-correlation analysis during a $0.3 \mu\text{mol L}^{-1}$ to $0.9 \mu\text{mol L}^{-1}$ PE concentration step in the right superfusion stream confirmed the asymmetric vasoconstriction under heterogeneous stimulation conditions (Fig. 6d). Magnitude and direction of the locally obtained displacement within the arterial wall are represented by color-coded arrows. Fig. 6e shows the temporal evolution of the inner and outer (side) wall positions as observed in center of the fixed artery segment. The measured displacement pattern for the left and right arterial (side) walls was asymmetric, with x vanishing at the drug-opposing (left) side and in vicinity of the axis of symmetry but increasing at the stimulated (right) side (Fig. 6f).

4. Discussion

The present study presents a new platform to assess resistance artery structure and function. Small arteries were loaded, immobilized and kept intact in microenvironments on a microfluidic device that accurately reflect the *in vivo* conditions of terminal arteries and allowed precise control of experimental conditions, *i.e.*, temperature (37°C), transmural pressure (45 mmHg) and the luminal and abluminal chemical milieu (*i.e.*, drug concentrations). Application of sub-atmospheric pressure through the fixation channels reversibly immobilized the arteries on a microfluidic chip (Fig. 1) and reliably separated luminal and abluminal fluid streams (Fig. 2).

Compared to conventional pressure myography systems, the presented method significantly reduces the technical demand related to preparation of a microvessel and provides the technology for routine investigation of functionally even more relevant^{40,41} pre-capillary arteries with diameters $<50 \mu\text{m}$. The facilitation of artery handling combined with the significantly reduced time required for on-chip fixation enables higher throughput and automation of experiments. The scalable format of our small artery-based method offers the opportunity to integrate all initial steps of cardiovascular drug development, encompassing target identification, target validation, drug design and testing on this experimental platform.

To compare the microfluidic platform with conventional pressure myography, resistance arteries hosted on the microfluidic chip were functionally tested in a spatially homogeneous microenvironment, keeping the inspection area at 37 °C and the transmural pressure at 45 mmHg. The arteries were per- and superfused with either a MOPS-buffered salt solution (acute experiments) or L-15 (Leibovitz)-based culture medium (culture experiments),³⁸ both simulating the metabolic environment of the *in vivo* milieu. In mouse mesenteric arteries, PE-stimulated vasoconstriction was virtually identical compared to results obtained using a conventional pressure myograph. The ACh dose–response relationship revealed well-maintained endothelial function. In fact, the conventional approach necessarily destroys parts of the endothelial layer through insertion of cannulae into the lumen and subsequent fixation with sutures. Since ECs extensively communicate *via* gap junctions and paracrine mediators;²⁶ the conventional procedures bear the risk to functionally compromise remote areas of the endothelial layer near the isolated ends of the vessel. In contrast, the EC layer remained fully intact during on-chip fixation, as demonstrated utilizing vessels, in which ECs selectively express GFP under a Tie2 promoter³⁶ (Fig. 3f and g).

The immobilization technique allowed for long-term on-chip fixation of pressurized resistance arteries. We adapted physical and chemical conditions (primarily flow rates, transmural pressure and culture media) that had previously been optimized for the use on classical pressure myographs³⁸ to establish an on-chip organ culture protocol. Functional results obtained from on-chip cultured mouse mesenteric arteries (*i.e.*, 24 hours of continuous per- and superfusion with L-15 culture medium at 45 mmHg³⁸) confirmed fully intact SMC and EC function. The ability to culture microarteries is a mandatory prerequisite for the transfer of gene transfection procedures onto the new platform.^{42–44}

Although *spatiotemporal* heterogeneity is assumed to be an important characteristic of the microenvironment surrounding resistance arteries, *in vivo*, current myography methods technically do not allow easy manipulation of the environment at discrete locations along the vascular wall to address this aspect. In contrast, conventional dose–response curves result from stepwise even changes in the organ bath drug concentration; regional heterogeneities are purposefully avoided. Yet, simulating regional heterogeneities is critical to understanding artery structure and function and how cell–cell communication coordinates vascular reactivity. We propose that the chip platform can provide an experimental environment needed to investigate these types of questions.

To illustrate this capability, we established a spatially heterogeneous microenvironment through unilateral application of PE (*i.e.*, only one half of the circumference was exposed). We hypothesized that a uniform constriction would result if the circumferentially arranged vascular smooth muscle cells of the wall behaved like a syncytium so that through communication all cells of the wall would contract. However, we found the constriction to be spatially restricted to the site of stimulation with PE. The vasoconstrictor response did not spread to the contralateral, non-stimulated side of the vessel. Our results are in agreement with previous evidence suggesting that not all responses conduct equally along the arterial wall^{45,46} and that initiated events may remain highly localized in the smooth muscle layer of resistance arteries.³⁹ Although a more detailed analysis of these findings is beyond the scope of our study, our data highlight an important advantage of the platform: the ability to analyze

small artery structure and function through exposure to well-defined heterogeneous environments. The precise manipulation of the artery's spatiotemporal environment on the microfluidic platform will allow us to address fundamentally new biological questions. Potential future applications of the presented method are pre-clinical pharmacological screening (drug development) and assessment of a patient's microvascular status in a clinical setting (personalized medicine).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Artery segment reversible on-chip loading and fixation. (a) Schematic representation of a resistance artery segment consisting of endothelial cells (ECs) lining the inner wall of the vessel and smooth muscle cells (SMCs) wrapped around the lumen. The artery microenvironment is characterized by its temperature, transmural pressure, P , as well as luminal and abluminal drug concentrations, C_i and C_o , respectively. (b) Schematic representation of the chip containing a microchannel network, an artery loading well and an artery inspection area. (c–e) Illustrations show reversible procedures for artery segment loading, fixation and inspection. The scale bar in (c) is 500 μm long. Microchannels E_1 and E_2 allow fixation of the two ends of the artery segment. (e) Perfusion of fixed small artery segment from microchannel A to microchannel D (luminal side). Superfusion of the artery abluminal side (outside) from microchannel B to microchannel C, while the loading well is sealed.

Fig. 2. Control of artery microenvironment. (a) Microfluidic device (footprint: 75 mm × 25 mm) with fluidic connections for perfusing, superfusing and fixation channels. A thermoelectric heater and a thermistor are attached to an optically transparent sapphire glass. The sapphire glass equally distributes heat in the horizontal plane while the TE element is operated with a proportional–integral–derivative (PID) controller to maintain physiological temperatures at the artery inspection area. (b) Numerically predicted temperature distribution across the device. (c–e) The culture media and drug containing superfusing streams B₁ and B₂ are fed using separate syringe pumps and are thoroughly mixed by diffusion, prior to contacting the outer artery wall. The inverted fluorescence micrographs illustrate the diffusive mixing process where the drug-containing stream B₂ was fluorescently labeled. Scale bars (c–e) are 100 μm.

Fig. 3.

Perfusion and superfusion flow rates, and transmural pressure are independently adjustable.

(a) Velocity and (b) concentration fields numerically obtained from a two-dimensional simulation of artery segment perfusion and superfusion (ESI[†]) blood vessel segment. (c) Superfusing stream fluorescently labeled and perfusing stream was clear. (d) Perfusing stream was fluorescently labeled and the superfusing stream was transparent. Diameter D of pressured artery segment indicated. (e) Fluorescent micrograph of artery segment (autofluorescence). (f) Confocal images of artery segment with GFP-expressing ECs in the inspection section of the chip and (g) at one of the fixation locations. Scale bars are 500 μm (a–e) and 100 μm (f and g).

Fig. 4.

Constriction of artery segment in spatially homogeneous microenvironment. (a) Experimental configuration for determination of artery outer and inner (luminal) diameter while phenylephrine (PE) was evenly applied on the abluminal side. (b) Two successive bright-field images of constricting artery segment. (c) Artery outer contour during constriction. (d) Time evolution of artery outer (red) and inner (green) diameter during constriction. (e) Top: cross-correlation of bright-field image pair shown in (b) that was obtained at a 0.14 s time separation resulted in two-dimensional displacement map (vectors indicate magnitude and direction of constriction in axial and radial direction). Bottom: radial displacement averaged along the artery. Window sizes of $77 \mu\text{m} \times 77 \mu\text{m}$ and $19 \mu\text{m} \times 19 \mu\text{m}$ were used in two subsequent correlation steps. (f) Fully automated acquisition of ten consecutive dose-response curves obtained by computer-controlled superfusion and evaluation of artery outer (red) and inner (green) diameter for one artery segment at $t_{DR} = 4 \times 3 \text{ min}$ and $t_W = 30 \text{ min}$.

Fig. 5.

Dose–response characteristics in homogeneous microenvironments. (a) Phenylephrine (PE) dose–response relationships for artery outer (●) and inner diameters (○) measured on the microfluidic device ($n = 6$), and using a conventional cannulation protocol (Δ, *i.e.*, pressure myograph). (b) Artery dilation obtained by abluminally applying ACh in PE-precontracted chip-hosted arteries ($n = 5$). Constriction and dilation are defined as $\Delta D = (D_{\text{max}} - D) D_{\text{max}}^{-1}$ where D_{max} is the diameter of the uncontracted artery. (c) Smooth muscle (stimulation with PE) and endothelial function (stimulation with ACh) after 24 h of on-chip culture. Mesenteric arteries constricted robustly in response to 1 $\mu\text{mol L}^{-1}$ PE and almost completely

dilated in response to $10 \mu\text{mol L}^{-1}$ ACh ($n = 5$). Error bars represent the standard error of the mean (SEM).

Fig. 6.

Artery constriction in spatially heterogeneous microenvironment. (a) Configuration consisting of drug-facing side (right side) and drug opposing side (left side). (b) Fluorescence micrograph (intensity inverted) confirming that abluuminally applied buffer solutions were strictly confined to one side (buffer on right side fluorescently labeled, scale bar indicates 500 μm). (c) Evolution of the artery outer contours for PE-containing buffer applied at the drug-facing side (replacing the fluorescently labeled buffer). (d) Two-dimensional (vectors indicate magnitude and direction of local constriction) and one-dimensional (average in the axial direction) evolution of artery shape as determined from cross-correlation of two subsequently acquired bright-field image frames that were obtained at a 0.14 s time separation. (e) Changes in the artery outer and inner wall positions as observed at the drug-facing and drug-opposing sides (all positions are relative to the position of the left inner wall). Window sizes of 77 $\mu\text{m} \times 77 \mu\text{m}$ and 19 $\mu\text{m} \times 19 \mu\text{m}$ were used in two subsequent correlation steps. (f) Steady-state positions of the artery outer wall with increasing PE concentration at the drugfacing and the drug-opposing side. Error bars represent the standard error of the mean (SEM).