Flow in the well: computational fluid dynamics is essential in flow chamber construction

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Abstract A perfusion system was developed to generate well defined flow conditions within a well of a standard multidish. Human vein endothelial cells were cultured under flow conditions and cell response was analyzed by microscopy. Endothelial cells became elongated and spindle shaped. As demonstrated by computational fluid dynamics (CFD), cells were cultured under well defined but time varying shear stress conditions. A damper system was introduced which reduced pulsatile flow when using volumetric pumps. The flow and the wall shear stress distribution were analyzed by CFD for the steady and unsteady flow field. Usage of the volumetric pump caused variations of the wall shear stresses despite the controlled fluid environment and introduction of a damper system. Therefore the use of CFD analysis and experimental validation is critical in developing

flow chambers and studying cell response to shear stress. The system presented gives an effortless flow chamber setup within a 6-well standard multidish.

Keywords Cell culture · Computational fluid dynamics · Endothelium · Venous macrovascular · Parallel plate flow chamber · Shear force

Abbreviations

ATR	Active test region
CFD	Computational fluid dynamics
HVEC	Human saphenous vein endothelial cells
HUVEC	Human umbilical vein endothelial cells

Introduction

In vivo, the endothelium is continuously exposed to shear stress forces exerted by blood flow. Much attention has therefore been focused to the effects of stress on cell growth and function. Morphological appearance of endothelium grown under non-flow conditions is modified after exposure to shear stress i.e. biomechanical forces. Endothelial cell responses to shear stress include modifications in cell turnover rate, changes in activation of ion channels, alterations in intracellular calcium concentration, intercellular signalling, gene expression or alteration in barrier function (Resnick et al. 2003; Sato et al. 2005).

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Variability in endothelial cell function is remarkable. There are differences of phenotype, antigen expression, cell size and growth, secretory function as well as G-protein expression depending on local conditions. (Thorin et al. 1998). The magnitude of shear stress can be roughly estimated by Poiseuille's law (Sumpio 1993).

The observation of endothelium *in vivo* remains more difficult. A number of *in vitro* flow chambers have been developed in an attempt to conduct controlled studies of the response of endothelial cells. Established fundamental models include the parallel-plate-chamber (Frangos et al. 1985; Krueger et al. 1971; Usami et al. 1993), the cone-disc-device (Dewey et al. 1981) or capillary tube geometries (Cooke et al. 1993). Some specialized systems have been described with small sized containers or filter supports (Minuth et al. 1992; Munn et al. 1994; Usami et al. 1993).

The relationship between the wall shear stress and the volume flow rate is thought to equal the relationship of two-dimensional, steady, fully developed flow between two infinite parallel plates (analytical solution) (Chung et al. 2003). Usually the chambers consist of two parts which are held together by vacuum or screws. Chamber variations during construction or assembly or influences from different pumps may affect flow-sensitive cell responses as previously mentioned (Li et al. 2005; McCann et al. 2005).

Our aim in the work presented here was to demonstrate the use of a flow chamber experimental setup based on a conventional tissue culture plate with optical accessibility and as a result to enable flow experiments on a larger scale. A region is defined where wall shear stress in *x*-direction does not deviate more than 5% from the intended target value. The apparatus introduced produces laminar flow in a standard (ANSI/SBS) cell culture 6 well multidish, thus permitting the continuation of cultivation without interruption. Problems associated with new flow chamber designs and experimental setups are addressed.

Different endothelial cell types were exposed to flow resulting in shear stresses of up to approximately 1 Pa (10 dyn/cm²) which is well in the physiological range (Sumpio 1993) with the objective of observing flow-differentiated cells for further usage: primary human vein macrovascular endothelial cells (HVEC)

and primary human umbilical vein endothelial cells (HUVEC, data not shown). The flow and the wall shear stress distribution in the flow chamber were analyzed by computational fluid dynamics (CFD).

Materials and methods

Flow system

Our apparatus forms a small test region using the flat surface of the bottom of a standard 6 well cell culture multidish. The entire chamber body has a diameter of 34 mm and is 34 mm high (see Fig. 1 where two chambers are placed in series). The test section has a height of 0.25 mm and a maximum width of 8 mm in the region where it is rectangular. The length of the rectangular region is 22 mm. The fluid enters the test section through an oval channel with a 5 mm × 45° chamfer. Above the channel there is a tube of a diameter of 3.8 mm, which is connected to the supply tubes by a Luer cone of 3 mm inner diameter. A Luer cone is also used at the outlet from the chamber, which is formed by a tube of 3.8 mm diameter, also with a 5 mm × 45° chamfer at the exit of the test section. The geometry and the sizes of the wetted surfaces are shown in Fig. 2.

To prevent bending, the bottom of the chamber is reinforced by a metal plate of 3 mm thickness. The chamber is made of clear acrylic glass, which allows direct microscopic observation. The chamber body and the multidish are held together by a metallic rack which also fits to the microscope stage for observation. Leakage of circulating medium is prevented by a silicone gasket. The assembled apparatus is shown in Fig. 1.

The medium circulates at variable flow rates driven by a peristaltic pump (B.Braun, Germany), which is connected to a damper system (air chamber). After crossing the flow channel the medium reaches a reservoir which is separated from atmospheric conditions through a filter to prevent contamination. See Fig. 3 for a scheme of the flow loop. The flow studies were conducted under culture conditions at 37 °C in a humidified CO₂ (5%) incubator. Therefore the whole experimental setup was placed in an incubator.

The pump was activated at least 24 h at a flow rate of 380 ml/h. This is equivalent to a wall shear stress of approximately 1 Pa or 10 dyn/cm² (see numerical

Fig. 1 Schematic view of the assembled apparatus

analysis section for details). For microscopic evaluation the apparatus was briefly attached to the microscope stage outside the incubator. After the experiment a light microscopic picture of a representative sample was taken. Cell shape was analyzed using the Zeiss Axiovision software package (Zeiss,

Fig. 2 Size of the computational domain. All values in mm

Germany) and described in terms of shape index (SI), where $SI = 4\pi \times \text{area} \times \text{perimeter}^{-2}$.

To visualize the laminar nature of the flow a small volume of dye ($0.5 \mu\text{l}$ Coomassie[®] brilliant blue) was placed on the bottom of the multidish at three different positions and was allowed to dry. The chamber was flushed with PBS at a pump flow rate of 380 ml/h and the distribution of the dye was observed.

To attenuate the peristaltic flow generated by the volumetric pump a disposable damper system was introduced. The damper system consists of an air filled hermetically sealed chamber followed by a variable flow restrictor as shown in Fig. 4. The fluid driven by the peristaltic pump moves into the air filled chamber which raises the pressure inside the sealed chamber. Then the fluid moves almost steady-going downstream into the actual flow chamber after the pressure is high enough to overcome the resistance set by the flow restrictor. Therefore the hermetically sealed chamber acts as an air chamber. To confirm its effect the reduction of pulsations was demonstrated by monitoring the time-dependent hydrostatic pressure using a transducer system (Medex, USA). The influence of the remaining pulsatility was calculated (see results and discussion section).

Cell culture

Human saphenous vein macrovascular endothelial cells (HVEC) were obtained from human saphenous vein by adaptation of the method described by Jaffe et al. (1973). Human umbilical vein endothelial cells (HUVEC) were purchased from Promocell (Germany).

Fig. 3 Schematic view of flow circuit with reservoir, damper, volumetric pump and flow chamber

Fig. 4 Damper system with air reservoir and restrictor

The endothelial identity of the isolated cells was confirmed by morphologic criteria and verified by immunohistochemical staining of factor VIII-related antigen and CD31 in a limited number of cases (not shown). In passage 1 or 2 the isolated cells were placed in a SBS standard six well microtiter plates (Nunc, Germany) which had been precoated with gelatine 0,75% (HVEC) with a density of $0.5\text{--}1.0 \times 10^5$ cells $\times \text{cm}^{-2}$ and were cultivated until the cells reached confluence. Endothelial cell basal culture medium

(Promocell, Germany) containing 10% FCS was changed three times a week. The same culture medium was used in the flow system.

Numerical analysis

Numerical simulations of the flow in the chamber were performed for steady and time varying boundary conditions. Under steady simulations the influence of the magnitude of volumetric flow rate and mesh resolution were analyzed. The unsteady simulation was used to examine the influence of a time dependent volumetric flow rate on the wall shear stress distribution and therefore to show the influence of the remaining pulsatility despite the use of a damper system.

Making use of the symmetry of the flowfield the steady simulation was performed in one half of the chamber. The symmetry of the flowfield has been verified with simulations in the full geometry, which was used for the unsteady simulation. The symmetry plane and the lower wall of the computational domain are shown together with the relevant dimensions in Fig. 2.

Computational grids

For the grid convergence study, three systematically refined block-structured grids were generated in the computational domain with a mesh generator. ANSYS-ICEMCFD. The grids are hexahedral cells,

Fig. 5 Surface mesh of the grid used in the unsteady simulations. **(a)** Bottom plane; **(b)** inlet section in the symmetry plane; **(c)** outlet section in the symmetry plane

as this type of cell is known to produce the most accurate result of the computed flow field near walls (Casey et al. 2000). The grid used for unsteady simulations is shown in Fig. 5. Details of the grid are shown as surface mesh on the bottom plate (Fig. 5a), the surface mesh in the symmetry plane close to the outlet (Fig. 5b) and close to the inlet (Fig. 5c).

Physical and numerical parameters

The steady simulations were conducted with volumetric flow rates up to $Q = 1.06 \cdot 10^{-7} \text{ m}^3/\text{s}$ (381.7 ml/h), leading to Reynolds numbers $Re_h = \rho Q / (\mu W)$ in the chamber up to 16.9. Here $W = 8 \text{ mm}$ is the maximum width of the chamber, μ the dynamic viscosity and ρ the density of the fluid. The respective values for these properties are those for our cell culture medium containing 10% FCS at a temperature of 37°C , $\mu = 7.860 \times 10^{-4} \text{ Ns/m}^2$ and $\rho = 1001.13 \text{ kg/m}^3$. The Reynolds numbers of the flow in the inlet and outlet tubes and the oval inlet channel are also in the range compatible with laminar flow. The unsteady simulations were conducted for a flow rate of $Q = 1.11 \times 10^{-7} \text{ m}^3/\text{s}$ (400 ml/h). The material properties of pure water at a temperature of 24.6°C are $\mu = 8.99 \times 10^{-4} \text{ Ns/m}^2$ and $\rho = 999.23 \text{ kg/m}^3$. The pressure measurements for the analysis of the

performance of the damper system were conducted with isotonic sodium solution (0.9% NaCl). The range of volumetric flow rates Q for the steady simulations has been determined from the well known relation between Q and the wall shear stress $\tau_{w,2D}$ for fully developed two dimensional flow between two parallel plates,

$$Q = \tau_{w,2D} W H^2 / (6\mu), \quad (1)$$

where $H = 0.25 \text{ mm}$ is the distance of the plates. The previously given value of $Q = 1.06 \times 10^{-7} \text{ m}^3/\text{s}$ corresponds to a target shear stress up to $\tau_{w,2D} = 1.0 \text{ Pa}$ in the constant cross section area of the test section. The flow rate has been prescribed as boundary condition at the small inlet tube in the steady simulations. The boundary condition at the outlet is a constant pressure while the velocity components are treated as fully developed with vanishing gradients in flow direction. At the walls the no slip condition is applied.

In the unsteady simulations the experimental time history of the static pressure was prescribed at the outlet. At the inlet the time history was computed by adding a constant dynamic pressure corresponding to the volumetric flow rate $Q = 1.11 \times 10^{-7} \text{ m}^3/\text{s}$ to the measured static pressure (see Fig. 6a). With these boundary conditions the static pressure and the volumetric metric flow rate can adapt to the varying

Fig. 6 (a) Measured pressure fluctuations around the mean pressure at the outlet ($p_2 - \langle p_2 \rangle$) and at the inlet ($p_1 - \langle p_1 \rangle$) for $Q = 400$ ml/h. (b) Comparison of the time histories of the

measured and computed difference of the static pressure at the inlet (p_1) and the time averaged static pressure at the outlet ($\langle p_2 \rangle$) for $Q = 400$ ml/h

static pressure at the outlet and the varying total pressure at the inlet at every time step in the unsteady simulation, still leading to a time average flow rate of $Q = 1.11 \times 10^{-7}$ m³/s. That this approach works very well can be seen in Fig. 6b where the measured and the computed static pressure at the inlet are shown. Both pressure histories are nearly equal.

The continuity and the momentum equations were solved with the double precision version of the flow solver FLUENT V6.1. FLUENT is a finite volume method that uses the midpoint rule for integration of the surface fluxes (FLUENT 2003). Time integration for the unsteady simulation is done with the implicit second order three time level method (Ferziger et al. 2002), which uses the implicit first order Euler method for the first time step. The steady simulations were run until the scaled residuals of the four equations solved reached the termination criterion of 10^{-6} . At six positions on the lower wall of the test section the values of τ_w at these positions for the used three grids are shown, see Fig. 7 and Table 1. For the unsteady simulations a constant time step of $\Delta t = 0.0011$ s was used.

The ATR was introduced by Chung et al. (2003) and defined as that region on the lower wall of the

Fig. 7 Position of the monitoring points $n = 1$ –6 used in the grid convergence study

chamber in which the computed wall shear stress in x -direction, $\tau_{w,x}$, does not deviate by more than 5% from the target value as resulting from Eq. 1 with the provided volume flow rate. In this work the ATR is defined for the modulus of the wall stress vector τ_w ,

$$100 \cdot |\tau_w(x,y) - \tau_{w,2D}| / \tau_{w,2D} \leq 5\% \quad (2)$$

Results and discussion

Experimental validation and distribution of Coomassie[®] blue

To confirm the assumed flow pattern a dye test using Coomassie[®] brilliant blue was conducted. The dye was applied to the bottom of a conventional tissue culture plate and dried. Figure 8 shows the dissolution of the dye after flushing with PBS. The distribution of dye shows that flow entering and passing the chamber is nonturbulent. The three spots disperse to non-interrupting streams within a narrow zone of PBS demonstrating the apparent laminar nature of the flow. This simple test can roughly demonstrate the existence of an uninterrupted flow in a fluid near a solid boundary in which the direction of flow at every point remains constant.

A more precise approach would include flow visualization with light-reflecting particle suspension and digital video recording for experimental validation of the computed results (Digital Particle Image Velocimetry, DPIV). But this technique was not available for this work presented here. The

Table 1 Coordinates of the monitoring positions $n = 1$ –6 at the bottom wall ($z = 0$ mm) in the flow chamber and wall shear stresses at these positions for the steady simulations with $Q = 381.7$ ml/h ($\tau_{w,2D} = 1.0$ Pa) using a fine grid G1 ($\tau_{w,1}$), amedium grid G2 ($\tau_{w,2}$) and a coarse grid G3 ($\tau_{w,3}$) and results of the grid convergence study for the wall shear stress of the fine grid G1 for the steady simulations with $Q = 381.7$ ml/h ($\tau_{w,2D} = 1.0$ Pa)

n	x (mm)	y (mm)	$\tau_{w,1}$ (Pa)	$\tau_{w,2}$ (Pa)	$\tau_{w,3}$ (Pa)	R	p	C	$U \times 10^3$ (Pa)	$\delta_1 \times 10^3$ (Pa)	δ_1 (%)	$\tau_{w,c}$ (Pa)
1	4	0	1.031	1.010	0.932	0.272	1.878	0.892	1.021	-7.103	-0.684	1.039
2	11	0	1.014	0.993	0.915	0.271	1.885	0.898	0.973	-6.979	-0.683	1.021
3	16	0	1.035	1.014	0.934	0.267	1.903	0.914	0.919	-7.125	-0.683	1.043
4	4	3.75	0.957	0.929	0.834	0.298	1.748	0.786	2.516	-9.442	-0.974	0.969
5	11	3.75	0.975	0.947	0.850	0.297	1.752	0.790	2.508	-9.605	-0.973	0.988
6	16	3.75	0.954	0.926	0.829	0.294	1.764	0.799	2.352	-9.516	-0.985	0.966

R is the ratio of solution changes, p the order of accuracy, C the correction factor and U the uncertainty of the improved error estimate. $\delta_1 \delta'_{11} = \delta_1 / \tau_{w,1}$ is the relative improved error estimate and $\tau_{w,c} = \tau_{w,1} - \delta_1$ the corrected wall shear stress of the fine grid solution

Fig. 8 Distribution of Coomassie® brilliant blue

computational analysis was performed using CFD simulations without Fluid-Solid Interaction (FSI). The CFD solutions however rely upon physical models of the real processes (e.g. turbulence, compressibility, chemistry, multiphase flow). The solutions that are obtained through CFD can only be as accurate as the physical models on which they are based. Solving equations on a computer invariably introduces numerical errors as round-off error (error due to finite word size available on the computer) and truncation error (error due to approximations in the numerical models). The truncation errors will go to zero as the grid is refined – so the mesh refinement is one way to control the truncation error. We used three systematically refined block-structured grids which were generated in the computational domain with a mesh generator.

In clinical research the CFD outputs are extremely sensitive to the acquired in vivo geometry. The geometry of the presented flow chamber is well described and measurable. Variations due to poor geometric data though can generate as much as 60% variation in hemodynamic parameters obtained using CFD, especially in complex flow regions. In addition, the validity of CFD results relies heavily on both temporal and spatial boundary conditions. These conditions are well controlled in our study but are not routinely available in clinical practice (Hoi et al. 2006). See Numerical Analysis section for details.

Cell response to shear stress

After initiation of flow and therefore application of shear stress HVECs and HUVECs tended to align themselves in the direction of flow. This process appeared to be time and shear stress dependent. Shear stress of approximately 1 Pa ($Q = 380$ ml/h) was applied for up to 24 h. Human saphenous venous endothelial cells and human umbilical venous endothelial cells (not shown) became elongated and spindle shaped as confirmed by microscopy. The cell-axis converged with the direction of flow. Figure 9 shows the schematical and microscopic view of cell elongation. The mean shape index of 152 (control) and 115 (flow) respectively evaluated cells dropped from 0.75 (SD 0.16) to 0.55 (SD 0.17). All the cells analyzed were from the same field of view and a representative sample. However not all cells became aligned after 24 h of exposure to 1 Pa

Fig. 9 Response of HVEC to shear stress, (a) 3d after reaching confluence; (b) 24 h after exerting approximately 1 Pa of shear stress generated by pulsatile pump with downstream air chamber. Black arrow indicates flow direction

generated by the pulsatile pump with downstream damper. As a result of the exposure to shear stress a limited number of cells seemed to lose their close contact in the process of rearrangement as seen in phase contrast microscopy. Occasionally some cells became detached and were flushed away while preserving the aspect of a homogenous cell layer. In the above mentioned shear stress range denudation did not occur. Ives et al noticed a resisted reorientation under flow for different endothelial cell types, nevertheless after a long period of time alignment occurred even at a relatively low shear stress level (Ives et al. 1983). The CFD-data suggest significant remaining shear stress and pressure gradients at both inlet and outlet areas. Inside the ATR exist lower gradients as intended, though.

The described and needless to say almost every flow chamber consists of different shear-stress zones at some points (e.g. borders). The presented chamber has remarkable spatial variations of the shear stress level at inlet and outlet areas due to the approaching flow characteristic. Inside the ATR however variations are predominantly due to remaining temporal variations. These variations could lead to sustained activation of genes. This is distinctive at areas of high shear stress gradients where endothelial cell turnover is also accelerated. The behaviour of these stimulated endothelial cells is comparable to that of sparse/subconfluent cells which are elongated, highly motile

and sensitive to growth-factor stimulation. After establishing close contact they loose the ability to respond to growth factors and switch to a resting condition where junctional proteins may play a critical role. It has been shown that after vascular damage and disruption of intercellular contacts, endothelial cells regain the ability to respond to growth stimuli and to migrate into the wounded area (Dejana 2004; Fisher et al. 2001). Several proteins participate in endothelial cell-to-cell communication and transfer a complex array of intracellular signals. For instance, VE-cadherin contributes as well to the endothelial cell response to shear stress as to the formation of endothelial adherens junctions. From a mechanosensory complex, formed by VE-cadherin, which functions as an adaptor early responsiveness to flow is conferred (Liebner et al. 2006).

This demonstrates that through cell-to-cell communication effects outside the ATR can still play a significant role even if inside the ATR shear stress gradients are remarkable low.

Steady wall shear stresses

Six local values of the wall shear stress τ_w , that are given in Table 1, are summarised in Table 2 for the computed case with volumetric flow rate of $Q = 381.7$ ml/h corresponding to the target shear

Table 2 Mean ($\langle\tau_w\rangle/\langle\tau_{w,2D}\rangle$), maximum ($\langle\tau_{w,max}\rangle/\langle\tau_{w,2D}\rangle$) and minimum ($\langle\tau_{w,min}\rangle/\langle\tau_{w,2D}\rangle$) normalised wall shear stresses at the six monitoring positions from the unsteady simulation with $\Delta t = 1.1 \times 10^{-3}$ s and $\langle Q \rangle = 400$ ml/h

n	$\langle\tau_w\rangle/\langle\tau_{w,2D}\rangle$	$\langle\tau_{w,max}\rangle/\langle\tau_{w,2D}\rangle$	$\langle\tau_{w,min}\rangle/\langle\tau_{w,2D}\rangle$
1	1.038	1.152	0.866
2	1.019	1.131	0.849
3	1.041	1.155	0.867
4	0.955	1.063	0.792
5	0.976	1.086	0.809
6	0.955	1.062	0.792

stress $\tau_{w,2D} = 1.0$ Pa. For the considered volumetric flow rate the flow is laminar within the entire chamber. At the end of the oval inlet pipe most of the fluid directly expands into the flow chamber in the direction of the outlet. A small portion only flows in negative x-direction first and then along the side wall towards the outlet. Therefore, the flow in the main part of the flow chamber develops again rapidly towards the flow field which is encountered in parallel plate flow chamber. This can be seen from the distribution of the streamlines in Fig. 10(a) and b). These streamlines compare well with the

Fig. 10 Streamlines of the steady flow field (grid G1, $\tau_{w,2D} = 1.0$ Pa). (a) 3D view; (b) view from positive z-direction

experimental visualization of the flow by the use of Coomassie blue, which is shown in Fig. 8. Particle image velocimetry flow measurements were not done. At the prescribed volumetric flow rate $Q = 381.7$ ml/h, corresponding to a target wall shear stress of $\tau_{w,2D} = 1.0$ Pa the highest shear stresses are observed immediately behind the chamfer of the inlet pipe and in front of the chamfer of the outlet pipe. Due to the oval shape of the inlet pipe this high stress region has a shorter extent in x-direction than the corresponding region in front of the circular outlet pipe. The larger extension of the oval inlet pipe in y-direction leads to lower velocities of the fluid as compared to the outlet region. This can also be concluded from the larger space between the streamlines at the inlet in contrast to their closer spacing in the outlet, see Fig. 10.

In the central part of the flow chamber the wall shear stress distribution is homogeneous and close to the target value. Towards the side wall the shear stress is reduced. Also under the inlet and outlet pipe very small values of $\tau_w/\tau_{w,2D}$ are encountered as hardly any fluid motion exists (see also Fig. 10).

The corner points of the rectangle defined as ATR coincide with the monitoring positions $n = 1$, $n = 4$, $n = 6$ and $n = 3$, see Fig. 7 and Table 1. At positions $n = 1$ and $n = 3$, which lie on the symmetry line at $y = 0$ mm, the highest shear stresses in the ATR are obtained. The normalised stress values at positions $n = 1$ and $n = 3$ are below the upper limit of 1.05.

The results for the grid convergence study for the six local values of the wall shear stress τ_w are summarised in Table 1 for the computed cases with the volumetric flow rates of $Q = 381.7$ ml/h corresponding to the target shear stress $\tau_{w,2D} = 1.0$ Pa. All results display monotonic convergence as the grid convergence ratio R is between 0 and 1 (Stern et al. 2006).

Even when using the corrected values from the grid convergence study $\tau_{w,c}$, see Table 1, the rectangular ATR only contains shear stresses below the upper limit. The same is true for the lower limit of 0.95 which is reached close to the side wall of the chamber. There the normalised stress values of position $n = 4$ and $n = 6$ are already slightly higher than 0.95 on the fine grid G1, see Table 1. With the correction from the grid convergence study the values ($\tau_{w,c}$) are further increased well above the lower ATR limit of 0.95. The unsteady wall shear stress distribution within the rectangular ATR when using a

peristaltic pump was analyzed to judge the suitability of the defined region.

Damper system

There are different approaches to generating a stable time-independent flow profile. Short experiments with non-circulating medium generally lead to simpler experimental setups with e.g. the use of harvard-type syringe pumps or gravity as propelling forces. Disadvantages include limited observation times due to defined syringe volume or complicated experimental setups due to the generation of the pressure difference. This makes conduction of flow studies feasible to specialized workgroups only. When using a peristaltic pump the influence of remaining pulsatility is not neglectable as demonstrated. Pulsations should be as low as possible to establish a reliable correlation between the applied shear force and endothelial cell response. The damper efficiently reduces the oscillations in the flow loop generated by the peristaltic pump. Also bubble formation in the flow chamber is greatly reduced by the damper system which serves as an air trap. The measured time series of the static pressure just after the exit of the outlet pipe of the flow chamber is shown in Fig. 11a with and without damper in the flow loop. The periodicity in the signal caused by the rotational motion of the peristaltic pump is well visible. By the use of the damper the periodicity is not altered but pressure oscillations are substantially reduced. This can be also seen when looking at the intensity of the

pressure fluctuations, Fig. 11b, as a function of the volumetric flow rate. The intensity is defined as ratio of the root mean square value of the pressure fluctuations and the mean, i.e. time averaged, pressure, $p_{rms}/\langle p \rangle$. The inclusion of the damper leads to a drop in the intensity of the oscillation by more than an order of magnitude. For the case with $\langle Q \rangle = 400$ ml/h the intensity is reduced from 68.03 to 2.66% of the mean pressure. Similar reductions are obtained for all analyzed volumetric flow rates. How the remaining unsteadiness of the flow caused by the peristaltic pump affects the flow field and especially the shear stress distribution on the bottom plate of the flow chamber is described in the following section.

Unsteady wall shear stresses

Despite the use of damper systems remaining pulsatility was found in the system. Therefore unsteady simulations were performed for a volumetric flow rate of $\langle Q \rangle = 400$ ml/h as described earlier. The duration of the entire simulation comprised three periods of the oscillating pressure signal, cf. Fig. 6. The temporal variation of the volumetric flow rate is caused by the time dependent pressure difference over the entire flow chamber. In Fig. 12a the time histories of the non dimensional pressure difference, $\Delta p/\langle \Delta p \rangle = p_1 - p_2/(\langle \Delta p_1 \rangle - \langle \Delta p_2 \rangle)$, and the non dimensional volumetric flow rate, $Q/\langle Q \rangle$, are shown. While $Q/\langle Q \rangle$ in general follows the pressure difference, i.e. low volumetric flow rate for small pressure

Fig. 11 Reduction of the pressure fluctuations with the damper. (a) Time histories of the static pressure at the outlet (p_2) of the chamber with and without damper for $Q = 400$ ml/

h. (b) Intensity of the pressure fluctuations at the inlet into the flow chamber, $I_1 = p_{1,rms}/\langle p_1 \rangle$, with and without damper as function of the volumetric flow rate Q

Fig. 12 Results of the unsteady simulation. **(a)** Time histories of the non dimensional pressure difference, $\Delta p / \langle \Delta p \rangle = p_1 - p_2 / (\langle p_1 \rangle - \langle p_2 \rangle)$, and the non dimensional volumetric flow rate, $Q / \langle Q \rangle$. **(b)** Time histories of the non dimensional wall

shear stress, $\langle \tau_w \rangle / \langle \tau_{w,2D} \rangle$, at monitoring position $n = 1$ and the non dimensional volumetric flow rate, $Q / \langle Q \rangle$. The vertical broken lines indicate the times at which the non dimensional wall shear stress on the bottom wall is shown in Fig. 13

difference and vice versa, the high frequency oscillations in $\Delta p / \langle \Delta p \rangle$ are absent in $Q / \langle Q \rangle$.

The instantaneous volumetric flow rate again determines the magnitude of the wall shear stress on the bottom wall of the flow chamber. In Fig. 12b the time histories of the non dimensional wall shear stress at position $n = 1$, $\tau_w / \langle \tau_{w,2D} \rangle$, and the non dimensional volumetric flow rate, $Q / \langle Q \rangle$, are shown. Here $\langle \tau_{w,2D} \rangle$ is computed from Eq. 1 using the time averaged volumetric flow rate $\langle Q \rangle$. Wall shear stress strictly follows the change of the volumetric flow rate at the flow chamber inlet. This behaviour is the same for the wall shear stresses at the other monitoring stations and on the entire bottom plate of the flow chamber. $\tau_w / \langle \tau_{w,2D} \rangle$ at monitoring position $n = 1$ both exceeds the maximum allowable value of 1.05 for the ATR and falls below the lower limit of 0.95. In Table 2 it is shown that the maximum non dimensional wall shear stress at position $n = 1$ exceeds 1.15 and the minimum value is below 0.87. The mean non dimensional wall shear stress, $\langle \tau_w \rangle / \langle \tau_{w,2D} \rangle$, lies between the corresponding value obtained on the medium grid G2 and the corrected value from the grid convergence study for the steady simulation. The corresponding values at the other five monitoring positions behave in the same way.

This is noteworthy since by definition the ATR is that region on the lower wall of the chamber in which the computed wall shear stress in x-direction does not deviate by more than 5 % from the intended target value. The only reason for this is the remaining pulsatility and therefore temporal variations in the system. With time-dependent deviations of up to

15.2% the maximum tensile force on the cell within the ATR is about 11 dyn/cm² which is – as the pulsatility in general – well in the physiological range. While endothelial cells in vivo in large arteries are exposed to mean wall shear stress levels in the range of 2–4 Pa (20–40 dyn/cm²) with variations from negative values, through zero values at the edges of flow separation regions, and up to values of 4–5 Pa (40–50 dyn/cm²) endothelial cells subjected to pulsatile flow in vitro failed to respond at certain frequencies. This may be because the positive stimulus for response needs to be sustained (Davies 1995). Our system has a remaining pulsatility of approximately 6 Hz. The exact impact of this pulsatility on the cells in our experiments remains unclear. But it has been shown that oscillatory shear stress is a stronger stimulus than steady shear stress for inducing cell orientation (Owatverot et al. 2005).

In Fig. 13, the non-dimensional wall shear stress distribution on the bottom plate of the flow chamber, $\tau_w / \langle \tau_{w,2D} \rangle$ is shown at these four points in time. At $t = 0.044$ s the non dimensional shear stress within the rectangular ATR is well within the valid range. However at $t = 0.286$ s the high instantaneous volumetric flow rate leads to wall shear stresses above the upper limit of ATR. In contrast to that the wall shear stresses are below the lower ATR limit at $t = 0.319$ s, owing to the very low instantaneous volumetric flow rate. Finally, at $t = 0.418$ s nearly all values within the rectangular region again exceed the upper ATR limit. Nevertheless the relative distribution of the wall shear stress is not changed. Only the range is shifted to either higher or lower values, depending on

Fig. 13 Non dimensional wall shear stress distribution, $\langle \tau_w \rangle / \langle \tau_{w,2D} \rangle$, on the bottom surface of the flow chamber at $t = 0.044$, 0.286 , 0.319 and 0.418 s for $\langle Q \rangle = 400$ ml/h. The rectangular area shows the ATR chosen from the steady simulation

the instantaneous volumetric flow rate. This corresponds to the fact that the flow field in the chamber is qualitatively always the same despite the temporal variation of the volumetric flow rate. The flow field is therefore indistinguishable from the steady flow field shown in section (Steady flow field).

The investigation of the relationship between endothelial cell phenotype and function and fluid shear stress is important due to the role of the endothelium in disease development. The parallel plate flow chamber is a commonly used apparatus to elucidate this relationship. The experimental setup may be sometimes cumbersome and chamber variations due to construction or assembly as well as time-

dependent changes in pressure or flow rate may affect flow-sensitive cell responses (McKinney et al. 2006). The flow chamber described facilitates the application of shear stress to underlying adherent (e.g. endothelial) cells because the experiment can be set up easily in a standard cell culture multidish eventually without passaging the cells. Due to the compact design the whole experimental setup fits in an incubator and up to six runs can be performed simultaneously in every single well of a standard 6-well multidish. The introduced perfusion system ensures well defined flow conditions in combination with a straightforward experimental setup. The chamber construction enables the design as disposable article, greatly facilitating analysis of cells within a well of a multidish. A similar chamber was used by Freyberg and Friedl (1998) with a tissue culture plate as bottom wall. Contrary to the chamber presented here, they used a circular area of cell growth on the bottom wall, which was created by using at least two 0.25 mm high silicone sheets. Therefore the height of their test section is 0.5 mm and possessed two steps. Cells had to be preincubated with the silicone sheets in place which lead to a more complicated experimental setup. In addition the geometry of their inlet and outlet differed from the chamber presented here and no numerical or experimental analysis of the chamber was performed.

Unlike cone-and-plate devices parallel-plate chambers work in horizontal, upright or upside down positions. The ability of easy positioning facilitates the analysis of the experiment e.g. with upright or inverted microscopy. Using the metal rack we press the chamber onto the surface by mounting the holding device with screws and do not rely on a vacuum. The metal rack provides a fixed connection of the multidish and the flow chamber within to the mechanical stage of the microscope which enables localisation of accordant test regions before and after the experiment. Therefore localized effects of flow on e.g. cellular realignment or surface protein expression can be described. In contrast to many other chamber types the presented chamber has a fixed height. This ensures constant chamber geometry between experiments which barely depends on assembling errors. However we consider distortion of the chamber body by pressing it against the well bottom with a metal rack. As demonstrated elsewhere, shear stress varied by as much as 11% across the chamber area due to

assembling errors or manufacturing tolerances (McCann et al. 2005). When cells are seeded throughout the well unfavorable crimping of cells will occur while assembling the chamber in the well. This can be easily prevented by using a template for the active test region (ATR) or by giving the cell suspension in the assembled apparatus. A comparatively small region (ATR 0.96 cm² or 10% of well area) of the well is exposed to the target shear stress values. The facilitated experimental setup or the use of ATR templates compensates for this disadvantage in our opinion. Depending on cell type and density approximately 200.000 cells lie within the ATR providing material for further analysis. Despite accurate construction of the chamber the given boundary conditions lead to unfavourable remaining shear stress gradients.

For a steady flow the chosen rectangular area within the flow chamber is consistent with the requirements of the ATR defined by Chung et al. (2003). The maximal shear force exists outside the ATR right below the inlet and outlet ports of the perfusion chamber. This could lead to undesired effects in these regions with higher shear forces as described by (McKinney et al. 2006). As has been stated before, the usage of a peristaltic pump renders the flow within the flow chamber unsteady despite the usage of the damper. This unsteadiness is not negligible and accurately described in our study. Thus the remaining pulsatility leads to wall shear stresses at the monitoring positions which are outside the defined valid range of the ATR. This indicates that the rectangular area chosen from the steady simulation as ATR does not fulfil the criteria in the actual operation of the flow chamber when using the described peristaltic pump only.

If cells were grown over the entire surface of the well (i.e. outside the ATR) they are exposed to different shear stress values. Collected samples of supernatant medium may contain products generated by cells exposed to an undefined shear stress range from zero to the calculated maximal shear force. Nevertheless this observation does not limit the range of use of the present chamber but emphasizes the importance of accurately describing the velocity field in flow chambers when interpreting flow study results. McCann et al. (2005) and McKinney et al. (2006) demonstrated changes in cell response as a direct consequence of such variations. We noticed

however a quantitative change in the magnitude of the wall shear stress. Some cells became detached and were flushed away. Furthermore cell alignment in the given time at given shear forces was not as pronounced as seen in other studies. This could be evoked by endothelial cell dysfunction which is thought to be caused by large spatial shear stress gradients. LaMack et al. (2005) demonstrated that porcine aortic endothelial cells exhibit elevated permeability in the presence of large spatial shear stress gradients. Multiple mechanical stimuli induce magnitude-dependent morphologic responses (Owatverot et al. 2005). In our system different mechanical stimuli are well controlled but not negligible. Many effects of flow studies could be possibly attributed to poorly understood flow fields inside the chamber. The aim to setup a flow chamber with a well controlled flow field within a standard cell culture multidish is achieved. The spreading of CFD analysis leads to more sophisticated chamber geometries. Therefore further improvement of the flow channel, chamber design and following stabilization of the flow field, as by the intricate use of gravitational force to pump the fluid through the flow channel, are necessary.

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