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Review

Animal models of surgically manipulated flow velocities to study shear stress-induced atherosclerosis

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ABSTRACT

Atherosclerosis is a chronic inflammatory disease of the arterial tree that develops at predisposed sites, coinciding with locations that are exposed to low or oscillating shear stress. Manipulating flow velocity, and concomitantly shear stress, has proven adequate to promote endothelial activation and subsequent plaque formation in animals. In this article, we will give an overview of the animal models that have been designed to study the causal relationship between shear stress and atherosclerosis by surgically manipulating blood flow velocity profiles. These surgically manipulated models include arteriovenous fistulas, vascular grafts, arterial ligation, and perivascular devices. We review these models of manipulated blood flow velocity from an engineering and biological perspective, focusing on the shear stress profiles they induce and the vascular pathology that is observed.

1. Introduction

Atherosclerotic plaque development occurs preferentially at geometrically predisposed areas, like the inner curvature of the aortic arch and at arterial branch points. This site specific development occurs despite the fact that the entire vasculature is exposed to systemic risk factors like hypertension and hypercholesterolemia [1,2]. The specific plaque localization is caused by the local differences in blood flow velocities, and thus the shear stress, to which the vessel wall is exposed to. Besides blood flow-induced shear stress, the vessel wall is also exposed to wall strain. Shear stress is the frictional force generated by the blood flow on the vessel wall, parallel to the endothelium, while wall strain is generated by the blood pressure, perpendicular to the endothelium. Both forces play a role in either plaque initiation and/or development, but shear stress was most often correlated to atherogenesis [1,3,4]. This review focuses on the animal models in which the blood flow velocities, and thereby shear stress, have been manipulated in order to study its causal relation with atherosclerosis.

The SI-unit of shear stress is Pascal (Pa), which is equal to N/m^2 or 10 dynes/cm^2. Shear stress at the vascular wall, wall shear stress (WSS), can be calculated by combining data on flow velocity, blood viscosity and vessel geometry using advanced modeling techniques and is usually averaged over the cardiac cycle. The most commonly used mathematical technique to calculate shear stress is computational fluid dynamics (CFD). Different blood flow velocity patterns in the vasculature give rise to diverse average WSS levels (Box 1) varying over the whole vasculature and between species. These WSS levels can range from 3–14 Pa in mice, 1–16 Pa in rabbits and 1–2 Pa in humans [5].

The correlation between atherosclerotic plaque localization and different blood flow velocity patterns is caused by the response of endothelial cells (ECs) to alterations in shear stress. ECs react via mechano-transduction by changing gene regulation and, subsequently, cell phenotype. The process of mechano-sensing and -transduction has been extensively studied and reviewed [6–9]. It includes mechanical deformation and activation of cytoskeletal elements, primary cilia, the glycocalyx, the VE-cadherin/PECAM-1/VEGFR2 complex, integrins, ion channels, and G-protein coupled receptors [6–9]. The mechanical signal is subsequently converted into a biochemical signal. When exposed to a high unidirectional laminar shear stress, ECs are quiescent and align into the direction of the blood flow [4,10]. They express an anti-inflammatory and anti-oxidant gene profile controlled by a concerted action of the shear stress-responsive transcription factors of which Krüppel-like factor 2 (KLF2) and nuclear factor (erythroid-derived 2) -like 2

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Box 1
Blood flow velocity patterns and shear stress

Flow velocity patterns and the concomitant shear stress profiles, depend on the velocity profile itself, blood viscosity, lumen diameter and vessel geometry. A simplified schematic depiction of the different laminar flow and shear stress profiles is shown in Fig. 1. The different flow routes a blood particle can follow are depicted by the dotted streamlines. Blood flow is laminar in most cases, which means the blood flows in parallel layers, without intersecting. WSS is represented by the slope of the parabolic velocity profile depicted in black (Fig. 1A). In case of a stenosis, caused by for example a plaque, flow remains laminar but can separate distal to a stenosis, forming recirculating flow layers near the wall, resulting in low and/or retrograde shear stress at the wall (Fig. 1B). At a curvature, secondary flow can occur, meaning that flow is moving perpendicular to the flow direction (Fig. 1C transverse section). This is still considered laminar flow as parallel flow layers are present. The peak velocity shifts to the outer curvature of the vessel, resulting in a higher shear stress at the outer curvature compared to the inner curvature (Fig. 1C). At bifurcations, secondary flow is present similar to flow patterns seen at curvatures, resulting in particle movement parallel and perpendicular to the vessel wall (Fig. 1D). This is not to be confused with the term turbulent flow. With turbulent flow, particles do not flow in parallel layers but intersect, and show unpredictable multi-directional, multi-velocity streamlines. Turbulent flow is uncommon but can occur occasionally in the human descending aorta, behind the aortic valve leaflets during peak systole, in large arteries distal to a very severe stenosis and in aneurysms [37–39]. Fig. 1 summarizes the different flow velocity patterns at one point in time. However, due to cardiac contractions, flow in the arterial system is pulsatile and therefore flow velocities, and the time averaged wall shear stress may differ in magnitude and direction.

(Nrf2) are the most important [11–16]. An important function of KLF2 is regulating vascular tone via regulation of endothelin-1 (ET-1), a vasoconstrictor [16,17], and endothelial nitric oxide synthase (eNOS), producing the vasodilator nitric oxide (NO).

At regions of low or oscillatory shear stress, the endothelium is activated and shows a pro-inflammatory transcription profile. This pro-inflammatory profile is mainly regulated by activation of NF-κB and its transcriptional targets, resulting in expression of adhesion molecules, a decrease in NO, and a higher permeability to plasma macromolecules, which results in a dysfunctional endothelium that is prone to atherosclerotic plaque initiation [9,18,19].

The different cellular adhesion molecules expressed by the dysfunctional endothelium facilitate adhesion and migration of monocytes and lymphocytes [20,21] into the sub-endothelial layer. There, monocytes differentiate into macrophages, which will take up oxidized low density lipoprotein (oxLDL) and transform into foam cells [22]. These foam cells produce high levels of pro-inflammatory cytokines, chemokines, and growth factors, which attract more inflammatory cells and induce smooth muscle cell (SMC) proliferation and migration into the intima. This results in plaque growth. Initially, plaque growth will not affect lumen diameter due to compensatory outward remodeling [23]. Shear stress remains low and will therefore continue to activate pro-inflammatory pathways, including pathways related to extracellular matrix degradation (reviewed by Wentzel et al. [24]). This process in turn promotes further compensatory outward remodeling [23,25–27]. When outward remodeling is not sufficient anymore to avoid lumen protrusion of the growing plaque, the shear stress levels at the plaque will change [28].

During atherosclerosis development, plaques can develop into a stable plaque or a vulnerable plaque. While most atherosclerotic plaques are stable, some develop into a vulnerable or rupture prone plaque [29]. The American Heart Association (AHA) classification [29,30] describes the characteristics of the different stages of human atherosclerotic plaques. The vulnerable or rupture-prone plaques (AHA class IV, V and VI) often show an eccentric phenotype and are defined by a large, lipid-rich necrotic core, a thin fibrous cap infiltrated by inflammatory cells, and outward vascular remodeling [31]. Other common characteristics of human plaque vulnerability are intraplaque hemorrhage, neovascularization, and calcification [29,30,32–36].

Atherosclerotic plaque formation in humans is slow and spans over a period of decades. Furthermore, harvesting plaque tissue from humans is not straight forward, and thus molecular mechanisms involved in the generation and destabilization of plaques can be difficult to investigate. Therefore, several animal models for atherosclerotic plaque development including mouse, rat, rabbit, pig and primate are used. To initiate the atherosclerotic process in these animal models, risk factors like a high cholesterol level and inflammation are genetically or experimentally induced. Shear stress can be surgically manipulated in these animal models in order to study the causal relationship between shear stress and atherosclerosis initiation and progression. This surgical manipulation can also be used to accelerate the atherosclerotic process. Here we review these surgically manipulated flow models from an engineering and biological perspective.

2. Flow velocity manipulated models

2.1. Arteriovenous fistula models

The arteriovenous (AV) fistula model is one of the first animal models that used surgery to manipulate the flow velocities in order to study the correlation between shear stress and atherosclerosis [40]. For the creation of an AV-fistula, an artery is connected directly, end-to-side, to a neighboring vein, thereby bypassing the distal arterial and venous vascular beds (Fig. 2A). The AV-fistula is originally developed as a form of permanent vascular access for hemodialysis patients by inducing venous maturation. However, the development of venous neointimal hyperplasia often led to fistula failure. This observation triggered a series of studies with this model on vascular remodeling and maturation of the venous segment, and a few studies on plaque development.

Flow measurements and shear stress calculations in mice demonstrated that creation of an AV fistula resulted in elevated blood flow levels, and concomitantly shear stress, in the arterial segment.

The venous segment showed endothelial dysfunction, which was indicated by an upregulation of shear stress responsive pro-inflammatory genes and proteins. Furthermore, significant lumen narrowing occurred as a result of intimal hyperplasia with infiltrating macrophages and SMCs [41,42]. Flow velocities and shear stress changes in the venous segment were not reported. The hemodynamics at the site where the artery connects to the vein is so complex, that it is not possible to predict what happens at that site without local measurements.

Similar results for blood flow and shear stress levels were obtained in a dog and rabbit model, where flow velocities and shear stress in the arterial segment were also elevated, which led to