FLUIGENT
SMART MICROFLUIDICS™

Why is it important to control shear stress in your microfluidic experiments?

EXPERTISE REVIEW

INTRODUCTION

Mechanical forces are potent regulators of cellular structures and functions in both health and disease. As a result of their unique location, endothelial cells experience several mechanical forces: pressure, created by the hydrostatic forces of blood within the blood vessel; circumferential stretch generated by cardiac pulsation; and shear stress, the dragging frictional force created by blood flow. Of these forces, shear stress is particularly important as it stimulates the release of vasoactive substances and changes gene expression, cell metabolism, and cell morphology.

PHYSICAL CHARACTERISTICS OF FLUID SHEAR STRESS

The shear stress τ is defined as the ratio of the tangential force to the surface area to which it is applied, and is expressed as a pressure. Fluid shear stress correlates with fluid velocity and with viscosity. For a Newtonian fluid, for which the viscosity is constant, the shear stress depends on the viscosity of the fluid and the shear rate. Therefore, for Newtonian fluids it may be computed according to Newton's law with the following relation:

$$\tau = \eta \frac{\partial v}{\partial z}$$

where η is the viscosity (g/cm*s = Poise), $\frac{\partial v}{\partial z}$ is the velocity gradient or shear rate (s).

A flowing fluid displays a parabolic velocity profile (fluid velocity is fastest at the center than near the channel wall).

Figure 1: Representation of the flow velocity profile (left) and shear rate distribution (right) of a laminar flow inside a circular channel

However, the blood in a non-Newtonian fluid and its viscosity is not constant. An apparent viscosity can be attributed, i.e., the viscosity of a Newtonian fluid showing the same relationship between the flow rate Q and the pressure differential ΔP, according to the Poiseuille's law. In addition, the velocity profile is flattened. For small vessels with diameters less than 300µm, blood viscosity is lower than in larger vessels, so that shear stress may differ, due to change in apparent viscosity, depending on the location throughout the vascular tree.

IN VIVO FLUID SHEAR STRESS

Cells are submitted to a variety of mechanical forces on a daily basis. Endothelial cells (ECs) lining blood vessels are constantly exposed to shear stress resulting from the blood flow. Shear stress corresponds to the dragging force generated by the friction between a moving fluid and the cells forming the inner lining of a vessel. Other cells exposed to shear stress include kidney epithelial cells or endothelial cells forming the lymphatic vessels[1]. This influences their behavior and gene expression. Cells exposed to a rapid and unidirectional blood flow display a spindle-like shape and align their longitudinal axis parallel to the direction of blood flow[2] (Figure 2). This is caused by cytoskeletal remodeling to minimize strain generated by the shear stress[3&4].

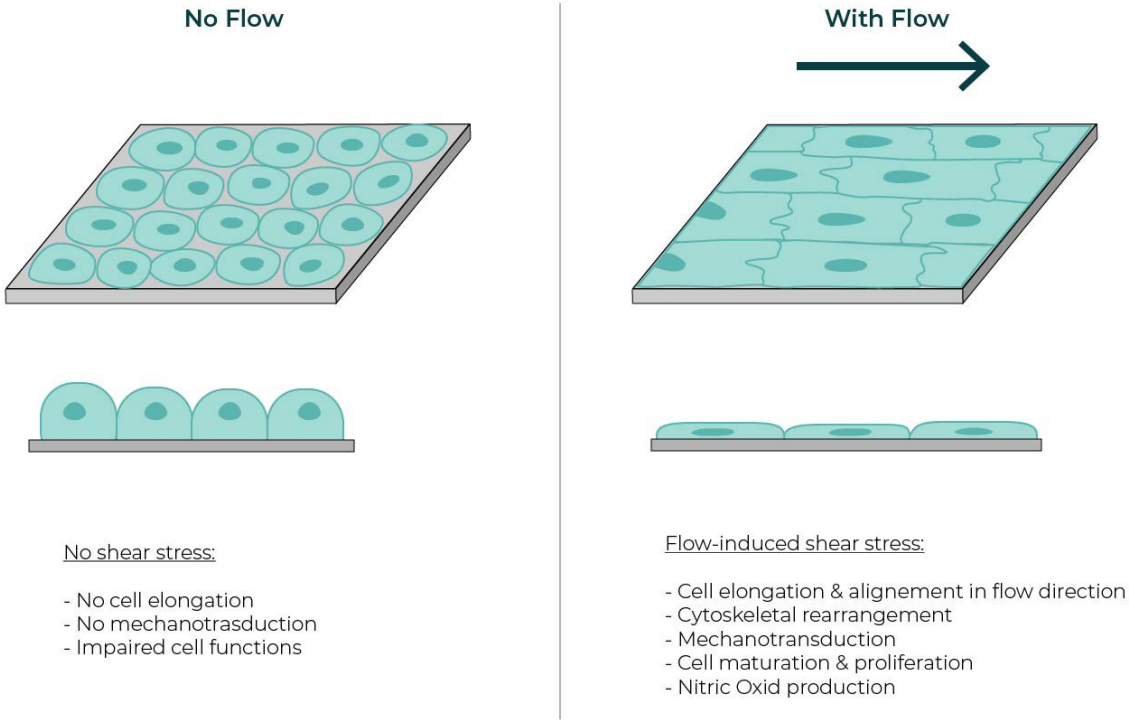


Figure 2: Cells sense and react to flow-induced shear stress

These mechanical forces cause morphological changes and trigger biochemical and biological events. Cells can sense mechanical cues (though their membrane receptors, ion channels, cytoskeleton and nuclear envelop proteins) and transduce them into molecular signaling cascades, a mechanism known as mechanotransduction. ECs respond to physical forces such as shear stress to maintain vascular homeostasis. Several investigations have revealed that shear stress regulates major EC functions including angiogenesis, vessel remodeling, and cell fate[5].

In vivo, the wall shear stress is ~1-2Pa (10-20 dyne/cm^2) in arteries, ~5 Pa in small arterioles[6], ~2 Pa in venules, and ~0.1 Pa (dyne/cm^2) in the vena cava[6-9]. Additionally, these average values should be taken as a range of order, since the values depend on where they have been measured and the mode of calculation. The physiological shear stress value is not uniform throughout the arterial network and may vary locally. Within the same vessel, shear stress levels and profiles can vary significantly due to geometric features including vessel curvature and branching.

Blood hemodynamics generates shear stress but also circumferential stretch which become naturally coupled *in vivo* and *in vitro*. ECs align parallel to shear stress and perpendicular to stretch; thus, axial shear stress and circumferential strain are expected to reinforce one another while shear stress and axial stretch would be expected to counteract one another. Differential coupling of signaling mechanisms and subsequent endothelial cell response may provide flexibility to the endothelial cells in terms of responding to varying types and degrees of shear stress and stretch that they may encounter. *In vivo*, pulsatile shear stress and cyclic hoop stretch are not synchronized, exhibiting a phase shift whose magnitude varies across the vascular tree. This phase shift was demonstrated to attenuate the synergistic effect, with altered production of vasodilators[10] and an increased expression of atherogenic genes when both stimuli are perfectly out of phase[11].

Acute changes in stretch and shear stress induce transient changes including the release of vasoactive agents and alterations in vessel diameter. Physiologic stresses and strains exert vasoprotective roles via the production of NO (Nitric Oxid) by the endothelium. NO stimulates the relaxation of vascular smooth muscle and regulates vascular resistance and blood pressure[12]. Perturbation of tissue stresses and strains can disturb the homeostatic oxidative balance and lead to vascular remodeling and possible dysfunction (e.g. altered vasorelaxation, tone, stiffness, etc.). Disrupted shear stress can drive abnormal endothelial cell behavior and vascular wall remodeling, causing inflammation that can be associated with cardiovascular disorders, including hypertension, atherosclerosis and stroke. These pathologies share mechanisms and processes including cardiovascular cell migration, hypertrophy, proliferation, apoptosis as well as changes in cell phenotype.

DIVERSITY OF FLOW-INDUCED SHEAR STRESS

Blood flow dynamics influence EC cytoskeletal organization and inflammatory state. Indeed, ECs are sensitive to small variations in magnitude, but also in the direction and regularity of blood flow-induced shear stress[13]. ECs experienced several types of shear stress:

- luminal flow within the vessel lumen that is parallel to the cell and acts on EC apical side
- pulsation across the endothelium produced by heartbeats which rather impacts cell–cell junctions
- interstitial flow resulting from liquid flowing among the interstitial space and acting on EC basal side.

Luminal - Steady laminar flow

From a pure physical aspect, Reynolds number (for a tube $R_e = pud/\mu$, with p the fluid density, u the flow velocity, and d the tube diameter) helps to predict flow patterns in different fluid flow situations. At low Reynolds number (typically Re < 1000), the flow is laminar: fluid flows in parallel layers, with no disruption between them, as opposed to turbulent flow. As the dimensions are in micrometer scale in most tissues and organs (e. g., capillaries are about 8-10µm in diameter), Reynolds number is low (typically < 10). Flows are mainly laminar, with a characteristic velocity flow profile (Figure 1).

Vascular flows are most typically uniaxial and laminar. In relatively straight vascular segments, flow streamlines are largely undisturbed and remain mostly parallel to the vascular wall[14] (Figure 3A). Steady laminar shear stress is a determinant of normal vascular function through the orchestration of NO production and anti-platelets aggregation mechanism. Steady wall shear stress is also important to downregulate pro-thrombotic molecules, such as tissue factor, an initiator of thrombus formation[15]. The major anti-coagulant cofactor (thrombomodulin) was also shown to be upregulated by high shear stress[16]. Low or reversing shear stress regions undergo an elevation of oxidative stress and predispose the vessel wall to atherosclerosis[17]. Low shear stress globally contributes to a high inflammatory and high prothrombotic state.

Luminal - Turbulent flow

Blood flow within vessels is generally laminar and streamlined. Abrupt changes in vessel geometry due to branching, sharp turns or stenosis can disturb the laminar blood flow, causing secondary flows in the form of vortices (Figure 3B). ECs lining those turbulent segments or stagnant are much rounder in shape and do not have a uniform orientation. Disturbed flows include turbulent flows and laminar flows with spatial shear stress gradients and/or secondary flows. Such disturbed flow patterns activate pro-inflammatory phenotypes in endothelial cells causing damages to the endothelial layer. Flow disturbance often correlates with the localization of vascular diseases including atherosclerosis, aortic valve calcification, and inflammation and thrombosis.

Pulsatile flow

Pulsatile flow is mainly observed in large vessels such as arteries. It is a consequence of rhythmic heartbeat. At each beat, aortic flow reaches a peak (systole), then diminishes to a low level (diastole) until the next beat. This produces pulsatile flow instead of a continuous flow. Though pulsatile, the flow remains laminar. The velocity flow profile varies as a function of time[18]. A typical flow rate curve of the artery for one heartbeat cycle displays two local maxima and a minimum, with positive and negative flow rate values[19]. As a result, the flow direction and the amplitude of the velocity flow profile vary as a function of time (figure 3A). This has an impact on the shear stress as it is derived from flow velocity. Pulsatile flow is usually performed in blood vessel-on-chip models to simulate the actual pulsatile blood flow in human circulation[20]. Within the microvasculature, this pulsatile behavior is significantly dampened and blood flow becomes quasi-steady.

Interstitial flow

Interstitial flow arises from fluid movement within the tissue surrounding the ECs, shearing them on their basal side (Figure 3C). This is mostly due to the movement of fluid through the extracellular matrix of tissues, where cells such as fibroblasts, immune tissue cells, and adipocytes can be found[21]. Fluid flow carries large proteins through the interstitial space and mechanically stimulates ECs. Several studies have demonstrated that shear flow induced by interstitial fluid was crucial for physiological cell responses such as cell differentiation[22-23]. Interstitial fluid flows at a lower velocity as compared to blood flow within vessels because of high resistance exerted by the extracellular matrix. Flow velocity profile and subsequent shear flow is also more difficult to define due to the complex architecture of the extracellular matrix and as the fluid moves around the cell-matrix interface in all directions. Interstitial flow significantly enhances vascular sprout formation, network extension, and the development of branching networks in a magnitude-dependent manner[24].

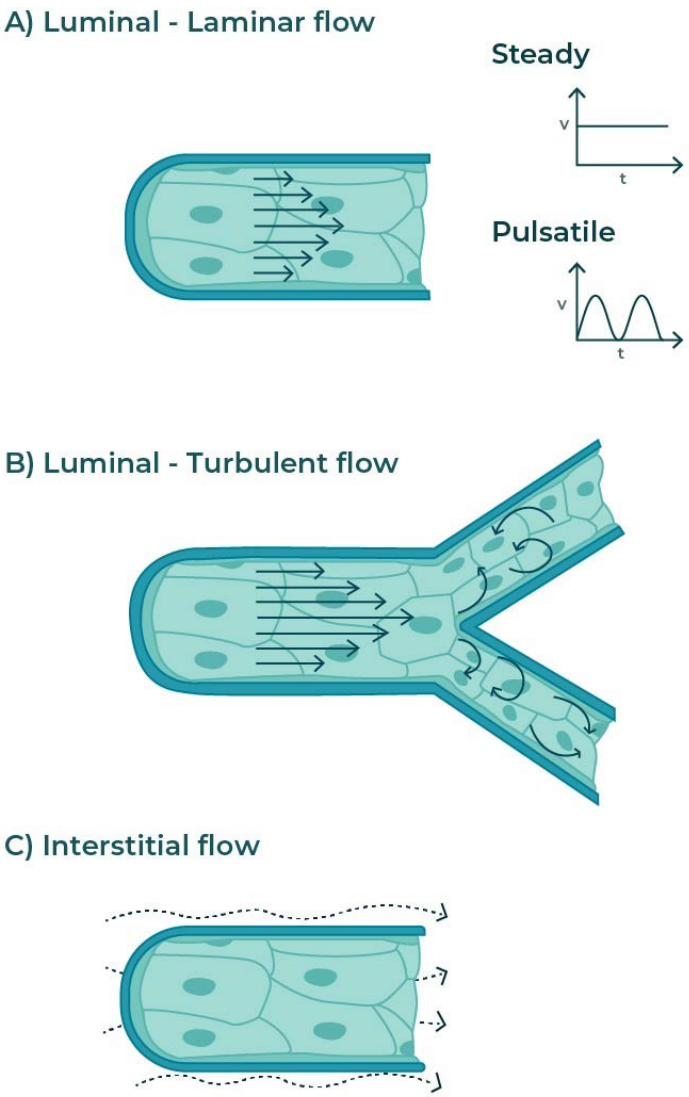


Figure 3: The different types of flow (Arrows represent the distribution of flow velocities)

STUDYING THE EFFECT OF SHEAR STRESS *IN VITRO* IN RELEVANT PHYSIOLOGICAL SYSTEMS

Traditionally, endothelial cells have been grown and studied in the absence of flow and mechanical stimulations on stiff substrates such as plastic or glass. These cells are not subjected to the physical forces that endothelial cells endure *in vivo*, thus the results of these experiments often do not mimic those observed in the body. The field of vascular biology now realizes that an intricate analysis of endothelial signaling mechanisms requires complex *in vitro* systems to mimic *in vivo* conditions.

In vitro platforms to apply defined mechanical stimuli to cells are critical to understanding how strains and shear stresses affect endothelial and other cells. Early studies have been performed using macro scale, custom built devices reviewed here[25]. For the past 10 years, the development of microfabrication and bioengineering approaches has given rise to microfluidic devices that offer the ability to control or alter the cell's physical environment.

These devices have been used to grow ECs under dynamic flow conditions to impose shear stress (by direct control of the fluid flow rate) and to analyze their responses at the cellular and molecular levels. Different systems have been developed to address specific aspects of how shear and strain affect EC functions. These range from microfluidic chips providing flow and stretch for investigating blood vessel biomechanics[28], generation of shear stress gradients[29] and integration of ridge-shaped obstacles within the microfluidic chip to create controlled disturbed flow patterns[30]. To modulate the shear-strain coupling, a versatile microfluidic platform based on collagen hydrogel actuated by flow was developed to provide a physiologically relevant mechanical stress environment to ECs[31].
These systems have demonstrated that ECs alter their morphology, function, and gene expression in response to shear stress. Microchannels mimicking human arteries and blood vessels were developed to investigate the influence of glucose and shear stress on endothelial cell apoptosis. The authors performed the experiments under pulsatile and static flow conditions. Under static conditions, glucose-treated cells induced 5.5-fold less apoptosis than when using pulsatile flow. This observation demonstrates the role of flow-induced shear stress in driving hyperglycemia-induced EC death.

In vitro models of the human microvasculature have been developed to study the effect of luminal flow on cancer cell migration[32]. This has contributed to a better understanding of the metastatic cascade process (extravasation and subsequent interstitial migration). Luminal flow significantly promotes the extravasation potential of tumor cells as compared to static conditions, with an average intravascular speed of tumor cells of ~ 12.5 µm/h under flow, as compared to ~ 9.4 µm/h under static conditions. This reveals the important role of fluid flow during metastatic extravasation and invasion[32]. In another study, a microfluidic device was developed to gain insights into how interstitial flow affects breast cancer cell invasion[33]. By performing live cell imaging under well-controlled flow conditions, the authors found that compared to static flow conditions, interstitial flow increases the percentage of cells that become migratory and increased their average migration speed.

Fluid flow within the tumor microenvironment can enhance tumor cell invasion by directing a subpopulation of tumor cells in the flow direction; i.e., towards the draining lymphatic vessels, a major route of metastasis[33]. These observations demonstrate how important it is to consider both laminar and interstitial flows in tumor models, as they affect tumor cell extravasation and invasion.

IMPLEMENTING SHEAR STRESS IN *IN VITRO* CULTURE SYSTEMS

Choosing the best fluid delivery instrument

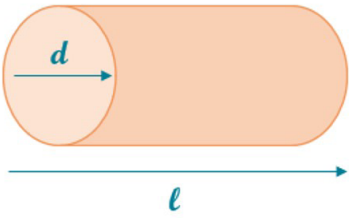
As described in this review, shear stress is a mechanical force that strongly influences cell morphology and behavior. Several microfluidic devices compatible with fluid perfusion and application of different shear stress strengths for the analysis of cellular functions now exist. To implement shear stress into microfluidic devices, it is essential to accurately control fluid delivery. The choice of flow control instrument is crucial. Available options include syringe pumps, peristaltic pumps or pressure-based flow controllers. A pressure-based system can provide well-controlled and steady flow rates as opposed to other devices such as peristaltic pumps. We have compared the impact of the perfusion system on endothelial cells seeded in microfluidic chips using either a peristaltic pump or pressure-based flow controllers. This test highlights the importance of flow stability in vascular models. It is important to choose an instrument providing instantaneous response time and preventing flow rate oscillations. Pressure controllers comply with these requirements as they offer the fastest response time and the most accurate flow control. We have compared the response time as well as the flow stability of high precision syringe pump and flow controllers.

Shear stress calculation in a microfluidic device

The shear stress applied on cells exposed to constant flow within a microfluidic chip can be determined using the continuity and Navier-Stokes equations. Following are the flow velocity profiles and shear stress equations for two common microfluidic channel geometries: circular and rectangular channels.

Circular channel

For a circular cross-section of diameter d, the fully developed velocity profile in cylindrical coordinates (r,Θ,z) follows the equation[34]:



$$u_z(r) = \frac{8Q}{\pi d^2}\left(1 - \left(\frac{2r}{d}\right)^2\right)$$

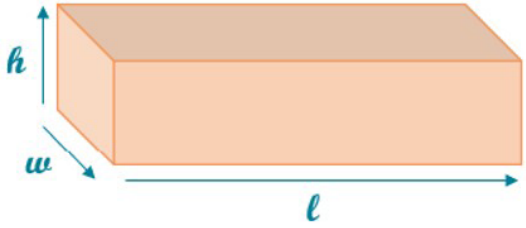
With Q the flow rate, and r the radial distance from the centerline of the channel. The distribution of flow velocities follows a parabolic profile, and the maximum velocity is for r = 0, at the center of the channel. It is of interest to determine the shear stress distribution at the channel wall (r = d/2), as this is where cells are located in the chip. To do so, the strain rate should be calculated by differentiating the flow velocity with respect to r. We get:

$$\tau_{\text{wall}} = \mu\gamma_{\text{wall}} = -\mu\frac{\mathrm{d}u_z}{\mathrm{d}r}\Big|_{r=d/2} = \frac{32\mu Q}{\pi d^3}$$

With μ the dynamic viscosity, Q the flow rate. This indicates that the shear stress remains constant along the channel walls and is a function of the viscosity, flow rate, and channel diameter. At constant channel diameter and viscosity, the higher the flow rate, the higher the shear stress.

Rectangular channel

In microfluidic devices with rectangular channels, the flow velocity profile and subsequent shear stress are more complex. Wall shear stress is not constant and varies across the top, bottom, and side walls of the channel. However, the geometry can be simplified by considering two infinite parallel plates instead of closed channels. Under this assumption, the shear stress follows the equation:



$$\tau_{wall} = \frac{6\eta Q}{wh^2}$$

With Q the flow rate, η the dynamic viscosity, w, and h the channel width and height, respectively.

SUMMARY OF *IN VIVO* SHEAR STRESS VALUES

Physiological shear stress values range from 0.1 to more than 100 dyne/cm² and depend on the tissue (e.g., kidney, lung, heart) or the vessel type for blood vessels (e.g., artery or vein). It also differs from one organism to another (e.g., mouse versus human). The shear stress is often measured in dyne/cm² (dyn/cm²) or in Pascal (Pa). Of note, 1 Pa = 10 dyn/cm².

Cell types	Shear stress value (Pa)	Shear stress value (dyn/cm²)
Arteries[35]	1-2 Pa	10-20 dyn/cm²
Human common femoral artery[36]	0.3-0.5 Pa	3-5 dyn/cm²
Carotid artery[36]	1.1-1.3 Pa	11-13 dyn/cm²
Veins[35]	0.1-0.6 Pa	1-6 dyn/cm²
Venules[35]	2-4 Pa	20-40 dyn/cm²
Arterioles[35]	6-8 Pa	60-80 dyn/cm²
Capillaries[35]	0.5-2 Pa	5-20 dyn/cm²
Glomerular capillaries[37]	0.1-9.5 Pa	1-95 dyn/cm²
Embryonic lung[38]	0.01-0.1 Pa	0.1-1 dyn/cm²
Airway epithelial cells[39]	0.05-0.3 Pa	0.5-3 dyn/cm²
Alveolar epithelial cells[40]	0.4-1.5 Pa	4-15 dyn/cm²
Mouse embryonic kidney[41]	0.04-0.5 Pa	0.4-5 dyn/cm²
Human kidney[42]	0.03-0.12 Pa	0.3-1.2 dyn/cm²
Cornelial epithelial cells[43]	0.4-0.8 Pa	4-8 dyn/cm²
Osteocytes[44]	0.8-3 Pa	8-30 dyn/cm²

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