Published in final edited form as:

Lab Chip. 2009 April 21; 9(8): 1085–1095. doi:10.1039/b812184b.

An easy to assemble microfluidic perfusion device with a magnetic clamp

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Abstract

We have built and characterized a magnetic clamp for reversible sealing of PDMS microfluidic chips against cover glasses with cell cultures and a microfluidic chip for experiments on shear stress response of endothelial cells. The magnetic clamp exerts a reproducible uniform pressure on the microfluidic chip, achieving fast and reliable sealing for liquid pressures up to 40 kPa inside the chip with <10% deformations of microchannels and minimal variations of the substrate shear stress in perfusion flow. The microfluidic chip has 8 test regions with the substrate shear stress varying by a factor of 2 between each region, thus covering a 128-fold range from low venous to arterial. The perfusion is driven by differential pressure, which makes it possible to create pulsatile flows mimicking pulsing in the vasculature. The setup is tested by 15 – 40 hours perfusions over endothelial monolayers with shear stress in the range of 0.07 - 9 dyn/cm². Excellent cell viability at all shear stresses and alignment of cells along the flow at high shear stresses are repeatedly observed. A scratch wound healing assay under a shear flow is demonstrated and cell migration velocities are measured. Transfection of cells with a fluorescent protein is performed, and migrating fluorescent cells are imaged at a high resolution under shear flow in real time. The magnetic clamp can be closed with minimal mechanical perturbation to cells on the substrate and used with a variety of microfluidic chips for experiments with adherent and non-adherent cells.

Perfusion chambers for experiments with live cells are among the most straightforward and versatile microfluidic devices. In addition to substantial reduction of the amounts of media and cells required for an experiment as compared to traditional flow chambers, microfluidic perfusion chambers offer numerous new capabilities. Those include the variation of composition of the perfusion medium across the microfluidic chip^{1, 2} and in time,³ the capture of flowing cells using microfabricated weirs or posts,^{3, 4} and generation of substrate coatings with customized micro-patterns.⁵ Microfluidic perfusion devices that are made of a cast PDMS chip sealed with a microscope cover glass also offer the advantages of compatibility with the standard high-resolution microscope objective lenses as well as low cost and disposability. Microfabrication makes it easy to produce tapered perfusion chambers, which generate varying shear stresses at the substrate.⁶ Chambers of this type were used to study the shear-dependent adhesion of lymphocytes to various substrates⁷ and to measure the strength of the substrate adhesion of fibroblasts⁸ and neutrophils.⁹

One of the applications of flow chambers is studies of shear stress responses of endothelial cells.¹⁰⁻¹³ Endothelial cells form a monolayer on the interior surface of blood vessels and

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sense shear stress generated by blood flow. Endothelial cells respond to shear stress with rearrangements, vascular remodeling, and alterations of vascular tone and vascular permeability.¹⁴⁻¹⁷ Shear stress can modulate many functions of endothelial cells including gene expression, cell adhesion, proliferation, differentiation, migration, and cytoskeletal alignment relative to the direction of flow.¹⁸⁻²¹ Previous experiments with endothelial cells in microfluidic devices included studies of static surface adhesion,^{22, 23} dynamics of surface adhesion from a flowing suspension,²⁴ and adhesion of neutrophils to endothelial monolayer.²⁵ Takayama *et al.*²⁶ built and tested a device where endothelial cells were exposed to pulsatile flows. The device had a relatively complicated construction, with the flow driven by on-chip peristaltic pumps and with the shear stress proportional to the pulsing frequency. Simon *et al.*²⁷ used a microfluidic device with a tapered perfusion chamber applying a ~10-fold range of shear stress to endothelium to study the shear stress-dependent protein transcription and membrane expression of cell adhesion molecules.

The loading of cells into a microfluidic device can be a delicate task, especially if the cell stock is small, cells are sensitive to hydrodynamic stresses, or a particular cell density on the substrate needs to be reached. To create a confluent culture of endothelial cells in a sealed microfluidic device, several consecutive steps are required: on-chip coating of the substrate by an extracellular matrix (ECM) protein to make cells adherent to the substrate; loading the microchannels with a cell suspension with subsequent washing of excessive cells; maintaining the culture until confluence is reached. Endothelial monolayers with a good degree of confluence (>75%) on glass and plastic surfaces can be created using this procedure,^{28, 29} but it may involve repeated cell loading and close monitoring of the quality of the monolayer. For extensive experiments in a biological laboratory, it would be advantageous to have a microfluidic platform compatible with a standard laboratory procedure for creating a confluent endothelial monolayer on a cover glass. The two main techniques that have been proposed to seal PDMS microchannel chips against wet substrates with cell cultures on them are mechanical clamping^{30, 31} and vacuum suction^{25, 27, 32-35}. However, the application of mechanical clamps usually involves poorly characterized and not completely reproducible mechanical stresses that may cause substantial deformations of microchannels, which may vary over the device. Sealing by vacuum suction is achieved by having the “wet” microchannel array with media and cells surrounded by a secondary network of “dry” microchannels connected to a source of vacuum.^{25, 27, 32, 34, 35} This technique was used for short term experiments with endothelial cells^{26, 28} and for long-term stem cell cultures.³⁵ Nevertheless, the application of vacuum might cause some slow changes in the gas content of the wet channel medium that may be difficult to detect and quantify.

Here we present a setup and technique to reversibly seal PDMS microfluidic chips against cover glasses with cell cultures by application of a controlled magnetic force. The setup (Figure 1) consists of a steel base holding the cover glass and a cover that houses a set of magnets and holds the chip. The magnetic clamp produces small deformations of the microchannels of the chip resulting in minimal variations of substrate shear stress, while providing reliable sealing up to pressures of 40 kPa inside the microfluidic device. The setup can be used with any type of microfluidic network of an appropriate size, as long as inlet and outlet ports of the network match those in the cover. Application of controlled differential pressures between the inlet and outlet makes it possible to generate flows with a broad range of speeds and substrate shear stresses, as well as pulsatile flows with well-defined characteristics. The sealing of the microfluidic devices with the magnetic clamp is simple and fast. The clamp can also be closed slowly by using a set of thumb screws in the cover (Figure 1), thus minimizing the flow and hydrodynamic stresses generated during the sealing.

To demonstrate the utility of the magnetic clamp setup, we used it for a series of proof-of-principle experiments on migration and alignment of confluent monolayers of Human

Umbilical Vein Endothelial Cells (HUVECs) under flows with various shear stresses. The microfluidic device had a microchannel network with 8 separate rectilinear test regions and with surface shear stress varying by a factor of ~ 2 between each region, thus covering a 128-fold range in the shear stress, from low venous to arterial. The endothelial monolayers consistently showed excellent viability during 15 hour perfusions, and the shear dependent alignment of cells was in agreement with previous data obtained using more conventional apparatuses. The experiments highlight the advantages of the proposed magnetic clamp setup compared with sealed microfluidic devices: the use of endothelial monolayers grown to confluence on cover glasses and the possibility to generate scratch wounds and to transfect cells in the monolayers using standard laboratory protocols.

Experimental

Design and fabrication of the magnetic clamp setup

The magnetic clamp setup consisted of two parts, the base and the cover (Figure 1), and had dimensions similar to those of a commercial perfusion device with a magnetic clamp, Chamslide™ (by LCI Corp., Seoul, Korea). The base was a $\varnothing 64$ mm, 3 mm thick disk made of grade 410 magnetic stainless steel. The base had a $\varnothing 29$ mm, 1 mm deep groove on its top side, a $\varnothing 15$ mm opening in the middle, and a 0.15 mm deep step around the opening to hold a circular 25 mm microscope cover glass (Figure 1d). The opening expanded at a wide angle toward the bottom of the base to allow good access of microscope objective lenses to the cover glass. The base had 3 ground steel pins, $\varnothing 3/16$ ", pressure-inserted into it. The cover was assembled out of two parts, a $\varnothing 64$ mm, 4.5 mm thick brass ring and a $\varnothing 38$ mm, 6 mm thick Plexiglas disk that was fastened to the ring with 4 screws (Figure 1a). The brass ring had 6 wells to house $\varnothing 1/4$ ", $1/8$ " thick Neodymium magnets (Magcraft™). Each well had one magnet permanently glued into it, and extra magnets could be added or removed to vary the magnetic force between the cover and the base. The ring had three holes matching the pins in the base to ensure concentric assembly of the setup and smooth sliding of the cover toward the base. The ring also had three threaded holes for M3-0.5 thumb screws (Figure 1a, d). The screws, which had rounded tips, supported the cover against the magnetic force and made it possible to set the distance between the cover and the base and to change the distance by small steps.

The Plexiglas disk had two pairs of juxtaposed holes drilled in its sides with short segments of steel hypodermic tubing inserted into the holes (Figure 1a, d). The steel tubing inserts were connected through lines of PVC tubing to reservoirs with the medium fed to the microfluidic device and drawn off from the device. The side holes were connected to $\varnothing 1.5$ mm vertical holes drilled 8.5 mm from the center that opened at the top of the disk to bubble traps, which were closed by plugs (Figure 1a, d). At the bottom of the disk, the vertical holes were connected to through-holes in an $\sim \varnothing 27$ mm, 5 mm thick flat-parallel slab of a transparent soft silicone rubber that was bonded to the Plexiglas disk and used as a cushion between the disk and the PDMS microchannel chip (Figure 1c). The soft cushion (Shore A durometer 20 vs. 50 for the microchannel chip) converted the magnetic force pulling the cover toward the base into a uniform pressure applied to the top of the chip. The chips used with the clamp had microchannels engraved on their bottom side facing the cover glass (Figure 1c, d). The chips had a diameter of ~ 24.5 mm, matching standard 25 mm microscope cover glasses, and the inlet and outlet through-holes in the chips (8.5 mm from the center) were aligned with those in the cushion (Figure 1c, d). The PDMS chips had a relatively small thickness of ~ 1.2 mm to achieve a low bending modulus for uniform distribution of the pressure from the cushion over the chip surface and for good contact between the chip and the cover glass.

To fabricate a cushion, four $\varnothing 1.5$ mm, 4.8 mm tall stainless steel posts were glued to the surface of a silicon wafer at distances of 8.5 mm from a common center. The wafer was placed onto a leveled horizontal surface and a silicone pre-polymer (10:1 mixture of base and activator of

XP-565 rubber by Silicones Inc., High Point, NC) was slowly poured onto the wafer, until the posts were completely submerged and the surface of the pre-polymer on top of them was flat. The silicone was slowly cured on a leveled substrate in a 50 °C oven and subsequently separated from the wafer. The vertical holes produced by the pins were opened at the top, and the silicon cast was punched by a sharpened steel tube, ~Ø27 mm, that was centered with respect to the holes. A completed cushion had flat upper and lower surfaces, and its thickness was uniform to within ~0.1 mm. To bond the cushion to the Plexiglas disk, a droplet of PDMS pre-polymer (Sylgard 184 by Dow Corning) was dispensed onto the center of the disk, the cushion was placed on top of the droplet and pressed against the disk, and the assembly was baked for 90 min in a 80 °C oven to cure the PDMS. (The disk and the cushion had 4 matching holes each, but only two juxtaposed holes were connected to the PDMS chips used in the present study, Figure 1c.) The disk, cushion, and PDMS chip were all optically clear and had flat-parallel surfaces, making the setup compatible with brightfield and phase-contrast microscopy.

Design and fabrication of microfluidic chips

The microfluidic chips used in the study (Figure 2) had a network of 75 µm deep microchannels and were cast of PDMS (Sylgard 184 by Dow Corning) using a photo-lithographically fabricated master mold. To produce the mold, a 5" silicon wafer was spin-coated with a 75 µm thick layer of a UV-curable epoxy (SU8-2050 by Microchem), exposed to UV-light through a specially designed photomask (photo-plotted at a resolution of 20,000 dpi), and developed. 15 g of PDMS pre-polymer was poured onto the mold, and the mold was placed onto a leveled horizontal surface in a 65 °C oven for 30 min to allow the pre-polymer to reflow, spread in an even layer, and slowly cure. To complete the curing, the mold was placed into an 80 °C oven for 1 hr. The cured PDMS cast was separated from the mold, individual chips were punched from the cast with a ~Ø24.5 mm sharpened steel tube (the cast had 24.5 mm rings engraved in it for concentric punching; Figure 2), and the inlet and outlet holes were punched in the chips with a gauge 14 luer stub. To increase the hardness and Young's modulus of the chips, they were backed for 30 min in a 150 °C oven. Completed chips had a thickness of ~1.2 mm that was typically uniform to better than 0.05 mm.

The microchannel network (Figure 2) had four separate lines of channel connecting the inlet and the outlet. Each channel line had a 0.6 mm wide and a 1.2 mm wide rectilinear segments, which served as test regions to study shear stress responses of endothelial cells. The mean flow velocity and substrate shear stress, τ , in two test regions of each channel line differed by a factor of 2 because of the 2-fold difference in the test region widths. The device also had 100 µm wide resistance channels that were designed to provide a 4-fold difference in the total hydrodynamic resistance between different channel lines and 4-fold variation of the volumetric flow rate through them. Altogether, the device was designed to have 2-fold variation of τ between test regions with consecutive numbers (Figure 2), and a total 128-fold range in τ . Numerical modeling with FemLab (COMSOL) indicated that for a fully developed laminar flow in a 75 µm deep channel line, the ratio of τ in internal regions (away from the side walls) of the 0.6 mm and 1.2 mm wide rectilinear channels is 2.06 (slightly greater than 2 due to side wall effects). The modeling also showed that the substrate shear stress in both channels is reduced near the side walls. Nevertheless, τ is within 5% of its maximal value (attained in the middle) in a 0.47 mm wide internal area of the 0.6 mm wide channel and in a 1.06 mm wide internal area of the 1.2 mm wide channel. Therefore, cells on the substrate in each of the two internal areas are expected to experience a nearly uniform mechanical stimulus.

Cell culture

Human Umbilical Vein Endothelial Cells (HUVECs) (Cambrex) were grown in endothelial basal medium (EBM-2) containing endothelial growth factor supplements (EGM-2 bullet kit, Cambrex). Circular 25 mm, #2 cover glasses were rinsed in methanol, spin-dried, treated for

5 seconds with air plasma using a laboratory corona treater (BD-20AC, by Electro-Technic Products, Inc., IL), and coated with fibronectin by placing a droplet of a 5 $\mu\text{g/ml}$ fibronectin solution onto a cover glass and incubating it for 1 hr. The cover glass surface was subsequently blocked by 30 min incubation under a 1% solution of heat-denatured BSA. An estimated 0.5×10^6 HUVEC cells were plated on the fibronectin-coated cover glass and incubated for 48-72 hours to form monolayer.

Experimental system and procedure

The medium fed to the inlet and drawn off from the outlet was kept in identical plastic syringes (10 or 60 cc for tests of flow in the device and 140 cc for extended perfusions with endothelial monolayers), which were held upright with the luer connectors at the bottom.³⁶ The syringes were connected to the device inlet and outlet through blunt hypodermic needles and ~1 m long segments of PVC tubing with the inner diameter of 1 mm. The syringe connected to the outlet was held at a height of ~10 cm above the level of the device to ensure positive pressure everywhere inside the device and prevent formation of air bubbles. The syringe connected to the inlet was attached to a stage sliding along a vertical rail. The flow in the microfluidic device was driven by a differential hydrostatic pressure between the inlet and outlet, $\Delta P = \rho g \Delta h$ (where $\rho = 1 \text{ g/cm}^3$ is the density of the medium), that was set by adjusting the difference between the levels of the media in the two syringes, Δh , and was controlled within 5 Pa (0.5 mm in Δh). The syringes were covered at the top to reduce evaporation but were normally vented to the atmosphere (not sealed).

Measurements of flow velocity and channel deformation in the device were performed on a Nikon Diaphot inverted fluorescence microscope equipped with a 100W mercury light source. A Sony XCD-X900 IEEE 1394 camera (1280×960 pixel CCD array, 7.5 frames/s) or a Marlin-F033b IEEE 1394 camera (640×480 pixel CCD array, 60 frames/s) were used for video microscopy. Flow velocities in the microfluidic device were measured with aqueous suspensions of 2 μm green fluorescent tracer particles. The microscope was focused at the mid-plane of microchannels, and the maximal flow velocities, v_{max} , were evaluated by measuring the lengths of longest streaklines produced by the beads. The fluorescence light source in the channel deformation tests was a high power blue LED (Royal Blue Luxeon V by Lumileds; central wavelength 455 nm) inserted into a modified Nikon lamp house. The LED was powered by a regulated DC supply and provided stable fluorescence illumination (<1% variation of intensity over several hours).

The experiments with endothelial cells were performed on a Nikon TE2000 inverted fluorescence microscope equipped with a cooled digital camera (CoolSnapHQ, by Roper Scientific) and an environmental enclosure. Time-lapse images of cells in various regions were acquired in parallel using a computer-controlled XYZ-motorized stage (LUDL 99S000). The stage was programmed to move in loops with stops at the positions of interest and was interfaced with the image acquisition using a routine in QEDinVivo (Media Cybernetics, Bethesda, MD).

The environmental enclosure maintained the temperature at $37 \pm 0.5 \text{ }^\circ\text{C}$, which was appropriate for endothelial cells. The temperature stabilization also minimized the drift of focus in the microscope. In addition to the microscope enclosure, we used a stage enclosure, which was placed onto the motorized microscope stage on top of the magnetic clamp setup. Continuous flow of humidified air with 5% CO_2 through the stage enclosure created a stable and nearly optimal gas environment around the PDMS microfluidic chip that helped in maintaining cell viability. The syringes with perfusion media connected to the inlet and outlet were kept outside of the microscope enclosure. To prevent formation of gas bubbles in the device due to reduction of gas solubility caused by heating of the medium from room temperature to $37 \text{ }^\circ\text{C}$, the syringe connected to the inlet was warmed to $\sim 37 \text{ }^\circ\text{C}$ by using a heat strip and a temperature controller.

Before an experiment was started, air with 5% CO₂ was bubbled through the perfusion medium in the inlet syringe to set the gas content of the medium and to bring its pH to 7.4. To maintain both the pH and the gas content constant during prolonged perfusions, a slow stream of air with 5% CO₂ was continuously blown above the medium surface in the inlet syringe.

Before the magnetic clamp was assembled, the engraved surface of the PDMS chip was treated with air plasma (to make it more wettable) and the back side of the chip was attached to the cushion on the clamp cover. A syringe with ~60 mL of perfusion medium was connected to the inlet, and a syringe with a small amount of the medium was connected to the outlet through ~1 m long lines of PVC tubing. Once the flow of the medium reached the cover of the magnetic clamp, filled the bubble traps, and flooded the surface of the PDMS chip, the bubble traps were closed with plugs, and both tubing lines were clamped to stop the flow through them. The cover glass with endothelium monolayer and with a thin layer of the perfusion medium on top of it was placed onto the base of the clamp. The thumb screws in the cover of the clamp were completely retracted, the holes of the cover were aligned with the pins of the base, and the cover was allowed to slide down under the action of magnetic force and gravity. The setup closing was accompanied by flow of the medium squeezed from between the chip and the cover glass. To prevent damage to the endothelium due to hydrodynamic stresses generated by the flow, it was important to unclamp the inlet and outlet PVC tubing lines before closing of the setup, thus providing a relatively low-resistance escape route for the medium. To separate the cover from the base after completion of a perfusion assay, the thumb screws were turned clockwise in a cyclic order, thus lifting the cover and gradually reducing the magnetic force.

Results

Magnetic force, quality of sealing, and channel deformation

A major concern about clamping as a method for sealing a microfluidic device is that it may cause substantial deformations of microchannels that would be inconsistent between different tests and would significantly alter the pattern of flow in the device. In the proposed magnetic setup, the clamping force is reproducible and depends only on the number of magnets and on the distance, d , between the cover and the base of the clamp. To measure the magnetic force between the cover and the base, the clamp was firmly attached to the top of an electronic balance, and the cover was slowly pulled upwards by three soft springs until it detached from the base. The magnetic force was quantified as the difference in readings of the balances between the time before the pulling started and a time immediately before the cover detached from the base. The measurements were done with different numbers of magnets in the wells of the cover (always the same number in each of the 6 wells) and at different d (Figure 3a). The magnitude of d was set by placing spacers of known thickness (brass shim) between the cover and the base with no other contact between the two parts. We chose $d = 0.7$ mm as a standard value for the setup, with 1 – 5 magnets per well providing clamping forces of 0.27 – 0.95 Kg (Figure 3a, inset) and pressures of 5.6 – 20 kPa on the 24.5 mm circular PDMS chips.

The quality of sealing produced by the clamp is measured by the maximal internal pressure a sealed device can withstand without leaking. To quantify this “burst” pressure, we sealed the PDMS chip (Figure 2) against a plain #2 cover glass, filled the sealed device with water, connected the inlet and the outlet to the same regulated source of compressed air, and gradually increased its pressure until the microfluidic device leaked. As expected, the burst pressure increased with the number of magnets (Figure 3b). Somewhat surprisingly, the values of the burst pressure were systematically higher than the pressure exerted onto the PDMS chip by the clamp cover, e.g., 28 kPa vs. 15 kPa for 3 magnets and 40 kPa vs. 20 kPa for 5 magnets. The added resistance to the pressure might have originated from adhesive forces between the surfaces of the cover glass and PDMS chip.

To evaluate the deformation of microchannels caused by the pressure exerted onto the chip by the clamp, we filled the device with a solution of fluorescent dye (6 ppm by weight of fluorescein isothiocyanate, FITC, by Sigma, St. Louis, MO) in a pH7 phosphate buffer and measured the intensity of its fluorescence. At a constant microchannel height of 75 μm , the fluorescence intensity of 0 – 8 ppm FITC solutions, which was measured with a 6.3 $\times$ /0.2 objective, was a linear function of concentration. The fluorescence intensity of a 6 ppm solution of FITC measured in three microchannels with depths of 20, 100 and 200 μm was a linear function of the channel depth within $\sim 3\%$ measurement error. Therefore, fluorescence intensity of a 6 ppm FITC solution in the proposed device was expected to be a linear function of the local channel height, h . The measurements were done in four 1.2 mm wide channels (test regions 1, 3, 5, and 7; Figure 2), four 0.6 mm wide channels (test regions 2, 4, 6, and 8), and four 100 μm wide channels (resistance channels in different areas of the chip). To have a reference point for the original channel height of 75 μm (no channel deformation), we first bonded the PDMS chip to a dry cover glass, filled the chip with the FITC solution, and measured fluorescence in the channels without the cover applied. The test was subsequently repeated with the clamp and 1 – 5 magnets in each well of the cover (Figure 3c). The sagging of the channel roofs was minimal near the channel walls and maximal in the middle of the channels. As expected, the sagging increased with both the number of magnets and channel width. Nevertheless, even for the 1.2 mm wide channels, the roof sagging in the middle of the channels was only $\sim 5\%$ of the channel height with 2 magnets (burst pressure of 16.5 kPa) and remained limited to $\sim 9\%$ with 5 magnets (burst pressure of 40 kPa). The height reduction was about half as large for the 100 μm wide resistance channels (Figure 3c).

To evaluate the influence of the channel deformation upon substrate shear stress, τ , during perfusion, we measured the maximal velocity of flow, v_{max} , in test region 7 (1.2 mm wide; Figure 2) at a differential pressure $\Delta P = 2$ kPa between the inlet and outlet. The measurements were performed with 2 – 5 magnets and the obtained values of v_{max} were used to calculate the surface shear stress as $\tau = 4\eta v_{\text{max}} / h$, where $\eta = 0.01$ Ps was the viscosity of water at room temperature (20 $^{\circ}\text{C}$) and h was the channel height measured in the previous test (Figure 3d). The augmentation of the magnetic force from 0.52 to 0.95 Kg (cf. Figure 3a, inset) and the resulting reduction of the channel height by $\sim 4\%$ (cf. Figure 3b) had little effect on τ ($\sim 1\%$ variation). The change in τ was so small most likely because the rate of flow through the test regions primarily depended on the flow resistance of 100 μm wide channels, which suffered about half as much roof sagging as the test regions themselves (Figure 3c).

Based on the results of the above tests, we chose to use 3 magnets per well. With a given number of magnets, the clamping force can only vary as a result of changes in the thickness of the PDMS chips used with the setup, which cause variations in d . The chip thickness variations are easy to limit to < 0.2 mm, resulting in d within 0.5 – 0.9 mm and the clamping force in a range of 0.66 – 0.98 Kg (pressure 14 – 20 kPa; Figure 3a). From the data shown in Figure 3c and 3d, the mean sagging of the microchannel roofs in this range of clamping force is expected to be < 7 μm and < 6 μm for 1.2 mm and 0.6 wide test regions, respectively, and is expected to lead to a minimal variation in τ in the test regions. The lowest value of the clamping force, 0.66 Kg, corresponds to a burst pressure of ~ 24 kPa, which is almost an order of magnitude higher than the pressure $\Delta P = 2.75$ kPa required to generate a substrate shear stress of 9 dyn/cm^2 (see below), a value characteristic for arterial blood flow. Therefore, the magnetic clamp achieves reliable sealing and makes it possible to perform consistent perfusion experiments with sufficiently high levels of surface shear stress.

Shear stresses in different test regions

We measured maximal flow velocities, v_{max} , in the test regions of the device at a differential pressure $\Delta P = 2.75$ kPa at the room temperature by feeding to the inlet water seeded with tracer

particles (2 μm fluorescent polystyrene beads). Streaklines produced by the particles were photographed under fluorescence illumination, and the value of v_{max} was obtained by dividing the maximal length of the streaklines (measured at the central axis of the channel) by the exposure time. The substrate shear stress in internal areas of the test regions was then calculated as $\tau = 4\eta v_{\text{max}} / h$. The value of v_{max} was highest in test region 8, where it was 19 mm/s, corresponding to a Reynolds number $Re = v_{\text{max}}\rho h / \eta = 0.14$, where $\rho = 1 \text{ g/cm}^3$ is the density of water. This relatively low value of Re indicated a stable laminar flow with a linear dependence of τ on ΔP . The uncertainty of τ was estimated as $\sim 4\%$ and was due to uncertainties in measurements of the streakline lengths and of the microchannel heights. The values of τ measured in consecutive test regions of the device closely followed a geometric progression with the common ratio of 2, deviating from the progression by $<13\%$ and spanning a total range of 127 (Table 1), essentially the same as the design target value of 128. The shear stresses in the test regions, $0.07 - 9 \text{ dyn/cm}^2$, were chosen to cover a range from low venous to arterial shear stresses in human circulation. We note that for a laminar flow driven by a given differential pressure, ΔP , the substrate shear stress, τ , is independent of the viscosity of the fluid. For example, for a flow in wide and shallow rectilinear channel of length L , it is $\tau = \Delta P h / (2L)$. Therefore, the results on τ obtained at the room temperature (Table 1) were directly applicable to experiments with endothelial cells at 37°C , when the fluid viscosity was $\sim 30\%$ lower.

Minimizing hydrodynamic stresses during sealing of the device

Because endothelial cells had strong adhesion to the substrate, no special care needed to be taken when sealing the device other than opening the connections of the microfluidic chip to the inlet and outlet medium reservoirs. Nevertheless, to avoid unwanted exposure of cells to high shear stresses during the device sealing and for potential perfusion assays with weakly adherent cells, it is desirable to have a means to minimize hydrodynamic stresses during sealing of the device. To reduce stresses during the sealing, the three thumb screws in the cover were set to an initial distance of $d = 2 \text{ mm}$ between the chip and cover glass. After the clamp cover was placed in its initial position, the thumb screws were turned counterclockwise by equal angles in a cyclic order, leading to a gradual approach of the chip to the cover glass. For two parallel disks (the chip and cover glass) approaching each other at a constant speed, v_z , the flow of the liquid squeezed out from the space between the disks produces substrate shear stress, $\tau = 3\eta v_z / z^2$, that is proportional to the radial coordinate, r , and inversely proportional to the square of the distance between the disks, z . (For the microfluidic device, this dependence is expected to be modified starting from distances on the order of the microchannel height, $h = 75 \mu\text{m}$). Therefore, the speed of approach was reduced as the distance, z , became smaller. Steps in z as small as $10 \mu\text{m}$ were made by turning a single screw (0.5 mm pitch) by $\sim 20^\circ$.

To evaluate τ , we deposited onto the cover glass an aqueous suspension of $10 \mu\text{m}$ red fluorescent polystyrene beads (with a density of 1.05 g/cm^3 and fast sedimentation) and tracked their motion under a microscope. The beads stayed at the substrate, but did not adhere to it. Therefore, their speed was expected to be proportional to τ . The tracking of beads in the downstream part of test region 3 with $r \approx 5 \text{ mm}$ (the highest value for the entire area of test regions) indicated that their speed never exceeded $100 \mu\text{m/s}$, as the microfluidic chip approached the cover glass (Figure 4). A subsequently started flow (time $> 60 \text{ sec}$ in Figure 4), which was driven by a differential pressure $\Delta P = 2.75 \text{ kPa}$ and produced a substrate shear stress $\tau = 0.25 \text{ dyn/cm}^2$ (cf. Table 1), resulted in a collective motion of the beads at $\sim 150 \mu\text{m/s}$. Therefore, during the sealing of the device, the substrate shear stress was limited by $\sim 0.17 \text{ dyn/cm}^2$. This level of shear stress is expected to be sufficiently low even for weakly adherent cells such as human embryonic kidney cells and HL-60 cells and, when applied for a short interval, should cause minimal perturbation to neuronal growth cones.³⁷ After the device was sealed, the number of beads in the field of view was reduced by less than a third compared

with the initial number of beads. Therefore, for some experiments, it may be practical to close the clamp immediately after cells from the deposited suspension sediment onto the cover glass, without letting the cells spread and attach to the substrate. In addition, the magnetic clamp setup may be suitable for experiments with non-adherent cells that would be confined by weirs or traps after the device is sealed.^{3, 4}

Pulsatile flow

Blood flow in the vasculature and the shear stress at the endothelium are inherently pulsatile. The ratio between the pulsing and constant parts of the shear stress varies over the vasculature, being generally higher in arteries than in veins, and the pulsing frequency varies in time as the heartbeat period changes. Pulses in the shear stress are believed to be an important part of the mechanical stimulus experienced by endothelium in-vivo.³⁸ Therefore, a close emulation of in-vivo flow conditions may require generation of shear stress pulses of controllable duration and amplitude. To generate a pulsatile flow in the microfluidic device, a rubber plug with a segment of PVC tubing was inserted into the syringe feeding the inlet, and the other end of the tubing was connected to the outlet of a solenoid valve with a low flow resistance and 7 ms switching time (P251SS-012-D by Ingersoll-Rand). One inlet of the valve was connected to a regulated source of compressed air set at a pressure $P_1 = 1.4$ kPa and the other inlet was vented to the atmosphere. The valve was periodically switched on and off using a home-made driver and a square wave signal from a function generator. In addition, the inlet syringe was set at a level 28 cm above the outlet syringe, generating a constant differential pressure $\Delta P_0 = 2.8$ kPa between the inlet and outlet. The resulting differential pressure was $\Delta P_0 + \Delta P(t)$, where $\Delta P(t)$ was equal to P_1 , when the valve was on (connecting the syringe to the pressurized air), and to zero, when the valve was off (venting the syringe to the atmosphere). Importantly, the period of pulsing and the ratio between the pulsatile and static parts, $P_1/\Delta P_0$, could be independently varied and controlled. We note that the characteristic time of establishment of a laminar flow profile, estimated as the viscous diffusion time $t_{vd} = h^2\rho/\eta$, was 0.006 sec. Therefore, for physiologically relevant pulsing frequencies (0.5 – 3 Hz), variations of the substrate shear stress, τ , were expected to follow the variations of the driving pressure with a minimal delay. Hence, τ in a given test region was expected to be proportional to $\Delta P_0 + \Delta P(t)$ with the same coefficient of proportionality as between the values of τ and ΔP in Table 1, and the flow in the microchannels was expected to have a developed laminar profile (with $\tau = 4\eta v_{\max} / h$) at practically all times.

A pulsing period of ~1 s characteristic for human circulation was easily achievable in the microfluidic device (Figure 5). When the solenoid valve was connected to the inlet syringe through a short and wide tubing segment, frame by frame analysis of the streakline images taken at a rate of 60 frames/s indicated that transitions between flow states corresponding to the driving pressures of ΔP_0 and $\Delta P_0 + P_1$ occurred within less than 30 ms. Abrupt changes of τ on time scales much shorter than the heartbeat period may not be physiological. To produce more gradual variations of shear stress, the solenoid valve was connected to a 10 cc inlet syringe by a long segment of thin tubing with a substantial resistance to air flow, R . The volume of the syringe above the liquid surface was used as a pneumatic capacitance, C , for the flow of air into and from the syringe, making a pneumatic equivalent of an electronic delay RC -circuit. The transition time, $t_{RC} = RC$, was adjusted by varying the length of the tubing segment and its resistance, R . The time dependence of the shear stress in the pulsatile flow with t_{RC} set at ~100 ms shows oscillations with more gradual changes in τ that more closely emulate the pulsing in blood circulation (Figure 5). The transition time could be extended further by increasing the value of R (not shown). For experiments with endothelial cells, when the perfusion medium must be saturated with air with 5% CO₂, the pressurized gas supplied to the inlet syringe is changed from air to a mixture of 5% CO₂ and 95% air.

Experiments with endothelial cells

To further test the magnetic clamp and microfluidic chip and to demonstrate their utility for live cell applications, we used them for a series of preliminary experiments with endothelial cells under shear flow, including experiments on the alignment of endothelial monolayer and healing of a scratch wound in it. In addition, we tested the feasibility of the transfection of cells in the monolayer using a standard laboratory protocol and the suitability of the setup for real-time imaging of fluorescently tagged cells under shear flow.

The endothelium monolayer and the microchannel network were examined immediately after the clamp was closed and the device was sealed. We consistently found no visible damage to the endothelium at the bottom of the microchannels, no clogging of the relatively narrow resistance channels by cells, and no detached cells in the microchannels. Cells near side walls of the test regions did not suffer any detectable damage (or viability problems) from possible motion of the chip in the plane of the cover glass (twist) during the setup closing. Moreover, many cells immediately adjacent to the walls, whose parts were cut by the PDMS chip, rapidly recovered and became motile. We examined the viability and shear stress response of endothelial cells during prolonged perfusion experiments. The flow was driven by a constant hydrostatic pressure $\Delta P = 2.75$ kPa and cells in all 8 test regions of the device, exposed to shear stresses $\tau = 0.07 - 9$ dyn/cm² (Table 1), were photographed every 10 min using a 10 $\times$ objective (see Supplementary Movie). The alignment along the flow direction was strong at the highest shear stress (9 dyn/cm²) and became weaker at lower shear levels, with the cells oriented nearly randomly at $\tau = 1$ dyn/cm² (Figure 6). Both results were in agreement with the previous reports.
19, 26, 39, 40

A total of 6 experiments were performed under nearly identical conditions, with the flow applied for ~ 15 hr. Endothelial cells consistently showed good viability in all test regions, and the degree of their alignment along the flow was qualitatively reproduced at all of the shear stresses tested. Good cell viability in all test regions was also found in a single 40 hr perfusion experiment. Because the goal of these experiments was a proof of concept, no quantitative analysis of the dependence of the cellular alignment on τ was performed, and no attempt was made to achieve a maximal variation of the degree of alignment by adjusting the range of τ . Upon completion of perfusion assays, after the clamp was disassembled and the PDMS chip was separated from the cover glass, endothelial cells on the substrate in the test regions remained intact. Consequently, the cells could be stained with various markers, fixed, and inspected again under a high resolution microscope. Supplementary Figure S-1 shows micrographs of endothelial monolayer with fluorescently stained actin filaments in all eight test regions of the device. A potential problem of the present microfluidic chip, especially in low flow rate test regions, is that cells might be influenced by soluble factors released by endothelium in upstream regions, where the shear stress is different. This problem can be resolved by building a chip with a single test region per channel line connecting the inlet and outlet and by limiting the endothelium monolayer to the test region area.

The migration of endothelial cells plays an important role in vascular regeneration and remodeling.⁴¹ The endothelium monolayer wound healing assay tests the migration of endothelial cells towards a “wound”, which is an area without cells, and the subsequent closure of the wound by a newly formed cell monolayer.⁴² We made wounds by scratching endothelial monolayer using a 200 μ l pipette tip (Figure 7, top left). The wound healing was tested in the microfluidic device under a flow with $\tau = 5$ dyn/cm² directed perpendicularly to the scratch. The wound with a width varying between 100 and 200 μ m was largely closed after 4 hr and completely healed after 6 hr of perfusion (Figure 7a). The healing time was in general agreement with previous reports.^{42, 43} The analysis of migration trajectories of individual cells (supplementary Figure S-2) indicated that cells from the upstream edge of the wound migrated downstream at an average speed of 18 μ m/hr compared with 11 μ m/hr upstream migration

speed of cells from the downstream edge of the wound. The faster migration in the downstream direction was in agreement with the previous reports.⁴⁴

To demonstrate the feasibility of transfection of cells in the monolayer using a standard protocol and of the subsequent visualization of fluorescently tagged cells under shear flow, we used a plasmid encoding fluorescent protein pmAKAR3.⁴⁵ This protein has two separate fluorescent domains, cyan and yellow (CFP and YFP), and a phosphorylation target specific to protein kinase A (PKA). The distance between the two fluorescent domains is reduced when the target is phosphorylated. Therefore, this protein can serve as a Förster resonance energy transfer (FRET) reporter of intracellular PKA activity.⁴⁵ We performed transient transfections of endothelium monolayer using FuGENE 6 transfection reagent (Roche Diagnostics) and a protocol provided by the manufacturer. A mixture of the plasmid and FuGENE 6 reagent diluted with a serum-free medium (100 μ L) was directly pipetted onto a cover glass with a confluent endothelial monolayer. The fraction of cells that were transfected and became fluorescent was 12-15% (typical values from three assays). No cells with a comparable level of fluorescence were observed with negative control transfection. Perfusion assays were performed 24 hours post-transfection.

Immediately before the cover glass with the endothelium monolayer was sealed against the microfluidic chip in the magnetic clamp setup, a scratch wound was produced in the endothelium, as described above. After the microfluidic device was sealed, a perfusion flow through it was started. Transfected cells were imaged under the flow using fluorescence microscopy of CFP with a 20 $\times$ /0.75 objective and the migration of fluorescent cells during the wound closing was tracked in real time (Figure 7b). Whereas pmAKAR3 was only used as a fluorescent tag in these preliminary experiments, the strong fluorescent signal and high resolution of the cell images indicated that the proposed experimental procedure could be used to perform FRET microscopy of this protein. The FRET microscopy would make it possible to measure the levels of activity of PKA in individual cells (including those migrating towards a wound) with a sub-cellular resolution in real time under various shear stresses.

Discussion

The sealing of a PDMS microfluidic chip against a cover glass using a clamp is a delicate task that requires application of a measured and reproducible force that is uniformly distributed over the chip. Excessive force would cause large deformations of the microchannels, whereas insufficient force would not provide reliable sealing, and an unevenly distributed clamping force would prove excessive in some areas, while insufficient in others. In the proposed magnetic clamp setup, a well-controlled, adjustable, and reproducible clamping force is generated by applying a certain number of identical magnets and by using microfluidic chips with well-defined, uniform thickness. To convert the clamping force into a uniform pressure onto the chip, a transparent cushion made of a soft silicone rubber is used.

The soft cushion is a major advantage of the proposed setup compared with a commercial perfusion chamber sealed by magnets (ChamlideTM, by LCI Corp.). The use of the cushion limited the sagging of the roofs of the widest microchannels (1.2 mm) to <7 μ m (Figure 3b) at the largest force applied (5 magnets) with small variations over the chip (SD $\approx$ 1.5 μ m), while providing reliable sealing that was resistant to pressures of up to 40 kPa inside the device. A cushion of the same size made of hard PDMS (Shore A durometer 55 vs. 20) resulted in $\sim$ 33% larger sagging of the channel roofs at the same number of magnets. In addition, setups with hard cushions frequently leaked during the perfusion experiments, which was most likely due to less efficient sealing between the cushion and the microfluidic chip and reduced tolerance to slight tilts of the cover with respect to the base. The pressure inside the microfluidic device during the perfusion experiments with endothelial cells did not exceed 4 kPa, and the device

would be reliably sealed with only one magnet per well (burst pressure of ~ 8 kPa) resulting in the 1.2 mm channel roof sagging by only ~ 3 μm (Figure 3b). Furthermore, the roof sagging was smaller for narrower channels (Figure 3c). So, with one magnet per well, the height of 100 μm wide channels was only reduced by ~ 1.5 μm . Further reduction of the channel deformation without reduction of the device burst pressure might be achieved by using thinner chips made of a harder formulation of PDMS. Therefore, the proposed magnetic clamp can potentially be used for PDMS chips with microchannel heights substantially less than 75 μm , making it suitable for a broad range of microfluidic applications.

Another important element of the proposed clamp setup is the pins in the base and matching holes in the cover of the clamp. The pins and holes make the closing of the setup simple and safe by ensuring the concentricity of the assembly and by preventing the motion of the chip in the plane of the cover glass. (Such motion might destroy the cell culture at the bottom of the flow channels.) Together with the thumbscrews, the pins also make it easy to disassemble the setup and to separate the PDMS chip from the cover glass without damaging cells on the substrate. Lastly, unlike the Chamlide™ chamber or other commercial perfusion systems (e.g. by GlycoTech Inc., MD), where the flow channel is a slit in a silicone gasket between the base and the cover, the proposed setup uses microfabricated PDMS chips. The only special features of the chips are relatively small thickness (1.2 mm), fixed size (~ 24.5 mm), and fixed inlet and outlet positions (8.5 mm from the center), allowing for much flexibility in the microchannel networks.

The use of microfabricated PDMS chips instead of gaskets makes it possible to adjust the flow resistance between the inlet and outlet, enabling parallel channel lines with different substrate shear stresses in channels with identical cross-sections. The microfluidic chip used in this study (Figure 2) had a network with 8 rectilinear test regions covering a 128-fold range in shear stress, a substantial enhancement compared with the previous perfusion chambers that generate either a single shear stress¹⁰⁻¹³ or ~ 10 -fold range of shear stresses.^{6-9, 27} In addition, the control of microchannel resistances makes it possible to drive the flow through the device by applying a differential pressure rather than by controlling the volumetric flow rate, Q , through the device with a syringe pump or peristaltic pump as in the previous microfluidic experiments with endothelium.²⁵⁻²⁷ The driving by pressure reduces the influence of channel deformations on the substrate shear stress, τ . Indeed, for a wide and shallow channel, the dependence of τ on the channel height, h , is quadratic for flow with a constant volumetric rate ($\tau \propto Q / h^2$), but linear for a flow driven by a constant differential pressure ($\tau \propto \Delta P h$; see above). The dependence of τ on the clamping force in the microfluidic device used in the study was further reduced by the relatively low deformability of the 100 μm wide resistance channels (Figure 3c). The variation of τ with the clamping force was very small ($< 3\%$; Figure 3d), indicating that the proposed setup, microfluidic chip, and the flow control method can be reliably used for shear stress response assays.

The use of the differential pressure also makes it easy to generate pulsatile flows with periods of ~ 1 s, which emulates pulsing in the vasculature. Such pulsatile flows would be difficult to achieve with syringe pumps of the type used in the endothelium perfusion assays in the vacuum-sealed devices.^{25, 27} The magnitudes of the constant and pulsing parts of τ are independently adjustable, mimicking a variety of physiological and pathological conditions. Because of the fast response of the flow to the pressure switching (~ 0.03 s), it is possible to achieve small pulsing periods. The addition of the pneumatic delay RC-circuit to produce pulses with smoothed shapes renders the microfluidic setup a simple and inexpensive alternative to bulky rotational systems (such as cone-and-plate) with oscillating angular velocity.⁴⁶⁻⁴⁸

The experiments with endothelial cells (Figure 6-8) demonstrated the advantages of the magnetic clamp setup compared with sealed microfluidic devices. Confluent endothelial

monolayers were grown on microscope cover glasses using a standard laboratory protocol, making them easy to prepare and reducing to a minimum concerns about consistency between the proposed microfluidic perfusion assay and the established assays in perfusion chambers¹⁰⁻¹³ and rotational systems.⁴⁶⁻⁴⁸ Multiple cover glasses with confluent cultures can be prepared in advance, reducing the anxiety of the operator. After a perfusion experiment is completed and the device is disassembled, cells can be accessed again for staining, inspection, and possibly, off-chip analysis. The magnetic clamp setup is particularly advantageous for the scratch wound assays. The generation of scratch wounds in the endothelium with its subsequent exposure to a shear flow is simple using the clamp, but would be difficult in a pre-assembled, sealed microfluidic device. In addition, the magnetic clamp makes it possible to transfect cells in the endothelium monolayer using a commercial reagent kit and a standard protocol optimized by the manufacturer, whereas efficient transfection in sealed microfluidic devices might require a series of trials and the development of a special new procedure.

The magnetic clamp setup offers an alternative to the vacuum sealing technique introduced previously.^{25, 27, 32, 34, 35} A disadvantage of the magnetic clamp is that it requires a special setup, which needs to be machined and assembled. On the other hand, the proposed setup does not require a source of vacuum. Moreover, while somewhat bulky, the mechanical parts of the clamp make it possible to reduce to a minimum the hydrodynamic stresses generated during sealing of the device (Figure 4), thus making the setup compatible with weakly adherent cells. Another disadvantage of the proposed setup is the geometrical constraints it imposes on the microfluidic networks: the alignment of the inlets and outlets with the holes in the clamp cover and the fitting of the microchannel networks onto 25 mm cover glasses. However, for many practical cases, these constraints are relatively minor. The plexiglas disk and the cushion in the cover of the clamp (Figure 1a-c) both have four matching holes, making them compatible with PDMS chips with up to four ports (inlets and outlets), and the number of the holes and ports could be further increased. In addition, no space needs to be allocated for a vacuum suction channel network, which can have a substantial footprint.^{29, 31}

In our own preliminary experiments with a pilot microfluidic perfusion device made of PDMS and sealed by vacuum (not shown), the endothelial monolayer looked healthy in the beginning, but cells detached from the cover glass after 30 min to 8 hours of perfusion. To test for possible effect of the vacuum suction on the concentration of oxygen in the porous PDMS chip (which is initially saturated with air), we performed a two-dimensional numerical simulation of the diffusion of oxygen in the vertical cross-section of the chip with FemLab. The simulation used the geometrical parameters of the device (5 mm thick PDMS chip with 4 mm wide vacuum channels placed 4 mm apart) and the putative value for the diffusivity of oxygen in PDMS ($3.9 \cdot 10^{-9} \text{ m}^2/\text{s}$ ⁴⁹) and disregarded the perfusion of the medium through the device. The simulation indicated a gradual reduction of the concentration of oxygen in the area between the vacuum channels that occurred on a scale of tens of minutes. The depletion of oxygen was a possible reason for the detachment of the endothelial cells in the vacuum-sealed device, and the magnetic clamp circumvents this problem.

To summarize, we built and tested a microfluidic perfusion chip and a magnetic clamp setup that provides reliable sealing of the chip against cover glasses with cell cultures. The clamp is fast to assemble and disassemble, and practical variations of the clamping force have little effect on the shear stress at the glass substrate during the perfusion. The perfusion chip makes it possible to test the responses of cells to a 128-fold range of shear stress in a single experiment, and the results of experiments with endothelial monolayers agree with previous reports. With the clamp setup, it is simple to perform a widely used wound healing assay and to transfect cells on the substrate before exposing them to a shear flow. We demonstrated a transfection that can potentially be applied to monitoring the activity of an enzyme, PKA, under flow in real time using FRET microscopy. The clamp setup can be used with a variety of easily

interchangeable microfluidic chips made of PDMS for experiments in steady and pulsatile flows with endothelial cells, other strongly adherent cells and, possibly, weakly adherent and non-adherent cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Virginia VanDelinder for useful suggestions. These studies were supported by NIH grants HL078784 and AR27214 (M.H.G. and E.T.), NSF NIRT Grant No. 0608863 (A.G. and E.G.), the Wellcome Trust (077532), UCSD/SDSU Institutional Research and Academic Career Development Award (NIH GM 68524), and a fellowship from the American Heart Association (E.G.).

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Figure 1.

Magnetic clamp setup. (a) Photograph of assembled setup. View from the top. (b) Photograph of the cover (viewed from the bottom) separated from the base. (c) Close-up view of the central part of the cover with silicone cushion and PDMS chip. (d) Schematic drawing of cross-section of assembled clamp setup. Red arrows indicate the perfusion flow.

Figure 2. Layout of microchannels in the microfluidic device. Numbers 1 – 8 indicate different test regions that are 1.2 and 0.6 mm wide rectilinear channels.

Figure 3.

Mechanical tests of the clamp setup. (a) Magnetic force (in Kg) as a function of the distance between the cover and the base, d , measured for different numbers of magnets per well: 1 (black diamonds), 2 (grey circles), 3 (black triangles), 4 (grey triangles), and 5 (black squares). Each data point shows a mean value of 4 individual measurements. *Inset*: magnetic force as a function of the number of magnets for $d = 0.7$ mm. Error bars are sums of standard deviations (SD) and measurement uncertainties. (b) The pressure inside the microfluidic device at which it starts leaking (burst pressure) as a function of the number of magnets per well for $d = 0.7$ mm. Each data point is an average of 4 individual tests. Error bars are SD. (c) Height, h , of 100 μm wide resistance channels (black circles), 0.6 mm wide test regions (grey squares), and 1.2 mm wide test regions (black triangles) measured in the middle of the channels as a function of the number of magnets per well for $d = 0.7$ mm. Each data point is an average of 4 measurements taken in different areas of resistance channels, test regions 2, 4, 6, and 8, and test regions 1, 3, 5, and 7, respectively. Error bars are SD. (d) Substrate shear stress, τ , in test region 7 at a driving pressure $\Delta P = 2$ kPa for different numbers of magnets per well. Error bars are measurement uncertainties.

Figure 4.

Mean speeds of 10 μm polystyrene beads at the substrate in test region 3 during a final stage of closing of the magnetic clamp (time = 0 – 56 sec) and after the setup is closed and a pressure $\Delta P = 2.75$ kPa between the inlet and the outlet is applied (time > 60 sec). Each data point is based on the mean absolute displacement of beads in the region of interest (40 beads on average) between two consecutive frames taken with a 0.2 s interval. To calculate the mean speed, the mean displacement is divided by 0.2 s.

Figure 5.

Substrate shear stress, τ , in test region 8 of the microfluidic device (Figure 2), as a function of time, when the driving pressure, ΔP , is switched between 2.8 and 4.2 kPa with a period of 1 sec. τ at a given time was calculated as $4\eta v_{\max} / h$, with the value of $v_{\max}$ obtained from the analysis of the streaklines in the corresponding video frame. Blue and red curves represent the pressure switching with and without a pneumatic delay RC -circuit, respectively.

Figure 6. Micrographs of endothelial cells in test regions 5 (top) and 8 (bottom) of the microfluidic device after 15 hours of perfusion at shear stresses of 1 dyn/cm² (top) and 9 dyn/cm² (bottom). Both micrographs are taken with a 10× objective using phase-contrast imaging.

Figure 7.

(a) Phase-contrast micrographs showing the healing of a scratch wound in endothelium monolayer at different times. A flow with a substrate shear stress of 5 dyn/cm^2 was started at time 0, immediately after the device was sealed. The flow direction was vertically down. Scale bar $200 \mu\text{m}$. (b) Real-time imaging of an endothelial cell expressing fluorescent protein pmAKAR3 at the upstream edge of a scratch wound under a flow with a substrate shear stress of 10 dyn/cm^2 . The flow was directed from top to bottom and was started at time 0. The images were taken with a $20\times/0.75$ objective lens under fluorescence illumination. Initial boundary of the wound is indicated by a gray line. Nearby cells of the confluent endothelium (above the boundary) that did not get transfected with pmAKAR3 are invisible under the fluorescence illumination. Scale bars $20 \mu\text{m}$.

Table 1
Shear stress at the substrate in internal areas of 8 test regions of the microfluidic device measured at $\Delta P = 2.75$ kPa. Values of the relative shear stress are normalized to the shear stress in test region 1.

Test region, #	1	2	3	4	5	6	7	8
Shear stress, dyn/cm ²	0.070	0.139	0.246	0.52	0.98	1.97	4.2	9.0
Shear stress, relative	1.0	1.99	3.51	7.4	14.0	28.1	60	127