BIOCELL
2020 44(2): 137-145

Tech Science Press

The roles of focal adhesion and cytoskeleton systems in fluid shear stress-induced endothelial cell response

Khawar Ali SHAHZAD[1,2,#]; Zhongjie QIN[3,4,#]; Yan LI[1,2,*]; Delin XIA[3,4,*]

[1] School of Pharmacy, Taizhou Polytechnic College, Taizhou, 225300, China
[2] Bone Tissue Engineering Research Center of Taizhou, Taizhou, 225300, China
[3] Department of Oral and Maxillofacial Surgery, The Affiliated Stomatology Hospital of Southwest Medical University, Luzhou, 646000, China
[4] Orofacial Reconstruction and Regeneration Laboratory, The Affiliated Stomatology Hospital of Southwest Medical University, Luzhou, 646000, China

Key words: Cytoskeleton, Endothelial cells, Fluid shear stress, Focal adhesion

Abstract: Focal adhesions are polyproteins linked to extracellular matrix and cytoskeleton, which play an important role in the process of transforming force signals into intracellular chemical signals and subsequently triggering related physiological or pathological reactions. The cytoskeleton is a network of protein fibers in the cytoplasm, which is composed of microfilaments, microtubules, intermediate filaments, and cross-linked proteins. It is a very important structure for cells to maintain their basic morphology. This review summarizes the process of fluid shear stress transduction mediated by focal adhesion and the key role of the cytoskeleton in this process, which focuses on the focal adhesion and cytoskeleton systems. The important proteins involved in signal transduction in focal adhesion are introduced emphatically. The relationship between focal adhesion and mechanical transduction pathways are discussed. In this review, we discuss the relationship between fluid shear stress and associated diseases such as atherosclerosis, as well as its role in clinical research and drug development.

Introduction

Mechanical stimulation plays an important role in the process of cell growth and tissue remodeling. Cells are subjected to hydrostatic pressure, mechanical tension, fluid shear stress, and other stress stimuli *in vivo* (Sigaut *et al.*, 2018). Fluid shear stress (FSS) due to blood fluid flow through the canalicular spaces is regarded as the principal mechanical stimuli for vascular endothelial cells (Dewey *et al.*, 1981). The vascular endothelial cells are flat polygonal cells that constitute the inner wall of vascular blood vessels and play an important role in maintaining their integrity and normal blood flow to keep long-term patency (Yamamoto and Ando, 2015). Endothelial cells sense external mechanical stimuli through mechanoreceptors, convert mechanical signals into chemical signals by mechanotransduction, and respond to mechanical stimuli by regulating gene expression and protein synthesis (Kunnen *et al.*, 2018). The endothelial cell mechanoreceptors and mechanotransducers include focal adhesion and cytoskeleton, vascular endothelial growth factor receptor kinase (VEGFR2), G protein-coupled receptor, and ion channels (Hahn and Schwartz, 2009). Although the mechanism through which endothelial cells sense and transfer external mechanical stimuli into intracellular biochemical signals is not fully understood, more and more pieces of evidence have suggested that focal adhesions and the cytoskeleton are the main pathways (Boldock *et al.*, 2017; Gong *et al.*, 2017; Li *et al.*, 2005).

Focal adhesions (FAs) are mechanical stimulatory receptors that function as a mediator to connect the cell membrane and the cytoskeleton with the extracellular matrix, which in turn can govern stimulus responses of the cell against flow shear stress. Compared with other mechanical stimulatory receptors, FAs and the cytoskeleton are probably the primary pathways through which force signals penetrate the cell membrane (Min and Schwartz, 2019; Schwartz, 2010). The downstream signal pathway of FAs is activated when endothelial cells are exposed to FSS, and a mechanical signal is transduced into a biochemical pathway by the cytoskeleton-integrin system. The cytoskeleton is the network structure of protein fibers in eukaryotic cells that not only plays an important role in maintaining cell morphology and the orderliness of the internal structure but also participates in many important life activities (Schwartz, 2010). The vascular system of the human body is very complex. Morphology of vascular tubes is characterized as straight, curved, and bifurcated. In different parts of the body, the shape, diameter, and internal blood flow velocity of the vascular tubes change; therefore,

*Address correspondence to: Yan Li, Email: 55397507@qq.com; Delin Xia, Email: xiadelin@136.com
#These authors contributed equally to this work
Received: 31 August 2019; Accepted: 25 January 2020

fluid shear stress (FSS) of the corresponding blood flow on the vascular endothelial cells will also change. At the bifurcation of blood vessels or in the vessel valves and heart valves, the blood flow changes from stable advection to turbulence, and the corresponding FSS changes from the original laminar shear force to oscillatory shear force (Giddens *et al.*, 1993). The endothelial cells respond to different FSS, which can regulate the physiological or pathological activities of endothelial cells. It has been shown that stable laminar flow can activate endothelial cells, promote the release of NO, inhibit coagulation, prevent the formation of endothelial cell dysfunction (ECD) (Gimbrone and García-Cardeña, 2016) and atherosclerotic plaque (Chiu *et al.*, 2009), and restrain the expression of YAP (yes associated protein) (Xu *et al.*, 2016) to resist the inflammatory response. Turbulence changes the cytoskeleton and adhesion spot linked protein, which leads to the morphological changes of endothelial cells, thus destroying the integrity of endothelial cells (Gabriels and Paul, 1998), inducing endothelial cell aging (Warboys *et al.*, 2014), increasing oxygen stress (Harrison *et al.*, 2003), and promoting the permeability of lipoproteins to the vascular wall, which are related to the pathogenesis of atherosclerosis. With the further development of signal transduction in endothelial cell FAs and cytoskeleton systems, it has been found that endothelial cells can play normal physiological functions and prevent cardiovascular diseases by artificially regulating the FSS signaling pathway or providing appropriate FSS stimulation. Hence, we aimed to conduct a detailed literature review regarding the functional mechanism to understand how FSS activates the FAs signal pathways and working principle of the FAs-cytoskeleton system in FSS mechanotransduction.

Focal Adhesions (FAS)

FAs are termed as the physical connection of extracellular matrix (ECM) and cytoskeleton (CSK) generated by a multitude of protein interactions, including integrin, ECM protein, signal protein, and scaffolding protein, which are of great importance in the physiological process of cell adhesion, cycle adjustment, proliferation, and apoptosis. Integrin is a kind of trans-membrane di-polymer protein composed of α and β subunits in non-covalent form, whose extracellular section integrates with ECM protein ligands and whose intracellular section connects with the actin cytoskeleton via cytoskeletal proteins such as ankle protein, pile protein, and α actin (Baeyens *et al.*, 2016; DeMali *et al.*, 2003). When FSS acts on endothelial cells, integrins transfer mechanical stimuli to the cytoskeleton and then to distal cell membranes, intracellular matrix, and even to nuclei, which trigger a series of cascade reactions and regulate cell adhesion, proliferation, and differentiation. Similarly, endothelial cells can transfer intracellular and extracellular signals through this pathway, which can achieve bidirectional transmission of signals.

Fluid shear stress (FSS) activates the FAs signaling pathway

FSS activates the FAs signaling pathway mainly in two ways: (1) by triggering the rapid recombination of FAs and the formation and aggregates of integrins, and (2) by activating the FAs signaling proteins. FAs are not static aggregates of proteins on cell membranes; in fact, they are dynamic structures. It was found that when cells move on the matrix, they need to attach and separate the matrix coordinately in front and back of the cell, and FAs show different degrees of adhesion to the matrix (Harburger and Calderwood, 2009). Mott and Helmke (2007) found that FSS increased the formation of new FAs by inducing the expression of the integrin β subunit (Shan *et al.*, 2009). At the same time, endothelial cells can also rapidly recombine the mature FAs on the cell membrane to meet the needs of cell adhesion according to the cell density. The polarity of FSS will cause the axial alignment of endothelial cells, which stimulate the cytoskeleton to produce greater tension. Stress will transfer to the FAs along with the cytoskeleton network, leading to the conformational changes of integrin and activating the downstream signals. Albinsson and Hellstrand (2007) constructed a model to increase the mechanical load of the portal vein in mice, which showed that the FSS causes the biphasic activation of FAs (Mott and Helmke, 2007). When the endothelial cells are exposed to FSS, FAs appear by phosphorylation, and then the conformational rearrangement of integrins is carried out. The increasing expression of integrin β1 can up-regulate the activities of FAs and Rho and activate the focal adhesion system (Cui *et al.*, 2018).

FSS regulates signal protein phosphorylation in FAs and increases the activity of many protein kinases in endothelial cells. Some researchers explored human umbilical vein endothelial cells (HUVECs) for laminar FSS and found that shear stress both induced focal adhesion kinase (FAK) and phosphorylated an amino acid residue in its catalytic domain (Ngu *et al.*, 2010; Ruze *et al.*, 2018). In a study conducted by Shikata *et al.* (2005), there was found that FAK in adhesion plaques of human pulmonary endothelial cells preferentially phosphorylated at Y576, which was induced by 5–10 dyn/cm^2 FSS. A study on bovine aortic endothelial cells demonstrated that FSS increased FAs phosphorylation and enzyme activity are synergized with growth factor receptor binding protein (Grb). FSS-activated FAs may regulate the mitogen-activated protein kinase signaling pathway through the Grb2-SOS complex, mainly mediating the activation of ERK2 and junk1 (Li *et al.*, 1997). FSS can also induce the up-regulation of IL-8 and GRO-alpha by heparin sulfate and integrin β3 on the cell surface and ultimately increase the activation of FAs (Weiss *et al.*, 2017). The endothelial cells, in different tissues, express different integrin subtypes and heparin sulfate types on cell membranes, this might be the reason why the sensitivity of endothelial cells to FSS is different in different blood vessels.

It was found that both calcium-dependent and calcium-independent mechanisms mediate the dynamic interaction between integrins and proteins involved in mechanical conduction by analyzing the FSS-induced biochemical signals of endothelial cells. Calcium-independent mechanisms include FAs regulation pathway, and calcium-dependent pathways include activation of phospholipase C, hydrolysis of phosphatidylinositol 4,5-diphosphate (PIP2), intracellular calcium increase, activation of calcium-induced proteins and protein kinase C (PKC). Calcium-dependent signaling is achieved by small GTP binding proteins Rac and Rho, as well as calcium-dependent PKC and MAPK (King *et al.*, 2004; Schwartz and DeSimone, 2008). Therefore, FAs connect the

two pathways by regulating the activity of PIP2 and focal adhesion kinase. Both these molecules trigger the remodeling of cytoskeleton and induce the activation of other signals, which lead to endothelial cells FSS-induced responses.

Important proteins in FAs and intracellular signal transmission

Some proteins and signaling pathways involved in the formation of FAs play an important role in FSS-induced cell morphological changes and activation of intracellular signal transduction. Here below are given some important proteins and signaling pathways that are involved in the formation of FAs.

FSS-induced intracellular signal transduction by focal adhesion kinase (FAK)

FAK plays an important role in FAs as an FSS mechanotransducer and mechanoreceptor. Knocking down the FAK gene can result in the failure of cells to respond to FSS. FAK can simply be divided into three domains: the central catalytic domain in the middle segment, the FAT domain in the N segment, and the FERM domain in the C segment. The phosphorylation of Y576 and Y577 in the central catalytic domain is very important for the activation of FAK (Berk *et al*., 1995). The FAT domain is linked to the paxillin growth factor receptor and receptor binding protein (Grb2) in FAs and interacts with each other to regulate the function of FAs. The FREM domain is associated with the regulation of the activation of FAK and SCR. When endothelial cells are exposed to FSS and integrin binds to the corresponding proteins in ECM, FAK is induced to phosphorylate at Y397 site, then FAK mediates the activation of Scr and phosphorylates other proteins in the FAs, such as paxillin and p130 (Parsons, 2003). At the same time, the active FAK also provides binding sites for Fyn and phosphatidylinositol 3-kinase (PI-3K) and activates downstream signaling pathways through a variety of protein kinases (Frame *et al*., 2010; Scheswohl *et al*., 2008; Zebda *et al*., 2012). Different degrees and types of FSS can react to various kinds of FA responses. In short, when endothelial cells are exposed to stable laminar FSS, recombination of FAs and forming new mature FAK can be observed within 10 min. FSS induces polarization of the long axis of endothelial cells along the flow direction and even migration along the FSS direction (Yan *et al*., 2019). The turbulence can lead to irregular polarization and abnormal function of endothelial cells, including a decrease in the activity of KLF2 and nitric oxide synthase (eNOS) and release of inflammatory factors, which are important causes of atherosclerosis (Chistiakov *et al*., 2017). FAK is also involved in maintaining the integrity of vascular endothelial cells *in vivo*. A study on FAK-knockout mice showed that the integrity of endothelial cells was significantly decreased due to the absence of FAK. Further, the phosphorylation and distribution of vascular endothelial cadherin at y658 also showed abnormalities, which suggests that FAK plays an important role in maintaining the integrity of vascular endothelial cells (Zhao *et al*., 2010).

FAK activation is closely related to the assembly of FAs. The traditional "integrin aggregation" model suggests that binding of integrins to ligands will naturally lead to Tyr (Y397) autophosphorylation and activation of FAK. But some researchers have observed that the ligand linking to integrin can induce Y397 autophosphorylation only by stimulation of external traction, whereas binding of integrins and their ligands in cell suspensions can merely result in Y861 phosphorylation but not Y397 (Shi and Boettiger, 2003). FSS can bring about FAK activation. Endothelial cells from different sources can utilize the phosphorylation of tyrosine at different sites of FAK to manifest their sensitivity difference to stress stimulation (Zebda *et al*., 2012). Phosphorylated FAK can provide binding sites for a variety of proteins, such as Fyn, Src, and PI-3K, which mediate multiple kinase activation and activation of downstream signaling.

Rho protein

Under FSS stimulation, the cytoskeleton can adjust its orientation, which is characterized by cell-type specificity (Makino *et al*., 2006). Studies have demonstrated that inhibiting cytoskeletal remodeling of endothelial progenitor cells under FSS stimulation impedes their differentiation (Cui *et al*., 2012). Rho protein family significantly contributes to the rearrangement of cytoskeleton under FSS stimulation. Phosphatase in FAs has a bidirectional regulatory function on Rho activity. A study conducted by Tzima *et al*. (2001) investigated that Rho activity of endothelial cells is temporarily reduced at the same time when FSS stimulated integrins are activated, which is essential for cytoskeletal rearrangement (Tzima *et al*., 2001). The change of Rho activity might be linked to phosphatase PTPa. In another study, von Wichert *et al*. (2003) observed that when the cells were spread onto the point of FN, PTPa bound with integrin, and simultaneously RhoA activity went through two stages, i.e., temporary drop and gradual rise. Activity fluctuations are caused by integrin aggregation by inducing PTPa's action on Src and Fyn, respectively (von Wichert *et al*., 2003). Qi and colleagues have discovered that low-level shear stress in blood vessels promote the migration and apoptosis of vascular smooth muscle cells by inhibiting the segregation of Rh-GDP to restrict factor-alpha expression, which leads to atherosclerosis. Thus, abnormal FSS-induced atherosclerosis is associated with Rho activity, while normal level shear stress is crucial in maintaining vascular health (Qi *et al*., 2008).

The signaling cascade reaction of Fyn, Src, ERK, JNK and PI-3K

FSS induces ECM binding to integrins and initiates the autophosphorylation of FAK on y397. The binding of FAK is activated by autophosphorylation and activation of Src proteins. The activated Src reacts with FAK to regulate y925 phosphorylation, which provides binding sites for proteins containing SH2 and mediates downstream signaling pathways. Fyn is a member of the Src kinase family, which can reach the FAPs with the help of caveolin-1 and beta subunit integrin or through FAK. Fyn can raise Src and activate the extracellular signal-regulated kinase (ERK). If FSS is produced by turbulence, the activation of the vascular endothelial cell signaling pathway will eventually induce the production of inflammatory factors, which is the basis of atherosclerosis (Hahn and Schwartz, 2009). The activation of Src is also regulated by PECAM-1. Tzima *et al*. (2005) examined that PI-3K, AKT, and SRC were not activated in endothelial cells using PECAM-1-/-, which suggested that the activation of SRC requires PECAM-1 (Tzima *et al*., 2005). A study on bovine aortic endothelial cells (BAEC) also demonstrated that

lack of PECAM-1 inhibited the expression of COX-2 and release of PGI2, which in turn impaired the function of vascular endothelial cells and prevented the regulation of larger FSS (Russell-Puleri *et al.*, 2017). The integrity of protein tyrosine kinase (PTK) and actin skeleton also play a key role in FSS-induced activation of ERK and JNK1. The results of a study showed that genistein and cytochalasin B were used to inhibit PTK and actin microfilaments in monolayer bovine aortic endothelial cells. After 10 and 15 min of FSS stimulation, the activity of JNK1 was significantly inhibited (Shikata *et al.*, 2005; Takahashi *et al.*, 1997). FSS-activated PI-3K can mediate the activation of nitric oxide synthase (eNOS) in endothelial cells. PI-3K binding with protein tyrosine phosphatase (PTP) forms PI-3K-PTP complex and activates Grb2. The phosphorylated Grb2 induces the formation of signaling complexes including Gab1, PTP, and protein kinase A (PKA), and then mediates the phosphorylation of eNOS at Ser 1177 site. The activated eNOS releases NO, thus relaxing blood vessels and regulating FSS in the cardiovascular system (Boo *et al.*, 2002; Dixit *et al.*, 2005).

FAK autophosphorylation induces binding and activation of Src protein, in addition to protein aggregation, the activated Src can also phosphorylate other proteins such as p130cas and FAK (Bidwell and Pavalko, 2010). Phosphorylation and transformation of p130cas affect the sensitivity of FAP to stress stimuli. FAK phosphorylation at multiple sites can further enhance its activity and provide docking sites for other proteins containing SH2 domains. For example, the phosphorylation of Y925 FAK can gather growth factor receptor binding protein-2 (Grb2) to form FAK-Grb2-SOS complex and activate signal molecule ERK and C-Jun N-terminal kinase (JNK) on the MAPKs lower pathway. In osteoblasts, FSS can notably promote the formation of this compound (Surapisitchat *et al.*, 2001).

FAK activated PI-3K can activate the nuclear factors (nuclear factor, KB, NE-KB) in the cytoplasm. NE-KB is usually deactivated by the binding of JKB (IkappaB) family proteins such as IKB-α and IKB-β. Mohan reported that in FAK osteoblasts, although FSS can promote IKBα/β phosphorylation, it cannot induce its degradation; therefore, NF-KB cannot be activated (Mohan *et al.*, 1997). However, if the cells are transfected with FAK cDNA, then NF-KB can be activated. The activation of FSS-induced PI-3K does not rely on the specific extracellular matrix, but the activation of the downstream signaling protein NF-KB has a matrix specific feature (Orr *et al.*, 2006). The matrix specific activation of NF-KB is related to atherosclerosis under the action of FSS. Moreover, FSS is quite crucial in the process of integrin activation and interaction of FAK, Shc, and PI-3K, which facilitates the activation of signaling pathways (Lee *et al.*, 2010).

Cytoskeleton

The cytoskeleton (CSK) is a major component of the cell. When cells cling to the surrounding environment, they produce intracellular contractile forces to adapt to different force stimuli of the environment (Ladoux and Nicolas, 2012). The CSK is a network of protein fibers in the cytoplasm, which is composed of microfilaments, microtubules, intermediate filaments, and cross-linked proteins. It is a very important cell structure for maintaining its basic morphology. Microfilaments, myosin, and actin aggregate, forming stress fibers that transmit skeletal tension. Stress fibers also participate in activating mechanical stimulatory receptors in the cell membrane. Integrin of FAs links actin of cytoskeleton with the extracellular matrix to form a transduction system by combining CSK, ECM, and FA, which is the basic material to transduce mechanical signals of FSS into biochemical signals. The activation of the FSS signaling pathway is closely linked to the actin cytoskeleton, mainly in the following two aspects: (1) force stimuli felt by cell membrane is transmitted from FAPs to the cytoskeleton, resulting in skeletal rearrangement based on which skeletal tension occurs (Wang *et al.*, 1993), and (2) skeletal tension promotes integrin activation and enhances FAs lower signaling pathways.

FSS-induced morphological changes of endothelial cells are transduced by CSK. Cell elongation along the direction of FSS can avoid the maximum shear force and protect cells. In this process, actin microfilaments, microtubules, and intermediate fibers play an important role. The reorganization of actin microfilaments under the control of the Rho family is the basis of morphological changes (Ueki *et al.*, 2010). FSS can activate Cdc42 and Rac of the Rho family; Cdc42 can induce filamentous pseudopodia to drive cell morphological changes, and Rac can induce F-actin to produce lamellar pods that also aid to change the morphology of cell (Kohn *et al.*, 2015; Liu *et al.*, 2019; Yu *et al.*, 2018).

HUVECs were exposed to 10 dyn/cm^2 laminar flow stimulation, and monolayer endothelial cells were arranged along the laminar flow direction and elongated in different degrees within 12 h. The stress alignment along the FSS direction produced by HUVEC CSK occurs before that of the cell body by atomic force microscopy (Steward *et al.*, 2015). Shear stress-induced fibers recombine within 24 h and line up in the direction of the shear axis. Interestingly, during this process, actin rearranges only part of the stress fiber and pushes the cell membrane to elongate in the direction of the shear axis (Noria *et al.*, 2004). Malek and Izumo (1996) found that chelating intracellular calcium ions and inhibiting PTK activity could inhibit FSS-induced CSK remodeling and prevent endothelial cell alignment along FSS direction. The complete microtubule structure also affects the alignment response of endothelial cells along the direction of FSS. Microtubules (MTS) can be used as a storage and delivery system of cytokines to regulate the actin assembly and cell morphology, as well as insertion of stress fiber into the focal adhesion region to regulate the recombination and resolution of FAK (McCue *et al.*, 2004). They can also influence the recombination of actin and change the cell morphology through cross-linking with microfilaments (Wang *et al.*, 1993).

It was found that nocodazole destroyed tubulin, prevented endothelial cells from aligning along the FSS direction, and inhibited the recombination of actin stress fibers (Malek and Izumo, 1996). On the contrary, when treated with paclitaxel, the alignment and the morphological changes of endothelial cells along the shear direction were weakened, and the reorganization of actin fibers along the shear direction was inhibited but not blocked (Malek and

Izumo, 1996). The effect of paclitaxel on MTS is related to the increase of microtubules stabilized by detyrosination (Kerr *et al.*, 2015). The abundance of microtubules stabilized by detyrosination is an important factor of mechanical activation, which affects the mechanical sensitivity and response of cells to shear stimulus, rather than the density of MTS (Lyons *et al.*, 2017).

Also, MTS is an important component of primary cilia, which has been proved to be a mechanoreceptor of endothelial cells, regulating Ca^{2+} signal transduction and NO production under shear stress (Goetz *et al.*, 2014). There are relatively few studies related to the effect of FSS on intermediate fibers. It is generally believed that microtubule recombination induced by FSS affects the distribution of intermediate fibers (McCue *et al.*, 2004) and that vimentin is actively recombined in the process of endothelial cell migration. Its function may be related to transport proteins (Nieminen *et al.*, 2006). Besides, intermediate fibers also participate in the transfer of actin tension to PECAM-1 and activate the downstream signal pathway. By measuring PECAM-1 tension, it was found that the tension increased significantly from almost no tension after FSS stimulation (Conway and Schwartz, 2015). It is speculated that this phenomenon is a mechanical manifestation of stimulus signal cascade amplification, but the connection between vimentin, PECAM-1, and the control of the upstream signaling pathway are not clear.

The force stimulation of FAPs and cytoskeleton responses are up to down and complementary. Hayakawa *et al.* (2008) studied that force stimuli sensed by the top surface of FAs transfer from actin stress fibers to the basal cell surface, that act on force receptors, and stress fiber is the main intracellular stress transfer device. At the same time, the stress fiber tension is the fundamental condition for mechanical stimulus to be activated. The recombination of the stress fiber is closely related to the local adhesion. It was observed by super-resolution optical microscopy that the topmost layer of the local adhesion is VASP/Zyxin protein complex and α actin (Noria *et al.*, 2004). Zyxin can bind to the EVH1 domain of vasodilator-stimulated phosphoprotein (VASP) to regulate the recombination and extension of the stress fiber. VASP binds g-actin at an appropriate salt concentration to promote nucleation and stabilize the actin core, increasing the rate of actin polymerization (Hüttelmaier *et al.*, 1999). While, Zyxin binds to the microfilaments in combination with alpha-actin and polymerizes at the end of the stress fiber, whereas VASP mediates the incorporation of actin into the stress fiber, resulting in remodeling and elongation of the stress fiber (Ariza Jimenez *et al.*, 2019; Hüttelmaier *et al.*, 1999; Moody *et al.*, 2009). However, interestingly, interference with VASP/Zyxin complex or separating VASP from focal adhesions did not prevent FSS induced actin assembly (Malek and Izumo, 1996), but another study suggested that inhibition or knockout of VASP and Zyxin would lead to defects in the whole cell mechanics (Oldenburg *et al.*, 2015). Currently, there is no conclusion, but it is speculated that this situation may be related to vinculin (Di Cio and Gautrot, 2016).

FSS can generate integrin aggregation and rapid FAs reorganization, which promotes cytoskeleton polymerization through a variety of proteins and strengthen the skeletal tension. Skeletal tension can boost the binding site spreading of integrin-ligand, regulate integrin conformation, changing ligand binding from a low state to a high state of activity, employing inside to outside activation. Additionally, Hayakawa and colleagues have revealed that compared with separate cell membranes, cell membranes connected by FAs and actin cytoskeleton were hardened, which is associated with intracellular molecular motors like myosin, and this kind of membrane state is conducive in maintaining its sensitivity to force stimuli (DeMali *et al.*, 2003; Hayakawa *et al.*, 2008). Friedland *et al.* (2009) have stated that disruption of actin cytoskeleton or inhibition of myosin-II motor may inhibit the transition of α5βt-FN to its stress state and hinder FAK-Y397 autophosphorylation (Friedland *et al.*, 2009). Mathur *et al.* (2000) reported that FSS stimulates actin skeleton transport in cells to produce tension, which is transmitted to the basal part of cells through stress fibers. FSS-stimulated basal surface integrin's highly active subtype α5β3 content and its expression increased, while the study on HUVEC found that FSS-stimulated basal surface integrin and ligand binding increased. On one side, the reorganization of focal adhesion and the aggregation of integrins can promote the reconstitution of cytoskeleton through various kinases, on the other side, the reconstitution of cytoskeleton can also change the conformation of integrins, which can transform the low-affinity state of integrin-ligand binding to high-affinity state, and stimulate downstream signaling pathways (Schwartz, 2010; Su *et al.*, 2016). The basal activity site of integrin is higher than the lumen activity site under FSS, which may be related to the prevention of atherosclerosis by cells themselves.

Relationship of FSS with Human Disease and Clinical Drug Research

It is well known that FSS participates in the genesis of atherosclerosis. Stability of FSS and endothelial cells can maintain blood flow to the steady-state and prevent the occurrence of atherosclerosis. However, in the region of the blood vessel bifurcations and heart valves, flow disturbance of FSS affects the normal function of endothelial cells. The endothelial cells showed increased permeability and uptake of low-density lipoprotein (LDL) (Caplan and Schwartz, 1973). Subsequently, the endothelial cells make physical and chemical modifications to the captured LDL and promote a large number of LDL to infiltrate in the lower layer of endothelial cells (Ross, 1993). The LDL-activated endothelial cells also release and amplify growth factors and chemokines to recruit macrophages and promote the proliferation of smooth muscle cells and the formation of a large amount of extracellular matrix. This is the first change that can be detected in the life cycle of atherosclerotic lesions (Gimbrone and García-Cardeña, 2016; Ross, 1993; Stary, 2000; Dali *et al.*, 2019). ECD can also reduce NO release and prothrombin activity, which are both inducing factors of atherosclerosis (Tabas *et al.*, 2015). By studying the signal transduction mechanism of FSS in EC, it can help us understand atherosclerosis and provide new ideas for clinical drug research.

After the endothelial cells sense the effect of FSS, the mechanical and chemical stimulation signals are transformed into the corresponding physiological responses of the

endothelial cells. The FSS realizes the regulation of signal pathway transmission, protein phosphorylation level and the change of actin skeleton morphology of the endothelial cells, to regulate the blood flow perfusion of the tissues (Barry *et al*., 2015; Chiu and Chien, 2011). Therefore, it might be an attractive direction for clinical drug research to study the signal pathway-related functions of EC mechanical stimulation. A large number of studies have shown that integrin and its signaling pathway are important for endothelial cells to transduce FSS stimulation. Through the study of the integrin signaling pathway, it has been shown that integrin kinase (ILK) can control the activity of eNOS in endothelial cells and affect the production of NO, and finally affect the vasodilation function (Herranz *et al*., 2012; Shafiei *et al*., 2015). In this context, the FSS signaling pathway as a drug target to regulate the synthesis of NO in EC has also been proposed for the treatment of acute coronary syndrome, portal hypertension after liver injury and the prevention of cardiac ischemia (Ley *et al*., 2016; Shafiei *et al*., 2015). PI-3K/Akt signaling pathway also activates eNOS and upregulates NO synthesis in the process of EC transduction of FSS. Drug research is also in progress by using this mechanism to prevent endothelial dysfunction caused by diabetes and early atherosclerosis prevention (Bhardwaj *et al*., 2014; Tariq and Zaigham, 2019; Margaritis *et al*., 2013).

Summary and Future Prospects

In this review, we summarized the research on FSS transduction in FAs and CSK of endothelial cells and introduced the role of important proteins and signal molecules in FSS transduction in FAs. At present, many mechanisms of endothelial cell transduction of FSS are still unclear, as most of the studies on endothelial cells are limited to a single mechanical stimulus. The effects of the extracellular matrix, mechanical stimulus, and chemical signals on endothelial cells are multidimensional *in vivo*. Hence, further extensive studies are required to better understand the FSS transduction in the FAs-CSK system. Further research on endothelial cell FSS transduction will significantly improve drug research and treatment procedures in cardiovascular diseases such as atherosclerosis.

Acknowledgement: This work was supported by Grants from the Innovative Research Team of Taizhou Polytechnic College (No. TZYTD-16-4), Natural Science Research General Project of Jiangsu Higher Education Institutions (No. 18KJD350002) and the Doctoral Research Foundation of Taizhou Polytechnic College (No. 1322819004).

Funding Statement: The author(s) received no specific funding for this study.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

References

Albinsson S, Hellstrand P (2007). Integration of signal pathways for stretch-dependent growth and differentiation in vascular smooth muscle. *American Journal of Physiology–Cell Physiology* **293**: C772–C782. DOI 10.1152/ajpcell.00622.2006.

Ariza Jimenez AB, Perez Ruiz E, Caro Aguilera P, Perez Frias FJ (2019). Pulmonary hypertension in children with down syndrome without cardiopathy. *Biomedical Letters* **5**: 13–14.

Baeyens N, Bandyopadhyay C, Coon BG, Yun S, Schwartz MA (2016). Endothelial fluid shear stress sensing in vascular health and disease. *Journal of Clinical Investigation* **126**: 821–828. DOI 10.1172/JCI83083.

Barry AK, Wang N, Leckband DE (2015). Local VE-cadherin mechanotransduction triggers long-ranged remodeling of endothelial monolayers. *Journal of Cell Science* **128**: 1341–1351. DOI 10.1242/jcs.159954.

Berk BC, Corson MA, Peterson TE, Tseng H (1995). Protein kinases as mediators of fluid shear stress stimulated signal transduction in endothelial cells: a hypothesis for calcium-dependent and calcium-independent events activated by flow. *Journal of Biomechanics* **28**: 1439–1450. DOI 10.1016/0021-9290(95)00092-5.

Bhardwaj P, Khanna D, Balakumar P (2014). Catechin averts experimental diabetes mellitus-induced vascular endothelial structural and functional abnormalities. *Cardiovascular Toxicology* **14**: 41–51. DOI 10.1007/s12012-013-9226-y.

Bidwell JP, Pavalko FM (2010). Mechanosomes carry a loaded message. *Science Signaling* **3**: pe51. DOI 10.1126/scisignal.3153pe51.

Boldock L, Wittkowske C, Perrault CM (2017). Microfluidic traction force microscopy to study mechanotransduction in angiogenesis. *Microcirculation* **24**: e12361. DOI 10.1111/micc.12361.

Boo YC, Sorescu G, Boyd N, Shiojima I, Walsh K, Du J, Jo H (2002). Shear stress stimulates phosphorylation of endothelial nitric-oxide synthase at Ser1179 by Akt-independent mechanisms: role of protein kinase A. *Journal of Biological Chemistry* **277**: 3388–3396. DOI 10.1074/jbc.M108789200.

Caplan BA, Schwartz CJ (1973). Increased endothelial cell turnover in areas of *in vivo* Evans Blue uptake in the pig aorta. *Atherosclerosis* **17**: 401–417. DOI 10.1016/0021-9150(73)90031-2.

Chistiakov DA, Orekhov AN, Bobryshev YV (2017). Effects of shear stress on endothelial cells: go with the flow. *Acta Physiologica* **219**: 382–408. DOI 10.1111/apha.12725.

Chiu JJ, Chien S (2011). Effects of disturbed flow on vascular endothelium: pathophysiological basis and clinical perspectives. *Physiological Reviews* **91**: 327–387. DOI 10.1152/physrev.00047.2009.

Chiu JJ, Usami S, Chien S (2009). Vascular endothelial responses to altered shear stress: pathologic implications for atherosclerosis. *Annals of Medicine* **41**: 19–28. DOI 10.1080/07853890802186921.

Conway DE, Schwartz MA (2015). Mechanotransduction of shear stress occurs through changes in VE-cadherin and PECAM-1 tension: implications for cell migration. *Cell Adhesion & Migration* **9**: 335–339. DOI 10.4161/19336918.2014.968498.

Cui LH, Joo HJ, Kim DH, Seo HR, Kim JS, Choi SC, Huang LH, Na JE, Lim IR, Kim JH, Rhyu IJ, Hong SJ, Lee KB, Lim DS (2018). Manipulation of the response of human endothelial colony-forming cells by focal adhesion assembly using gradient nanopattern plates. *Acta Biomaterialia* **65**: 272–282. DOI 10.1016/j.actbio.2017.10.026.

Cui XD, Guan XM, Zhang XY, Hong LI, Xin LI, Wang JY, Cheng M (2012). Role of F-actin cytoskeleton in differentiation of endothelial progenitor cells induced by laminar shear stress. *Journal of Medical Biomechanics* **27**: 548–555.

Dali Y, Abbasi SM, Khan SAF, Larra SA, Rasool R, Ain QT, Jafar TH (2019). Computational drug design and exploration of potent

phytochemicals against cancer through in silico approaches. *Biomedical Letters* **5**: 21–26.

DeMali KA, Wennerberg K, Burridge K (2003). Integrin signaling to the actin cytoskeleton. *Current Opinion in Cell Biology* **15**: 572–582. DOI 10.1016/S0955-0674(03)00109-1.

Dewey CF Jr., Bussolari SR, Gimbrone MA Jr., Davies PF (1981). The dynamic response of vascular endothelial cells to fluid shear stress. *Journal of Biomechanical Engineering* **103**: 177–185. DOI 10.1115/1.3138276.

Di Cio S, Gautrot JE (2016). Cell sensing of physical properties at the nanoscale: mechanisms and control of cell adhesion and phenotype. *Acta Biomaterialia* **30**: 26–48. DOI 10.1016/j.actbio.2015.11.027.

Dixit M, Loot AE, Mohamed A, Fisslthaler B, Boulanger CM, Ceacareanu B, Hassid A, Busse R, Fleming I (2005). Gab1, SHP2, and protein kinase A are crucial for the activation of the endothelial NO synthase by fluid shear stress. *Circulation Research* **97**: 1236–1244. DOI 10.1161/01.RES.0000195611.59811.ab.

Frame MC, Patel H, Serrels B, Lietha D, Eck MJ (2010). The FERM domain: organizing the structure and function of FAK. *Nature Reviews Molecular Cell Biology* **11**: 802–814. DOI 10.1038/nrm2996.

Friedland JC, Lee MH, Boettiger D (2009). Mechanically activated integrin switch controls $\alpha_5\beta_1$ function. *Science* **323**: 642–644. DOI 10.1126/science.1168441.

Gabriels JE, Paul DL (1998). Connexin43 is highly localized to sites of disturbed flow in rat aortic endothelium but connexin37 and connexin40 are more uniformly distributed. *Circulation Research* **83**: 636–643. DOI 10.1161/01.RES.83.6.636.

Giddens DP, Zarins CK, Glagov S (1993). The role of fluid mechanics in the localization and detection of atherosclerosis. *Journal of Biomechanical Engineering* **115**: 588–594. DOI 10.1115/1.2895545.

Gimbrone MA Jr., García-Cardeña G (2016). Endothelial cell dysfunction and the pathobiology of atherosclerosis. *Circulation Research* **118**: 620–636. DOI 10.1161/CIRCRESAHA.115.306301.

Goetz JG, Steed E, Ferreira RR, Roth S, Ramspacher C, Boselli F, Charvin G, Liebling M, Wyart C, Schwab Y, Vermot J (2014). Endothelial cilia mediate low flow sensing during zebrafish vascular development. *Cell Reports* **6**: 799–808. DOI 10.1016/j.celrep.2014.01.032.

Gong X, Zhao X, Li B, Sun Y, Liu M, Huang Y, Jia X, Ji J, Fan Y (2017). Quantitative studies of endothelial cell fibronectin and filamentous actin (F-actin) coalignment in response to shear stress. *Microscopy and Microanalysis* **23**: 1013–1023. DOI 10.1017/S1431927617012454.

Hahn C, Schwartz MA (2009). Mechanotransduction in vascular physiology and atherogenesis. *Nature Reviews Molecular Cell Biology* **10**: 53–62. DOI 10.1038/nrm2596.

Harburger DS, Calderwood DA (2009). Integrin signalling at a glance. *Journal of Cell Science* **122**: 159–163. DOI 10.1242/jcs.018093.

Harrison D, Griendling KK, Landmesser U, Hornig B, Drexler H (2003). Role of oxidative stress in atherosclerosis. *American Journal of Cardiology* **91**: 7–11. DOI 10.1016/S0002-9149(02)03144-2.

Hayakawa K, Tatsumi H, Sokabe M (2008). Actin stress fibers transmit and focus force to activate mechanosensitive channels. *Journal of Cell Science* **121**: 496–503. DOI 10.1242/jcs.022053.

Herranz B, Marquez S, Guijarro B, Aracil E, Aicart-Ramos C, Rodriguez-Crespo I, Serrano I, Rodríguez-Puyol M, Zaragoza C, Saura M (2012). Integrin-linked kinase regulates vasomotor function by preventing endothelial nitric oxide synthase uncoupling: role in atherosclerosis. *Circulation Research* **110**: 439–449. DOI 10.1161/CIRCRESAHA.111.253948.

Hüttelmaier S, Harbeck B, Steffens NO, Meßerschmidt T, Illenberger S, Jockusch BM (1999). Characterization of the actin binding properties of the vasodilator-stimulated phosphoprotein VASP. *FEBS Letters* **451**: 68–74. DOI 10.1016/S0014-5793(99)00546-3.

Kerr JP, Robison P, Shi G, Bogush AI, Kempema AM, Hexum JK, Becerra N, Harki DA, Martin SS, Raiteri R, Prosser BL, Ward CW (2015). Detyrosinated microtubules modulate mechanotransduction in heart and skeletal muscle. *Nature Communications* **6**: 8526. DOI 10.1038/ncomms9526.

King J, Hamil T, Creighton J, Wu S, Bhat P, McDonald F, Stevens T (2004). Structural and functional characteristics of lung macro- and microvascular endothelial cell phenotypes. *Microvascular Research* **67**: 139–151. DOI 10.1016/j.mvr.2003.11.006.

Kohn JC, Zhou DW, Bordeleau F, Zhou AL, Mason BN, Mitchell MJ, King MR, Reinhart-King CA (2015). Cooperative effects of matrix stiffness and fluid shear stress on endothelial cell behavior. *Biophysical Journal* **108**: 471–478. DOI 10.1016/j.bpj.2014.12.023.

Kunnen SJ, Malas TB, Semeins CM, Bakker AD, Peters DJM (2018). Comprehensive transcriptome analysis of fluid shear stress altered gene expression in renal epithelial cells. *Journal of Cellular Physiology* **233**: 3615–3628. DOI 10.1002/jcp.26222.

Ladoux B, Nicolas A (2012). Physically based principles of cell adhesion mechanosensitivity in tissues. *Reports on Progress in Physics* **75**: 116601. DOI 10.1088/0034-4885/75/11/116601.

Ley K, Rivera-Nieves J, Sandborn WJ, Shattil S (2016). Integrin-based therapeutics: biological basis, clinical use and new drugs. *Nature Reviews Drug Discovery* **15**: 173–183. DOI 10.1038/nrd.2015.10.

Li S, Kim M, Hu YL, Jalali S, Schlaepfer DD, Hunter T, Chien S, Shyy JYJ (1997). Fluid shear stress activation of focal adhesion kinase. Linking to mitogen-activated protein kinases. *Journal of Biological Chemistry* **272**: 30455–30462. DOI 10.1074/jbc.272.48.30455.

Li YS, Haga JH, Chien S (2005). Molecular basis of the effects of shear stress on vascular endothelial cells. *Journal of Biomechanics* **38**: 1949–1971. DOI 10.1016/j.jbiomech.2004.09.030.

Liu L, Jiang H, Zhao W, Meng Y, Li J, Huang T, Sun J (2019). Cdc42-mediated supracellular cytoskeleton induced cancer cell migration under low shear stress. *Biochemical and Biophysical Research Communications* **519**: 134–140. DOI 10.1016/j.bbrc.2019.08.149.

Lyons JS, Joca HC, Law RA, Williams KM, Kerr JP, Shi G, Khairallah RJ, Martin SS, Konstantopoulos K, Ward CW, Stains JP (2017). Microtubules tune mechanotransduction through NOX2 and TRPV4 to decrease sclerostin abundance in osteocytes. *Science Signaling* **10**: eaan5748. DOI 10.1126/scisignal.aan5748.

Makino A, Prossnitz ER, Bünemann M, Wang JM, Yao W, Schmid-Schönbein GW (2006). G protein-coupled receptors serve as mechanosensors for fluid shear stress in neutrophils. *American Journal of Physiology–Cell Physiology* **290**: C1633–C1639. DOI 10.1152/ajpcell.00576.2005.

Malek AM, Izumo S (1996). Mechanism of endothelial cell shape change and cytoskeletal remodeling in response to fluid shear stress. *Journal of Cell Science* **109**: 713–726.

Margaritis M, Antonopoulos AS, Digby J, Lee R, Reilly S, Coutinho P, Shirodaria C, Sayeed R, Petrou M, De Silva R, Jalilzadeh S, Demosthenous M, Bakogiannis C, Tousoulis D, Stefanadis C, Choudhury RP, Casadei B, Channon KM, Antoniades C (2013). Interactions between vascular wall and perivascular adipose tissue reveal novel roles for adiponectin in the regulation of endothelial nitric oxide synthase function in human vessels. *Circulation* **127**: 2209–2221. DOI 10.1161/CIRCULATIONAHA.112.001133.

Mathur AB, Truskey GA, Reichert WM (2000). Atomic force and total internal reflection fluorescence microscopy for the study of force transmission in endothelial cells. *Biophysical Journal* **78**: 1725–1735. DOI 10.1016/S0006-3495(00)76724-5.

McCue S, Noria S, Langille BL (2004). Shear-induced reorganization of endothelial cell cytoskeleton and adhesion complexes. *Trends in Cardiovascular Medicine* **14**: 143–151. DOI 10.1016/j.tcm.2004.02.003.

Min E, Schwartz MA (2019). Translocating transcription factors in fluid shear stress-mediated vascular remodeling and disease. *Experimental Cell Research* **376**: 92–97. DOI 10.1016/j.yexcr.2019.01.005.

Mohan S, Mohan N, Sprague EA (1997). Differential activation of NF-kappa B in human aortic endothelial cells conditioned to specific flow environments. *American Journal of Physiology–Cell Physiology* **273**: C572–C578. DOI 10.1152/ajpcell.1997.273.2.C572.

Moody JD, Grange J, Ascione MP, Boothe D, Bushnell E, Hansen MD (2009). A zyxin head-tail interaction regulates zyxin-VASP complex formation. *Biochemical and Biophysical Research Communications* **378**: 625–628. DOI 10.1016/j.bbrc.2008.11.100.

Mott RE, Helmke BP (2007). Mapping the dynamics of shear stress-induced structural changes in endothelial cells. *American Journal of Physiology–Cell Physiology* **293**: C1616–C1626. DOI 10.1152/ajpcell.00457.2006.

Ngu H, Feng Y, Lu L, Oswald SJ, Longmore GD, Yin FCP (2010). Effect of focal adhesion proteins on endothelial cell adhesion, motility and orientation response to cyclic strain. *Annals of Biomedical Engineering* **38**: 208–222. DOI 10.1007/s10439-009-9826-7.

Nieminen M, Henttinen T, Merinen M, Marttila-Ichihara F, Eriksson JE, Jalkanen S (2006). Vimentin function in lymphocyte adhesion and transcellular migration. *Nature Cell Biology* **8**: 156–162. DOI 10.1038/ncb1355.

Noria S, Xu F, McCue S, Jones M, Gotlieb AI, Langille BL (2004). Assembly and reorientation of stress fibers drives morphological changes to endothelial cells exposed to shear stress. *American Journal of Pathology* **164**: 1211–1223. DOI 10.1016/S0002-9440(10)63209-9.

Oldenburg J, van der Krogt G, Twiss F, Bongaarts A, Habani Y, Slotman JA, Houtsmuller A, Huveneers S, de Rooij J (2015). VASP, zyxin and TES are tension-dependent members of Focal Adherens Junctions independent of the α-catenin-vinculin module. *Scientific Reports* **5**: 17225. DOI 10.1038/srep17225.

Orr AW, Ginsberg MH, Shattil SJ, Deckmyn H, Schwartz MA (2006). Matrix-specific suppression of integrin activation in shear stress signaling. *Molecular Biology of the Cell* **17**: 4593–4935. DOI 10.1091/mbc.e06-04-0289.

Parsons JT (2003). Focal adhesion kinase: the first ten years. *Journal of Cell Science* **116**: 1409–1416. DOI 10.1242/jcs.00373.

Qi YX, Qu MJ, Long DK, Liu B, Yao QP, Chien S, Jiang ZL (2008). Rho-GDP dissociation inhibitor alpha downregulated by low shear stress promotes vascular smooth muscle cell migration and apoptosis: a proteomic analysis. *Cardiovascular Research* **80**: 114–122. DOI 10.1093/cvr/cvn158.

Ross R (1993). The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* **362**: 801–809. DOI 10.1038/362801a0.

Russell-Puleri S, dela Paz NG, Adams D, Chattopadhyay M, Cancel L, Ebong E, Orr AW, Frangos JA, Tarbell JM (2017). Fluid shear stress induces upregulation of COX-2 and PGI_2 release in endothelial cells via a pathway involving PECAM-1, PI3K, FAK, and p38. *American Journal of Physiology–Heart and Circulatory Physiology* **312**: H485–H500. DOI 10.1152/ajpheart.00035.2016.

Ruze A, Zhao Y, Li H, Gulireba X, Li J, Lei D, Dai H, Wu J, Zhao X, Nie Y (2018). Low shear stress upregulates the expression of fractalkine through the activation of mitogen-activated protein kinases in endothelial cells. *Blood Coagulation & Fibrinolysis* **29**: 361–368. DOI 10.1097/MBC.0000000000000701.

Scheswohl DM, Harrell JR, Rajfur Z, Gao G, Campbell SL, Schaller MD (2008). Multiple paxillin binding sites regulate FAK function. *Journal of Molecular Signaling* **3**: 1. DOI 10.1186/1750-2187-3-1.

Schwartz MA (2010). Integrins and extracellular matrix in mechanotransduction. *Cold Spring Harbor Perspectives in Biology* **2**: a005066. DOI 10.1101/cshperspect.a005066.

Schwartz MA, DeSimone DW (2008). Cell adhesion receptors in mechanotransduction. *Current Opinion in Cell Biology* **20**: 551–556. DOI 10.1016/j.ceb.2008.05.005.

Shafiei MS, Lui S, Rockey DC (2015). Integrin-linked kinase regulates endothelial cell nitric oxide synthase expression in hepatic sinusoidal endothelial cells. *Liver International* **35**: 1213–1221. DOI 10.1111/liv.12606.

Shan Y, Yu L, Li Y, Pan Y, Zhang Q, Wang F, Chen J, Zhu X, Yamada KM (2009). Nudel and FAK as antagonizing strength modulators of nascent adhesions through paxillin. *PLoS Biology* **7**: e1000116. DOI 10.1371/journal.pbio.1000116.

Shi Q, Boettiger D (2003). A novel mode for integrin-mediated signaling: tethering is required for phosphorylation of FAK Y397. *Molecular Biology of the Cell* **14**: 4306–4315. DOI 10.1091/mbc.e03-01-0046.

Shikata Y, Rios A, Kawkitinarong K, DePaola N, Garcia JGN, Birukov KG (2005). Differential effects of shear stress and cyclic stretch on focal adhesion remodeling, site-specific FAK phosphorylation, and small GTPases in human lung endothelial cells. *Experimental Cell Research* **304**: 40–49. DOI 10.1016/j.yexcr.2004.11.001.

Sigaut L, von Bilderling C, Bianchi M, Burdisso JE, Gastaldi L, Pietrasanta LI (2018). Live cell imaging reveals focal adhesions mechanoresponses in mammary epithelial cells under sustained equibiaxial stress. *Scientific Reports* **8**: 9788. DOI 10.1038/s41598-018-27948-3.

Stary HC (2000). Natural history and histological classification of atherosclerotic lesions: an update. *Arteriosclerosis, Thrombosis, and Vascular Biology* **20**: 1177–1178. DOI 10.1161/01.ATV.20.5.1177.

Steward R Jr., Tambe D, Hardin CC, Krishnan R, Fredberg JJ (2015). Fluid shear, intercellular stress, and endothelial cell alignment. *American Journal of Physiology–Cell Physiology* **308**: C657–C664. DOI 10.1152/ajpcell.00363.2014.

Su Y, Xia W, Li J, Walz T, Humphries MJ, Vestweber D, Cabañas C, Lu C, Springer TA (2016). Relating conformation to function

in integrin α5β1. *Proceedings of the National Academy of Sciences of the United States of America* **113**: E3872–E3881. DOI 10.1073/pnas.1605074113.

Surapisitchat J, Hoefen RJ, Pi X, Yoshizumi M, Yan C, Berk BC (2001). Fluid shear stress inhibits TNF-α activation of JNK but not ERK1/2 or p38 in human umbilical vein endothelial cells: inhibitory crosstalk among MAPK family members. *Proceedings of the National Academy of Sciences of the United States of America* **98**: 6476–6481. DOI 10.1073/pnas.101134098.

Tabas I, García-Cardeña G, Owens GK (2015). Recent insights into the cellular biology of atherosclerosis. *Journal of Cell Biology* **209**: 13–22. DOI 10.1083/jcb.201412052.

Takahashi M, Ishida T, Traub O, Corson MA, Berk BC (1997). Mechanotransduction in endothelial cells: temporal signaling events in response to shear stress. *Journal of Vascular Research* **34**: 212–219. DOI 10.1159/000159225.

Tariq H, Zaigham K (2019). Genetic contribution of GJB2 gene and DFNB2 locus to hearing impairment in Kashmiri and Pakistani families. *Biomedical Letters* **5**: 53–65.

Tzima E, del Pozo MA, Shattil SJ, Chien S, Schwartz MA (2001). Activation of integrins in endothelial cells by fluid shear stress mediates Rho-dependent cytoskeletal alignment. *EMBO Journal* **20**: 4639–4647. DOI 10.1093/emboj/20.17.4639.

Tzima E, Irani-Tehrani M, Kiosses WB, Dejana E, Schultz DA, Engelhardt B, Cao G, DeLisser H, Schwartz MA (2005). A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. *Nature* **437**: 426–431. DOI 10.1038/nature03952.

Ueki Y, Sakamoto N, Sato M (2010). Direct measurement of shear strain in adherent vascular endothelial cells exposed to fluid shear stress. *Biochemical and Biophysical Research Communications* **394**: 94–99. DOI 10.1016/j.bbrc.2010.02.115.

von Wichert G, Jiang G, Kostic A, De Vos K, Sap J, Sheetz MP (2003). RPTP-α acts as a transducer of mechanical force on α_v/β_3-integrin-cytoskeleton linkages. *Journal of Cell Biology* **161**: 143–153. DOI 10.1083/jcb.200211061.

Wang N, Butler JP, Ingber DE (1993). Mechanotransduction across the cell surface and through the cytoskeleton. *Science* **260**: 1124–1127. DOI 10.1126/science.7684161.

Warboys CM, de Luca A, Amini N, Luong L, Duckles H, Hsiao S, White A, Biswas S, Khamis R, Chong CK, Cheung WM, Sherwin SJ, Bennet MR, Gil J, Mason JC, Haskard DO, Evans PC (2014). Disturbed flow promotes endothelial senescence via a p53-dependent pathway. *Arteriosclerosis, Thrombosis, and Vascular Biology* **34**: 985–995. DOI 10.1161/ATVBAHA.114.303415.

Weiss D, Avraham S, Guttlieb R, Gasner L, Lotman A, Rotman OM, Einav S, Vinci MC (2017). Mechanical compression effects on the secretion of vWF and IL-8 by cultured human vein endothelium. *PLoS One* **12**: e0169752. DOI 10.1371/journal.pone.0169752.

Xu S, Koroleva M, Yin M, Jin ZG (2016). Atheroprotective laminar flow inhibits Hippo pathway effector YAP in endothelial cells. *Translational Research* **176**: 18–28.e2. DOI 10.1016/j.trsl.2016.05.003.

Yamamoto K, Ando J (2015). Vascular endothelial cell membranes differentiate between stretch and shear stress through transitions in their lipid phases. *American Journal of Physiology–Heart and Circulatory Physiology* **309**: H1178–H1185. DOI 10.1152/ajpheart.00241.2015.

Yan Z, Su G, Gao W, He J, Shen Y, Zeng Y, Liu X (2019). Fluid shear stress induces cell migration and invasion via activating autophagy in HepG2 cells. *Cell Adhesion & Migration* **13**: 152–163. DOI 10.1080/19336918.2019.1568141.

Yu H, Shen Y, Jin J, Zhang Y, Feng T, Liu X (2018). Fluid shear stress regulates HepG2 cell migration though time-dependent integrin signaling cascade. *Cell Adhesion & Migration* **12**: 56–68. DOI 10.1080/19336918.2017.1319042.

Zebda N, Dubrovskyi O, Birukov KG (2012). Focal adhesion kinase regulation of mechanotransduction and its impact on endothelial cell functions. *Microvascular Research* **83**: 71–81. DOI 10.1016/j.mvr.2011.06.007.

Zhao X, Peng X, Sun S, Park AY, Guan JL (2010). Role of kinase-independent and -dependent functions of FAK in endothelial cell survival and barrier function during embryonic development. *Journal of Cell Biology* **189**: 955–965. DOI 10.1083/jcb.200912094.