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# Exogenous and endogenous force regulation of endothelial cell behavior

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ABSTRACT

Endothelial cells live in a dynamic environment where they are constantly exposed to external hemodynamic forces and generate cytoskeletal-based endogenous forces. These exogenous and endogenous forces are critical regulators of endothelial cell health and blood vessel maintenance at all generations of the vascular system, from large arteries to capillary beds. The first part of this review highlights the role of the primary exogenous hemodynamic forces of shear, cyclic strain, and pressure forces in mediating endothelial cell response. We then discuss the emergent role of the mechanical properties of the extracellular matrix and of cellular endogenous force generation on endothelial cell function, implicating substrate stiffness and cellular traction stresses as important mediators of endothelial cell health. The intersection of exogenous and endogenous forces on endothelial cell function is discussed, suggesting some of the many remaining questions in the field of endothelial mechanobiology.



## 1. Introduction

Endothelial cells (EC) form the lining of every blood vessel in the body, from the large veins and arteries that transport blood to and from the heart, to the fine capillary networks that perfuse tissues. As such, ECs are in direct contact with flowing blood and control vascular tone, vessel permeability, and have anticoagulant properties. ECs also mediate pathological responses such as inflammation, angiogenesis during wound healing and tumorigenesis, hypertension, and atherosclerosis.

ECs adhere to the basal lamina and line all blood vessels as monolayers (Kalluri, 2003). In arteries and veins, the endothelial layer and basal lamina (*intima*) is surrounded by layers of smooth muscle cells (SMC) (*media*), and connective tissue (*adventitia*) (Gossl et al., 2003). The microvasculature consists solely of an intima and supporting pericytes, which are specialized SMCs that confer both stability (von Tell et al., 2006) and quiescence to capillaries (Antonelli-Orlidge et al., 1989). These large and small vessels range in diameter from approximately 2.5 cm in the aorta to 8 μm in the capillaries (Burton, 1954). Because of their unique location between flowing blood and a variably complex vascular wall, ECs are subjected to forces *in vivo* that act either directly on their apical membrane or through extracellular matrix (ECM)/adventitial layers (Fig. 1). Flowing pulsatile blood creates both shear and pressure stresses that act directly on the apical cell surface. ECs are also subjected to forces provided by residual hoop stress in the vessel wall, external forces from surrounding tissues that impinge vessels, e.g. muscle-mediated vessel contraction (Sheriff, 2005), and mechanical input from the local ECM stiffness. Collectively, these mechanical forces play a concerted role in mediating both normal and disease-related EC responses from the tissue to molecular level.

In this review, both exogenous force (those forces that are imposed on ECs) and endogenous forces (those forces generated by ECs) are discussed, with a special emphasis on the effects of the mechanical properties of the vessel wall and surrounding ECM on EC function in normal and disease states.

## 2. Hemodynamic forces on the endothelium

### 2.1. Shear stress

Shear stress is the frictional force imposed by blood flow. The magnitude of shear in the capillaries is approximately 0.5 Pa and averages 1–3 Pa in large arteries (Shaik et al., 2009). Shear stresses may approach 10 Pa at bifurcations or in tortuous vessels (Dewey et al., 1981) and during periods of increased cardiac output or hypertension (Nerem et al., 1993; Posch et al., 1999). Blood flow is typically laminar in straight portions of vessels and disturbed near tortuosities or bifurcations (Chien, 2007). It is one of the most studied hemodynamic forces because physiological shear stresses are largely considered atheroprotective, whereas atherosclerotic lesions are often associated with areas of disturbed flow.

Physiological shear stress in vessels promotes an atheroprotective environment characterized by a quiescent EC phenotype (Berk, 2008). Atheroprotective shear stress principally regulates

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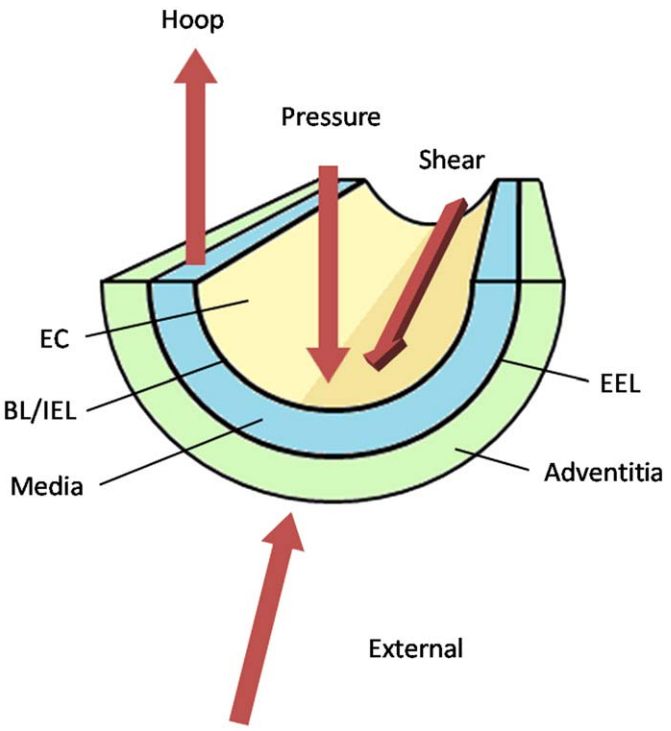


**Fig. 1.** Stresses in a typical elastic artery *in vivo*. A typical artery contains an intimal layer composed of an endothelial cell (EC) monolayer and its ECM, the basal lamina (BL). Surrounding the intima is the media, layers of circumferentially oriented smooth muscle cells (SMCs) separated by fenestrated sheets of elastin that is bounded by the internal and external elastic laminae (IEL/EEL) (dela Paz and D'Amore, 2009). The outermost adventitial layer consists primarily of a dense network of ECM fibers, innervations, and is perfused by the vasa vasorum. ECs experience shear (shown in the direction of flow) and pressure stresses from flowing pulsatile blood, tension from residual hoop stresses in the vessel wall, stiffness from the ECM, and external stresses from adjacent tissues.

arterial diameter (Davies, 1995) through the release of nitric oxide (NO) (Joannides et al., 1995), promotes anticoagulation by stimulating inhibitors of platelet aggregation (Diamond et al., 1989), and inhibits leukocyte adhesion and SMC proliferation (Traub and Berk, 1998). In response to atheroprotective flow, ECs also undergo morphological alignment in the direction of flow (Hastings et al., 2007), presumably to reduce their resistance to shear (Sumagin et al., 2008), with concomitant cytoskeletal polarization (Galbraith et al., 1998). Exposure to shear stress alters endothelial cell–matrix interactions, increasing recruitment of adapter proteins to focal adhesions, lamellipodial protrusion, and migration in the direction of flow (Li et al., 2002). Shear stress also mediates endothelial cell–cell connectivity, upregulating adherens junction proteins including vascular-endothelial cadherin and catenin family members (Noria et al., 1999; Ukropec et al., 2002; Kondapalli et al., 2004).

In contrast to the atheroprotective laminar flow found throughout most of the vasculature, flow at bifurcations or in tortuous vessels is characterized by reversals, low flow velocities, and flow separation that cause shear stress gradients that may contribute to the development of atherosclerotic lesions at these susceptible locations (Gimbrone et al., 1997; Dai et al., 2004; Davies, 2009). These disturbed flow profiles promote an activated EC phenotype that is pro-thrombotic, pro-inflammatory, and supports increases in EC proliferation and apoptosis (Paszkowiak and Dardik, 2003; Reinhart-King et al., 2008b). In contrast to the elongated morphology of cells in laminar flow, ECs under disturbed flow are polygonal in shape, show a decrease in cell and cytoskeletal alignment (Helmke, 2005), a decreased expression of genes associated with differentiated ECs (Hastings et al., 2007), and an upregulation of proteins associated with endoplasmic reticulum stress (Feaver et al., 2008). It is believed that disturbed shear stress contributes directly to the development of atherosclerotic lesions (Gimbrone et al., 1997; Dai et al., 2004; Davies, 2009).

There has been extensive research performed to identify the molecular mechanisms by which ECs mechanotransduce shear stress into a cellular response. A number of mechano-responsive elements have been identified including ion channels, the cytoskeletal network, integrins, G-protein coupled receptors and cell–cell junction complexes. While previous efforts have largely focused on identifying an individual receptor in ECs that first sense shear stress, more recent literature suggests that EC mechanotransduction of shear occurs through multiple pathways (Davies, 1995, 2009 for reviews).

### 2.2. Cyclic strain

Cyclic strain is the circumferential deformation of the blood vessel wall during distension and relaxation of the recurring cardiac cycle (Kakisis et al., 2004). Measurements of circumferential cyclic strain *in vivo* average 2% in the human thoracic aorta (Wedding et al., 2002) at a frequency close to 1 Hz with increases upwards of 30% strain on arterial walls in hypertension (Lee and Sumpio, 2004). Like shear stress, cyclic strain alters EC health and vessel remodeling. Notably, the effects of cyclic strain on EC mechanobiology are rate, duration, and species-specific.

Cyclic strain mediates the ability of ECs to remodel their ECM, proliferate, change shape, and signal to SMCs. Cyclic strain has been generally shown to increase EC proliferation (Sumpio et al., 1987), a response that may require cell–cell connections and vascular-endothelial cadherin engagement (Liu et al., 2007). Lower (6–10%) cyclic strain does not affect bovine aortic EC apoptosis while increasing strain to 15–20% stimulates apoptosis (Liu et al., 2003). Similar work with a human EC line showed 5% to inhibit, and 20% cyclic strain to induce, apoptosis (Kou et al., 2009). Human umbilical vein ECs (HUVEC) under 3–5% strain at 1 Hz increase inhibition of SMC proliferation (Baker et al., 2008) suggesting a paracrine role for the responses of ECs to mechanical stimuli.

Similarly to the effects of shear, cyclic strain induces EC morphological alignment. Typically, the actin cytoskeleton reorganizes and cells realign perpendicular to the stretch axis (Iba and Sumpio, 1991). Treatment with inhibitors of actomyosin contractility have shown that ECs subjected to cyclic strain exhibit a decrease in orientation angle (Ngu et al., 2008) with a reorientation of stress fibers parallel to the stretch direction (Kaunas et al., 2005). Both the activation of numerous focal adhesion adapter proteins (Katanosaka et al., 2008) and cell migration (Yano et al., 1996) increase. Cyclic strains modulate the endothelial ECM composition and matrix metalloproteinase (MMP) expression; see (Cummins et al., 2007) for a review. Interestingly, some of the signaling mechanisms by which ECs sense shear stress have also been implicated in the sensing of stretch (Shi et al., 2007). There are a number of excellent reviews which address these and other strain-initiated signaling responses (Kakisis et al., 2004; Pradhan and Sumpio, 2004; Cummins et al., 2007).

### 2.3. Pulsatile pressure

Pressure within the vasculature ranges from approximately 120/100 mmHg/mmHg in the aorta to 0–30 mmHg in the microcirculation (Guyton and Hall, 2000), and patients are diagnosed as borderline hypertensive at pressures of 130–149 mmHg systolic or