A multichannel acoustically driven microfluidic chip to study particle-cell interactions

Xue-Yan Wang,¹ Christian Fillafer,² Clara Pichl,¹ Stephanie Deinhammer,¹ Renate Hofer-Warbinek,³ Michael Wirth,¹ and Franz Gabor^{1,a)}

¹*Faculty of Life Sciences, Department of Pharmaceutical Technology and Biopharmaceutics, University of Vienna, Vienna A-1090, Austria*

²*Department of Mechanical Engineering, Boston University, Boston, Massachusetts 02215, USA*

³*Department of Vascular Biology and Thrombosis Research, Center for Physiology and Pharmacology, Medical University of Vienna, Vienna A-1090, Austria*

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Microfluidic devices have emerged as important tools for experimental physiology. They allow to study the effects of hydrodynamic flow on physiological and pathophysiological processes, e.g., in the circulatory system of the body. Such dynamic *in vitro* test systems are essential in order to address fundamental problems in drug delivery and targeted imaging, such as the binding of particles to cells under flow. In the present work an acoustically driven microfluidic platform is presented in which four miniature flow channels can be operated in parallel at distinct flow velocities with only slight inter-experimental variations. The device can accommodate various channel architectures and is fully compatible with cell culture as well as microscopy. Moreover, the flow channels can be readily separated from the surface acoustic wave pumps and subsequently channel-associated luminescence, absorbance, and/or fluorescence can be determined with a standard microplate reader. In order to create artificial blood vessels, different coatings were evaluated for the cultivation of endothelial cells in the microchannels. It was found that 0.01% fibronectin is the most suitable coating for growth of endothelial monolayers. Finally, the microfluidic system was used to study the binding of 1 μm polystyrene microspheres to three different types of endothelial cell monolayers (HUVEC, HUVEctert, HMEC-1) at different average shear rates. It demonstrated that average shear rates between 0.5 s^{-1} and 2.25 s^{-1} exert no significant effect on cytoadhesion of particles to all three types of endothelial monolayers. In conclusion, the multichannel microfluidic platform is a promising device to study the impact of hydrodynamic forces on cell physiology and binding of drug carriers to endothelium. © 2013 AIP Publishing LLC.

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I. INTRODUCTION

The endothelium constitutes the inner layer lining the blood vessels and as such is involved in a variety of physiological and pathophysiological processes, for instance, in the course of atherosclerosis,¹ diabetes,² and cancer.³ Thus, from a therapeutic point of view, it would be beneficial to deliver drugs or contrast agents to dysfunctional parts of the endothelium. To reach this aim, particulate drug delivery systems, such as drug-loaded nanoparticles or liposomes, have been frequently proposed. By modifying the surface of these carriers with specific target molecules, selective adhesion to diseased cells and tissues might be possible. Several strategies have been suggested: E-selectin-binding peptide modified N-(2-hydroxypropyl) methacrylamide

^{a)}Electronic mail: franz.gabor@univie.ac.at

(HPMA) polymer drug carriers⁴ and sialyl Lewis X-conjugated liposomes for targeting inflamed vasculature,⁵ anti-ICAM decorated PLGA nanocarriers⁶ and RGD-conjugated HPMA copolymer particles for targeting of tumor angiogenic vasculature,⁷ as well as leukocyte-mimetic anti-P-selectin and VCAM-1 grafted microparticles of iron oxide for MRI⁸ are some representative examples. However, it has also been shown that coating of colloids with polymers such as polyethylene glycol (PEG) prolongs the half life of particles in the blood stream.⁹ Thus, understanding the key parameters that either enhance adhesion or prolong residence in the circulatory system is of fundamental importance.

Currently, *in vitro* studies concerned with the adhesion of particles to endothelial cells are usually carried out under stationary conditions. *In vivo*, however, endothelial cells grow in a three-dimensional matrix and are constantly exposed to hydrodynamic forces caused by blood flow.^{10,11} Generally, hydrodynamic flow can be regarded as an external force acting on a cell. The exposure of endothelial cells to shear stress has indeed been shown to induce alterations in gene expression¹² and cytoskeletal re-arrangement.^{13,14} For instance, hydrodynamic flow strongly affects the interaction between leukocytes and endothelial cells occurring during recruitment of leukocytes in response to inflammation inside vascular walls.¹⁵ Therefore, it would be advantageous to have *in vitro* models at hand, which allow simulation of hydrodynamic flow conditions. Microfluidic systems offer promising solutions for this purpose. By using soft lithography, channel structures can be produced in arbitrary geometries and numbers at a sub-millimeter scale. In contrast to classical flow-through chambers, only minute amounts of samples are necessary and highly reproducible experimental conditions are ensured.¹⁶

In the present work a multi-channel acoustically driven microfluidic system based on surface acoustic wave technology (SAW) will be described. This device will be employed to mimic physiological conditions *in vitro*. A central advantage of this setup is that quantitative analysis of cell-bound fluorescence, luminescence, and/or absorbance can be carried out by using a standard microplate reader. First, the range of flow rates that can be generated reproducibly in the system will be assessed. Second, optimized cell culture techniques, especially channel coatings with extracellular matrix components, will be described that allow cultivation of three different types of endothelial cells inside microchannels. Finally, as a proof-of-concept, the SAW-driven microfluidic device will be applied to investigate the adhesion of polymer microparticles to endothelial cell monolayers in the presence of hydrodynamic drag.

II. MATERIALS AND METHODS

A. Material

Sylgard[®]184 Silicone Elastomer Kit was obtained from Baltres (Baden, Austria). Yellow-green fluorescent carboxylated polystyrene particles with a diameter of 1000 nm were purchased from Polysciences Europe GmbH (Eppelheim, Germany). Human fibronectin was bought from BD Bioscience (Bedford, USA). All other chemicals were of analytical purity.

B. Fabrication of sterile microchannels

Microchannels composed of poly(dimethylsiloxane) (PDMS) were fabricated as described previously.¹⁷ Briefly, 10 g base and 1 g curing agent were mixed by vigorous stirring. After evacuation for 30 min to remove gas bubbles, the liquid pre-polymer was poured into pre-structured aluminium molds and hardened over night at 70 °C. After peeling the PDMS replicas from the molds, they were attached to predetermined spots on a glass plate (127 × 85 × 1.1 mm, length × width × height) and fixed irreversibly by gluing with liquid pre-polymer and heating to 100 °C for 30 min.

C. Characterization of multichannel microfluidic system

Each of the four identical microchannels on the glass plate was filled with water containing 0.25‰ fluorescent microparticles (3 μm, Duke Scientific Corp., California, USA).

According to Hodgson *et al.*, in order to guarantee efficient transmission of the SAWs into the microchannels, water was used as a couplant between the IDT and the glass plate.¹⁸ The system is operated by a radio frequency generator (SMB100A, Rohde&Schwarz GmbH, Austria) connected in series to a coaxial amplifier (LZY-1, Mini Circuits, Brooklyn, USA) and a fixed attenuator (VAT-3W2+, Mini Circuits, Brooklyn, USA). The radio frequency signal is distributed to the four IDTs by a 4-way divider (D1572-102, Werlatone, Brewster, USA). The whole setup was mounted on a Zeiss Axio Observer.Z1 (Göttingen, Germany). Due to the difficulty of directly measuring the power transduced into the SAW, the input power levels given herein simply correspond to the arithmetic sum of the powers of the generator and amplifier minus loss in the splitter. The values given should not be considered as the actual power transduced into the SAW. The input power was converted from dBm to the mW-scale according to Eq. (1).

$$P = P_{\text{ref}} 10^{\frac{x}{10}}, \quad (1)$$

where P is the power in [mW], P_{ref} is the reference power (1 mW), and x is the power in [dBm]. The flow velocity in the microchannels was determined from the translational velocity of the fluorescent particles by microscopy. Moreover, the corresponded average shear rate was calculated according to Eq. (2),

$$\gamma = \frac{V_{\text{max}}}{\frac{H}{2}}, \quad (2)$$

where γ is the average shear rate in [s^{-1}], V_{max} is the flow velocity measured in a laterally as well as vertically central region of the channel, which was the highest velocity due to the character of parabolic flow, and $H/2$ is the half-height of the microchannel (Figure 1(D)).

D. Cultivation of endothelial cells

Three different cell types were used: (i) primary human umbilical vein endothelial cells (HUVECs) isolated from human umbilical cords by treatment with collagenase Type I, (ii) human umbilical vein endothelial cells immortalized with human telomerase reverse transcriptase (HUVECtert),¹⁹ and (iii) immortalized human dermal microvascular endothelial cells (HMEC-1, ATCC cat no. CRL-10636).²⁰ The tissue culture flasks used for cultivation were coated with a 1% (w/v) aqueous solution of gelatin for 30 min prior to seeding the cells suspended in EndoPrime® Medium (EndoPrime Kit, PAA, Linz, Austria). All cells were grown in a humidified atmosphere with 5% CO_2 at 37°C and subcultured twice a week using trypsin-EDTA solution.

The PDMS microchannels were coated with several coating solutions (see below) before seeding the endothelial cells suspended in M199 medium supplemented with growth factors, heparin, and 20% fetal bovine serum (500 μl cell suspension per channel). Confluent cell monolayers were accomplished after 1 day cultivation by seeding of 2×10^5 cells mL^{-1} in case of HUVECs and HUVECtert or after 3 days cultivation by seeding of 3×10^5 cells mL^{-1} in case of HMEC-1. The cells were used for the experiments between passage 2 to 8 (HUVECs), 21 to 28 (HUVECtert), or 30 to 38 (HMEC-1).

E. Coating of PDMS microchannels

Three different coating solutions were applied: (i) gelatin (1% w/v in phosphate buffered saline; PBS), (ii) collagen (0.14 mg mL^{-1} in PBS), and (iii) fibronectin (0.1 mg mL^{-1} in PBS). Briefly, after incubation of the autoclaved microchannels with sterile PBS (500 μl per channel) for 30 min at room temperature, 300 μl of the coating solution was added. After incubation for 1 h the coating solution was removed from the microchannels. In the case of gelatin and fibronectin, the cells were seeded immediately. In the case of collagen, the microchannels were washed twice with cell culture medium (500 μl each channel) prior to seeding of cells.

FIG. 1. Multichannel acoustically driven microfluidic platform. The SAW-chip consists of four IDTs, which are structured on a piezoelectric substrate (PES) and connected to a high frequency source (HF-input). Four microchannels for the cultivation of cells are formed by attaching poly(dimethylsiloxane) (PDMS) structures to a glass plate dimensioned $127 \times 85 \times 1.1$ mm (length $\times$ width $\times$ height). Inspection windows in the aluminum block allow for microscopic observation during experiments (A). The positioning of the microchannels on the glass plate was kept constant by a preprinted template in order to guarantee the localization of the IDTs underneath the microchannels (B). The system is operated by a radio frequency generator connected to a coaxial amplifier (operated by a laboratory power supply, with an integral fan for cooling), and a fixed attenuator. The radio frequency signal is distributed to the SAW-Chip by a 4-way divider (C). Schematic presentation of cross-section of acoustically driven microfluidic platform for the studying of interaction between particles and cells under flow condition (D).

Furthermore, composite coating with collagen/fibronectin was investigated. After collagen-coating as described above, $300 \mu\text{l}$ of fibronectin solution (0.1 mg ml^{-1} in PBS) were added and 15 min later the cells were seeded. Confluence of the cell monolayer was checked microscopically.

F. Identification of suitable buffer systems for flow experiments

Three commonly used buffer media were tested: (i) isotonic HEPES/NaOH buffer pH 7.4, (ii) PBS, and (iii) PBS supplemented with $\text{Ca}^{2+}/\text{Mg}^{2+}$. After filling each microchannel with $500 \mu\text{l}$ buffer, flow was induced by acoustic streaming at a power of 25 dBm (average shear

rate $\sim 2.25 \text{ s}^{-1}$) for 15 min. Subsequently, the integrity of the cell monolayers was checked microscopically. M199 cell culture medium instead of buffer was used as a control.

G. Cytoadhesion of microparticles under flow conditions

To study binding of microparticles to endothelial cell monolayers, $1 \mu\text{m}$ fluorescent polystyrene microspheres were used. After removing the cell culture medium from the microchannel, and washing the monolayer once with $500 \mu\text{l}$ PBS each, $500 \mu\text{l}$ microsphere suspension ($100 \mu\text{g ml}^{-1}$ in PBS supplemented with $\text{Ca}^{2+}/\text{Mg}^{2+}$) were added and the monolayer was incubated for 15 min under flow conditions. In order to generate different flow velocities, certain input power levels from 17.5 dBm to 25 dBm corresponding to average shear rates between 0.49 and 2.25 s^{-1} were applied. After removal of non-bound microspheres by washing twice with $500 \mu\text{l}$ PBS supplemented with $\text{Ca}^{2+}/\text{Mg}^{2+}$, the cell-associated fluorescence was determined in a microplate reader (TECAN; Infinite M200; excitation: 440; emission: 485 nm). The corresponding amount of cell-bound particles was calculated from a calibration curve that has been acquired with a dilution series of microspheres in the PDMS channels.

H. Microscopy

To study the morphology and integrity of endothelial cell monolayers in microchannels, phase contrast images were acquired at defined cultivation intervals using a Zeiss Axio Observer.Z1 (Zeiss, Göttingen, Germany).

In order to visualize the distribution of microparticles on the endothelial cell surface at the end of the cytoadhesion studies and to confirm confluence of the cell monolayer even after exposure to flow, fluorescence microscopy was applied. For this purpose, the cells were fixed in ice cold methanol (-20°C) for 10 min at -20°C . After rehydration in PBS containing 1% BSA for 20 min at room temperature the cells were washed with the same buffer. Then, $300 \mu\text{l}$ polyclonal goat-anti-human VE-cadherin (C-19) antibody ($200 \mu\text{g ml}^{-1}$, Santa Cruz Biotechnology) in PBS containing 1% BSA was added to label vascular endothelial cadherin by incubation for 1 h at 37°C . After washing with the same buffer, $300 \mu\text{l}$ of a 1:200 dilution of rhodamine-labelled rabbit-anti-goat antibody (1 mg ml^{-1} , Abcam) and $0.5 \mu\text{g}$ Hoechst 33342 in PBS containing 1% BSA were added and stored for 30 min at 37°C . Finally, the cell layers were washed twice with the same buffer and fluorescence images were acquired.

I. Data analysis

Data were statistically analyzed using the Microsoft Excel[®] integrated analysis tool. The hypothesis test among two data sets was made by comparing two means from independent (unpaired) samples (t-test). Values of $p < 0.05$ were considered statistically significant.

III. RESULTS AND DISCUSSION

A. A multichannel acoustically driven microfluidic platform

Since hydrodynamic flow is expected to affect the adhesion of particles to cells, dynamic biopharmaceutical test systems are of highest interest especially due to the growing importance of micro- and nano-scaled drug delivery systems. It will be demonstrated that a multichannel acoustically driven microfluidic system can be used to study such hydrodynamic effects *in vitro*. SAW-induced streaming of liquids in miniature channels has already been described previously.^{16,17} In brief, exciting IDTs on a piezoelectric substrate (LiNbO_3) at an appropriate frequency generates a SAW. When this SAW couples into a liquid, it generates a pressure gradient therein that induces streaming. In contrast to classical flow systems, which rely on external pumps (e.g., syringe pumps) and tubing, acoustic streaming allows to minimize the risk of contamination. Furthermore, by eliminating the need for tubing, the system becomes simpler and avoids any dead volume. It is also possible to upgrade the device and parallelize multiple SAW pumps on a chip. In the present work, a platform was developed which can be used to

simultaneously pump liquid in four microfluidic channels (Figure 1). For this purpose, four IDTs were glued into a pre-structured aluminum block and connected with ports for cabling. As illustrated in Figure 1, a small inspection window was included for each channel which allows microscopic observation by inverted or upright microscopes during the experiment. This device holds glass plates ($127 \times 85 \times 1.1$ mm, length $\times$ width $\times$ height) on which four PDMS-microchannels with arbitrary geometry can be mounted. The dimensions of the glass plate were chosen in order to match the size of standard microplates as most plate readers allow specifying the exact location of read-out points on a microplate. Thus, the geometry of the PDMS-microchannels on the glass plate can be directly programmed into the software. Consequently, data are simply collected by reading the luminescence, absorbance or fluorescence at pre-selected points in the microchannel. All in all, the system offers the advantages of low risk of contamination, high versatility in cultivation of cells of different origin, qualitative evaluation by microscopy and quantitative assessment of bound material by microplate reading as well as simple operation.

B. Characterization of multichannel acoustically driven microfluidic system

To assess the reproducibility and the attainable range of flow velocities with parallelized SAW-pumps, flow velocity was measured in several different microchannels. The flow velocity was determined in a laterally as well as vertically central region of the channel by measuring the translational velocity of suspended microparticles via fluorescence microscopy. As shown in Figure 2, there is a clear relation between input power and average shear rate in the channels. According to Eq. (1), the total input power, which directly operated SAW-device, ranged from 25 mW (14 dBm) to 1400 mW (31.5 dBm). Since four transducers were operated in parallel, every transducer is supplied with a fourth of the total input power. Typically, flow velocities between 0.3 mm s^{-1} and 10 mm s^{-1} were generated corresponding to average shear rates between 0.15 s^{-1} and 5 s^{-1} with the present channel geometry. It is important to mention, however, that by down-scaling of the channel dimensions (e.g., decreasing channel width and height) clearly higher average shear rates (1–2 orders of magnitude) can be attained.

As demonstrated in Figures 2(A) and 2(B), the average shear rates determined in the four microchannels are widely identical over a period of at least 30 min. This underlines that constant flow velocities and shear rates can be generated in this parallelized multichannel platform. At input powers >1400 mW (31.5 dBm), the system heats up considerably which ultimately leads to evaporation of the water couplant between the chip surface and the glass plate. This strongly reduces the coupling efficiency of the SAW into the microchannels. When operation of the SAW-chips at such high input power levels is desired, it would thus be necessary to use low-viscosity oil or similar non-aqueous coupling fluids to reduce evaporation by heating.

In order to guarantee comparable pumping levels in consecutive experiments, the localization of the IDTs underneath the microchannels has to be kept constant. This can be achieved easily by using a preprinted template for positioning of the microchannels on the glass plate (Figure 1(B)). Furthermore, two framing bars were included on the SAW-chip to allow for fixation of the glass plate and to ensure reproducible positioning of the IDTs underneath the channels (Figures 1(A) and 1(B)). If these potential pitfalls are kept in mind, only slight variations of the flow velocities will be observed (Figure 2(C)). These results collectively underline that parallelized SAW-chips can generate a considerable range of flow velocities in microchannels with satisfactory precision and reproducibility. Moreover, not only constant flow can be generated. In principle, pulsatile flow is easily attained by modulating the amplitude of the high frequency signal. The latter is a highly interesting approach to mimic the flow patterns prevalent in the circulatory system.

C. Cultivation of endothelial cells in microchannels

In order to generate artificial blood vessels, growth of endothelial cell monolayers in the microchannels would be desirable. To approach this aim, three different cell types representing large vessel and microvascular endothelial cells were tested for cultivation in PDMS microchannels:

FIG. 2. Average shear rates generated by IDTs in PDMS-microchannels on microfluidic platform. Channels 1–4 represent the respective microchannels arranged on top of the four IDTs (Figure 1). Flow velocities of 10 particles per channel were determined after 15 min (A) and 30 min of operation (B), respectively. Flow velocities of 10 particles, generated by one of the four IDTs of the platform in a microchannel on four different glass plates on consecutive days, were determined after 15 min of operation (C). (SD ≤ 1.12 , $n = 10$).

primary HUVECs, immortalized HUVEctert, and immortalized HMEC-1. Primary HUVECs are isolated from human umbilical cords by treatment with collagenase type I²¹ and are widely used as a cell model for *in vitro* studies in flow chambers.²² HUVECs offer the typical advantages of primary cells but they can only be cultivated for about eight passages. Retroviral infection with human telomerase reverse transcriptase (hTERT) can extend their ability to replicate and thus their lifespan.^{19,23} It is supposed that immortalized HUVEC cell lines (HUVEctert) exhibit similar functional and morphogenetic characteristics like their primary parent cells. Furthermore, immortalized human dermal microvascular endothelial cells (HMEC-1) represent an interesting cell line for cultivation in flow channels since they exhibit similar morphologic, phenotypic, and functional characteristics compared to their endothelial parent cells.²⁰

As endothelial cells do not adhere and proliferate on a plain glass surfaces, coating of the channel with gelatine, collagen, fibronectin, and collagen/fibronectin mixtures can facilitate adhesion. Several different techniques and protocols have been reported in the literature for one and the same adhesion molecule so that the following results only pertain to the exact protocols as described above. Although gelatin is frequently employed for the coating of tissue culture flasks for HUVECs, it was found that it is not optimal for coating PDMS-microchannels (Figure 3(A)). Similarly, coating with collagen also led to rather insular attachment of cells (Figure 3(B)). In contrast, coating with fibronectin mediated regular attachment of HUVECs to the surface inside the PDMS microchannels (Figure 3(C)). Interestingly, sequential coating of the channels with collagen and fibronectin did not result in improved cell adhesion (Figure 3(D)). Similar results as described above were also found for HUVEctert and HMEC-1 cell lines. As illustrated in Figure 4, all three types of endothelial cells formed a confluent monolayer with elongated and polygonal cells on fibronectin-coated glass. Thus, coating of PDMS microchannels with fibronectin was used in all further experiments.

D. Selection of buffers suitable for experiments with endothelial cells under flow conditions

In order to identify a suitable medium for experiments under flow conditions, the effect of three different commonly used buffer media (isotonic HEPES/NaOH buffer pH 7.4, PBS, and PBS supplemented with $\text{Ca}^{2+}/\text{Mg}^{2+}$) on endothelial cells were investigated. For that purpose, the cell culture medium overlying confluent endothelial cell monolayers inside PDMS microchannels was replaced by different buffers followed by incubation under flow conditions at an average shear rate of 2.25 s^{-1} for 15 min and microscopic images of the HUVEctert monolayers were acquired (Figure 5). As is clearly visible, the cell monolayers incubated with $\text{PBS} + \text{Ca}^{2+}/\text{Mg}^{2+}$ exhibited no morphological changes as compared to the M199 medium. Interestingly, incubation with PBS or isotonic HEPES/NaOH pH 7.4 resulted in detachment of endothelial cells from the channel surface (Figures 5(C) and 5(D)). This effect was also

FIG. 3. Phase contrast images of primary HUVECs one day after seeding ($2 \times 10^5 \text{ cells ml}^{-1}$) in microchannels coated with gelatin (1%, A), collagen (0.14 mg ml^{-1} , B), fibronectin (0.1 mg ml^{-1} , C) and collagen/fibronectin ($0.14 \text{ mg ml}^{-1}/0.1 \text{ mg ml}^{-1}$, D), respectively.

FIG. 4. Phase contrast images of primary HUVECs (A) and HUVEctert (B) one day after seeding (2×10^5 cells ml^{-1}) as well as HMEC-1 (C) four days after seeding (3×10^5 cells ml^{-1}) in microchannels coated with fibronectin (0.1 mg ml^{-1}).

observed under stationary conditions (data not shown). Most probably, the lack of Ca^{2+} and Mg^{2+} ions in these media weakens the adhesion between endothelial cells as well as that to the growth surface and reduces the monolayers' resistance against hydrodynamic stress. In addition, although the zwitter-ionic buffer HEPES is relative chemically inert²⁴ and is commonly used for different cell culture experiments, its stability is still not well defined and it can form aggregates that results in decreased buffering capacity.^{25,26} Consequently, the instability of HEPES might contribute to monolayer detachment. Therefore, PBS + $\text{Ca}^{2+}/\text{Mg}^{2+}$ buffer was chosen as suitable medium for particle-monolayer interaction experiments.

E. Cytoadhesion of microparticles to endothelial cell monolayers under flow conditions

Finally, cytoadhesion of negatively charged $1 \mu\text{m}$ polystyrene microparticles was investigated under different flow conditions. Endothelial cell monolayers in PDMS microchannels were incubated with particles at different average shear rates between 0.49 and 2.25 s^{-1} . Non-bound colloids were removed by washing, and then each cell layer was checked for confluence under the microscope. As shown in Figure 6, most of the cell monolayers retained their confluence after flow incubation and only monolayers of full integrity were analyzed. As depicted in Figure 7, the average number of cell-bound particles decreased slightly with increasing flow velocity. Similar results were found for all three types of endothelial cells. This suggests that increasing hydrodynamic drag exerts no significant influence on the cytoadhesion of polystyrene microparticles in the rate of

FIG. 5. Differential interference contrast images of HUVEctert after 15 min incubation with M199 medium (A), PBS + $\text{Ca}^{2+}/\text{Mg}^{2+}$ (B), PBS (C), and isotonic HEPES pH 7.4 (D) under flow conditions (average shear rate 2.25 s^{-1}).

FIG. 6. Fluorescence microscopic image of cell-bound $1\ \mu\text{m}$ polystyrene microparticles (green) after incubation at an average shear rate of $1.13\ \text{s}^{-1}$ after two washing steps. The nuclei of HUVECs were stained in blue using Hoechst 33342 and VE-cadherin was immunostained in red.

average shear rates tested. Interestingly, similar results have been obtained for the interaction between Caco-2 cell monolayers (enterocytes) and $1\ \mu\text{m}$ polystyrene microparticles coated either with wheat germ agglutinin or with poly(ethyleneimine).²⁷ Charoenphol *et al.*,²² who modified the surface 0.5 and $2\ \mu\text{m}$ polystyrene particles with sialyl-Lewis A to target vascular selectin, have also made comparable observations. Their cytoadhesion studies were performed in DPBS buffer using a circular parallel plate flow chamber with shear rates between $100\ \text{s}^{-1}$ and $640\ \text{s}^{-1}$. In accordance with our work, no significant difference in adhesion levels of particles onto endothelial monolayers was observed.²² Interestingly, a lower binding rate of microparticles was observed in case of HMEC-1 monolayers as compared with HUVECs and HUVECtert. The latter two types of endothelial cells, however, revealed similar microparticle binding capacity. At the highest average shear rate of $2.25\ \text{s}^{-1}$, the binding efficiency of particles to HMEC-1 monolayer was only about 65% of that to HUVECs monolayer; at average shear rates of 0.5 , 1 , and $1.5\ \text{s}^{-1}$ the particle adhesion level on HMEC-1 monolayer was 79%, 73%, and 78% of that on HUVECs monolayer, respectively. This significant difference might be due to the different endothelial cell type. HUVECs were

FIG. 7. Cytoadhesion of $1\ \mu\text{m}$ fluorescent polystyrene microparticles on HUVECs, HUVECtert and HMEC-1 monolayers at different average shear rates between $0.5\ \text{s}^{-1}$ and $2.25\ \text{s}^{-1}$ in a multichannel microfluidic platform. ($n \geq 28$). Statistically significant differences were determined by student's t-test. *ns = not significantly difference. # = $p < 0.01$ compared to HUVECs-binding at the same average shear rate.

isolated from umbilical cords, which represent the cell type in large vessels, whereas HMEC-1 represent a microvascular cell type. According to the literature, not only the growth rate and differentiation status^{20,28} but also the cell surface structures and the amounts of cell adhesion molecules expressed are different for endothelial cells derived from microvascular vessels compared to large vessels according to their different functionality.^{29–32} This might be a possible reason why HUVECs exhibited higher binding of negatively charged 1 μm polystyrene particles than HMEC-1.

All in all, as a proof-of-concept, cytoadhesion studies with 1 μm negatively charged microspheres were performed in this novel multichannel microfluidic system. Three different types of endothelial cell monolayers retained their confluency under flow conditions which allows for successful mimicking of macro- and micro blood vessels. Due to the expected impact of surface properties, dimension and size of particulate drug carriers, it might be interesting to study cytoadhesive characteristics of different types of particles in further experiments. Another important parameter to be elucidated might be viscosity of the medium because the shear stress is the arithmetic product of shear rate and viscosity. Thus, further hydrodynamic studies with media of viscosity similar to blood and with suspended blood cells might bring us one step closer to physiological conditions.

IV. CONCLUSION

A.T. Florence has hypothesized that hydrodynamic effects could play a substantial role in the interaction between particles and cells or tissues *in vivo*, in particular in the circulatory system.³³ In order to shed some light on this issue, a multichannel microfluidic setup was developed and optimized that can be used to mimic physiological flow conditions in the lab. This chip-based system allows constant and pulsed flow. With the current channel geometry average shear rates up to 5 s^{-1} are easily generated. By integration of four identical SAW-pumps in one platform, parallelized experiments can be carried out, which greatly extends versatile applicability of this microfluidic system. In addition, the dimensions of our current setup match those of standard microplates. Thereby, channel-associated fluorescence, luminescence, and absorbance can be simply detected by using a standard microplate reader. Furthermore, the device is fully compatible with cell culture as demonstrated by successful cultivation of HUVECs, HUVEctert, and HMEC-1 monolayers inside the PDMS-microchannels. To achieve the latter, the channel surface was coated with a 0.01% solution of fibronectin. Moreover, the endothelial monolayers remained widely intact during incubation with buffers and microparticle suspensions under flow conditions. Studying the cytoadhesion of negatively charged 1 μm microparticles to endothelial cell monolayers under flow conditions revealed no significant effects of hydrodynamic drag at average shear rates between 0.5 s^{-1} and 2.25 s^{-1} for all three types of endothelial cells. However, most probably due to the differences between endothelial cells derived from large and small vessels, HUVECs and HUVEctert exhibited higher particle binding capacity as compared to HMEC-1. In conclusion, the developed multichannel microfluidic platform represents a promising device to elucidate the effect of shear forces on cell-binding of particles. Moreover, the system is not only limited to biopharmaceutical applications but is also suitable for general experiments on the effect of hydrodynamic forces in luminal compartments of the human body. Further studies with such dynamic *in vitro* test models are expected to improve our understanding of basic mechanisms in targeted drug delivery and cell physiology.

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