Shear Stress Kit

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Summary

Cardiovascular diseases and, in particular, those with ischemic etiology developed by atherosclerosis are leading causes of death[1]. Atherosclerosis is a chronic inflammatory disease of the arterial vessels characterized by the formation of intimal lesions (atherosclerotic plaques) in the vasculature and it lies behind of the main vascular pathologies as myocardial infarction and stroke[2].

Atherosclerosis is a complex multifactorial process including several biological factors such as hypercholesterolemia, hypertension, diabetes and inflammation. It is remarkable that even that these biological factors are systemic, atherosclerotic plaques mainly form near arterial bifurcations, branch ostia and curvatures[3,4].

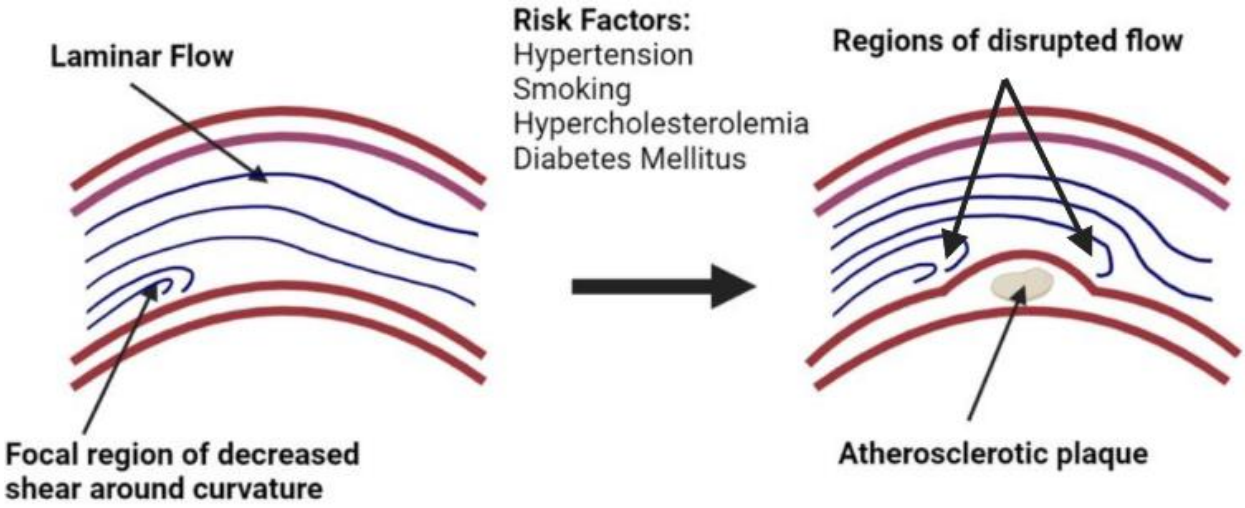

Figure 1: *Schematic representation of the effect of shear stress in the formation of atherogenic conditions. Reduced shear stress modifies cellular morphology and biochemical processes, with an essential role in atherogenic alterations. There is a higher chance of atherosclerotic plaque formation at these sites when there are systemic risk factors present, which, once formed, further disrupts flow and stimulates the expansion of the fibroinflammatory lipid plaque. Modified from [2]*

To understand these preferred localizations, it is necessary to study atherogenesis physical factors, such as flow-induced shear stress. Maintenance of a physiologic, laminar shear stress is known to be crucial for normal vascular functioning, differentiation and maintenance. Disturbed or oscillatory flows near arterial bifurcations, branch ostia and curvatures are associated with atheroma formation, including changes in endothelial gene expression, cytoskeletal arrangement, leukocyte adhesion as well as to the vasoreactive, oxidative and inflammatory states of the artery wall[3,4].

The difficulties to study shear stress effects on the endothelium are related with the limitations of 2D cultures to reproduce the 3D structure and flow conditions in arteries. To solve this situation, BFlow has developed vessel-on-a-chip models with differential wall shear stress conditions based in the flow speed and the bifurcation angle[5,6]. Recently, the use of vessel-on-a-chip has allowed to determine flow biomechanics of Circulating-Tumour Cells (CTC) accumulation at blood vessel's bifurcations[7].

BFlow's Shear Stress Kit allows performing experiments with endothelium under different controlled shear stress conditions. Bifurcations with three different angles will allow to simulate diverse shear stress conditions. The effects of these conditions on endothelial molecular biology, cell adhesion of circulating cells or particles can be analyzed with this kit.

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Content of the kit

- 5 bifurcated chips with 45º angle and 1 mm diameter circular channel. REF: B01_0008
- 5 bifurcated chips with 90º angle and 1 mm diameter circular channel. REF: B01_0007
- 5 bifurcated chips with 135º angle and 1 mm diameter circular channel. REF: B01_0009
- 6 meters of silicone tube (divided in 6 parts of 1 meter REF: B02_0009) (3 mm outer diameter and 1 mm inner diameter).
- 3 pairs of clamps (6 clamps). REF: B02_0001
- 9 inlet/outlet plugs. REF: B02_0006
- 9 chip connectors. REF: B02_0002
- 3 Y-connectors. REF: B02_0003

Additional materials (not provided)

- **Cell culture room fully equipped**, including cell culture incubator and laminar flow hood, autoclave, cell culture incubator for standard human cell conditions (37ºC temperature, 90% humidity and 5% CO_2), temperature-regulated water bath, inverted microscope with phase contrast, system to count the cells and centrifuge.
- **Cell culture reagents and materials**, including endothelial cells, cell culture medium, trypsin, cell culture material including flask, plates and/tubes, sterile PBS and centrifuge tubes.
- **Reagents and equipment for fluidics**, including fibronectin, forceps and peristaltic or syringe pump.

General considerations

- Chips were tested with the indicated reagents and cellular culture. The conditions have to be adapted to other experimental settings and cells.
- All material used for cell cultured has to be sterile. Chip plugs and tubes for the planned experiments have to be autoclaved in advance.
- Avoid forming bubbles into the channels. These bubbles can affect the integrity of the adhesive cell culture.
- To avoid cell damage, the injection of any cell suspension solution should be performed slowly and allowing the re-equilibration of the internal pressure of the circuit.
- The following protocol is based in HUVEC culture from Rodiño-Janeiro et al[8].

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Protocol of Vessel-on-a-chip cell culture

- Autoclaved channels are filled with fibronectin (5 µg/ml) in gelatin 0.02% from bovine skin to coat the surface and incubated during at least 3h in the cell culture incubator (37 ºC).
- Detach HUVEC at confluence with trypsin, pellet and resuspend in complete medium at a density of 1.5 x 10^6 per ml and seed them into the chip.
- Cells are incubated into the device for 4 hours. (Video)
- Turn upside-down the device and repeat the cells' seeding procedure with cells for the other side of the channel. Close the inlets and the outlets with autoclaved plugs. Incubate other 4 hours to cover the whole channel surface.
- One end of the silicon tube for the inlet is introduced in the medium reservoir. The tube is mounted in the peristaltic pump and the tube is primed with cell culture medium (until one drop start to form in the other end) and then, the tube is inserted in the inlet (using a forceps). (Video)
- The silicon tubes for the outlets are inserted in the corresponding outlet and the other end is placed in a reservoir (shared with the inlets if the model has recirculariza tion). The tubes are clamped to avoid any flow in the next step.
- The full system is placed in an incubator (if the incubator has an outlet port, the bomb can be placed outside). Once placed, remove the clamps.
- The pump is turned on at the desired flow rate.
- Keep the system during the duration of the experiment.

Shear stress and flow conditions

As previously showed in Otero-Cacho *et al.*[6], the area of recirculation is affected by the bifurcation angle. Numerical simulations (figure 2) show the velocity inside of the bifurcation with different inlet flow rates and angles. Reynolds number predicts the behavior in fluid flow, being low Reynolds number flows mainly laminar and high Reynolds number flows tend to be turbulent.

A moderate Reynolds number (200) represented in figure 1 shows the differential flow area with low velocity (in blue scale) with different angles in channels with the same characteristics that BFlow's Shear Stress kit.

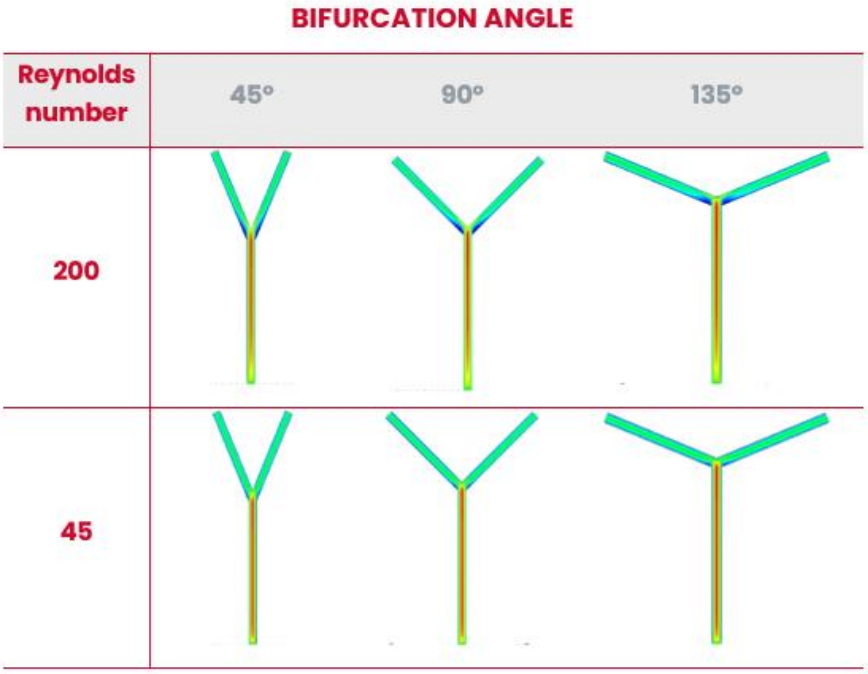

Figure 2: Differential flow velocity regions at moderate and low Reynolds number with different bifurcation angles. *Numerical simulation data in three different bifurcation angles (45°, 90° and 135°) at two flow rates: Moderate Reynolds number of 200 (14ml/min) and Low Reynolds number of 45 (6 ml/min). The color of the velocity corresponds to a scale from 0 to 0.6 m/s in the case of moderate Reynolds number and from 0 to 0.12 m/s in the case of low Reynold number (blue the minimum and red the maximum). FBS 10% physical conditions were used in the simulations (density: 1006.881 Kg/m^3, Dynamic viscosity: 0.00149 Pa*s)*

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