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Shear stress and the endothelium

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Shear stress and the endothelium. Vascular endothelial cells (ECs) *in vivo* are influenced by two distinct hemodynamic forces: cyclical strain due to vessel wall distention by transmural pressure, and shear stress, the frictional force generated by blood flow. Shear stress acts at the apical cell surface to deform cells in the direction of blood flow; wall distention tends to deform cells in all directions. The shear stress response differs, at least partly, from the cyclical strain response, suggesting that cytoskeletal strain alone cannot explain it. Acute shear stress *in vitro* elicits rapid cytoskeletal remodeling and activates signaling cascades in ECs, with the consequent acute release of nitric oxide and prostacyclin; activation of transcription factors nuclear factor (NF)κB, c-*fos*, c-*jun* and SP-1; and transcriptional activation of genes, including ICAM-1, MCP-1, tissue factor, platelet-derived growth factor-B (PDGF-B), transforming growth factor (TGF)-β1, cyclooxygenase-II, and endothelial nitric oxide synthase (eNOS). This response thus shares similarities with EC responses to inflammatory cytokines. In contrast, ECs adapt to chronic shear stress by structural remodeling and flattening to minimize shear stress. Such cells become very adherent to their substratum and show evidence of differentiation. Increased adhesion following chronic shear stress has been exploited to generate vascular grafts with confluent EC monolayers, retained after implantation *in vivo*, thus overcoming a major obstacle to endothelialization of vascular prostheses.

HEMODYNAMIC FORCES ACTING AT THE VESSEL WALL

Cyclical strain

Endothelial cells (ECs) *in vivo* are influenced by two key hemodynamic forces: transmural pressure gradients and wall shear stress. In blood vessels *in vivo*, these forces vary in a pulsatile fashion, except in locations where the cardiac pressure curve has been dampened by high upstream resistances. Transmural pressure gradients result in vessel wall distention and development of wall tension, as described by Laplace's law [1]:

$$T = \Delta P \times r$$

where ΔP is the transmural pressure gradient and r is the radius. Wall tension is thus directly proportional to the transmural pressure gradient and vessel diameter. The distensibility of the vessel wall is determined by its thickness, composition, and degree of smooth muscle contraction [1]. Because wall tension rises with increasing vessel diameter, for any given transmural pressure gradient, tension is greatest in vessels that are highly distensible. Tension develops in all structural components of the vessel wall, including extracellular connective tissue and the cytoskeleton of constituent cells. Tension is transmitted to the cell cytoskeleton at points of cell-cell and cell-matrix adhesion, where the cytoskeleton connects to adhesion molecules on other cells or to matrix molecules via several transmembrane proteins. In highly distensible vessels, the degree of cell cytoskeletal deformation and tension due to transmural pressure gradients can be very large.

Shear stress

Endothelial cells also are subject to the frictional force shear stress generated by blood flowing past their apical surfaces. This force is determined by the mean fluid flow rate, its viscosity, and the physical dimensions of blood vessels. For Newtonian fluids (defined as those for which flow velocity does not alter viscosity) flowing in rigid containers with unvarying internal geometry, uniform velocity gradients develop such that fluid velocity is least at the stationary vessel wall and rises with increasing distance from it. Fluid flow with this characteristic uniform velocity gradient is termed laminar flow because the fluid can be viewed as a series of molecular layers (laminae) slipping past each other with increasing velocity as the center of the container is approached (Fig. 1). In a uniform, rigid cylinder, shear stress (τ) at the vessel wall can be derived from Poiseuille's law [1] as:

$$\tau = 4Q \times \eta/\Pi \times r^3$$

where Q is the fluid flow rate, and η is the fluid viscosity. If mean flow rate is constant, the greater the resistance to flow, either because of high fluid viscosity or small vessel diameter, the greater the shear stress. Shear stress causes cell deformation (strain), which raises cytoskeletal tension, although the direction of the deformation differs from that

Key words: vascular prosthesis, intimal hyperplasia, differentiation, strain, nitric oxide, transcription factors, Ca^{2+}, mechanical-chemical coupling, diacylglycerol, phospholipase C, protein kinase C, mitogen-activated protein kinase, stress-activated protein kinase, nuclear factor κ B, platelet-derived growth factor B.

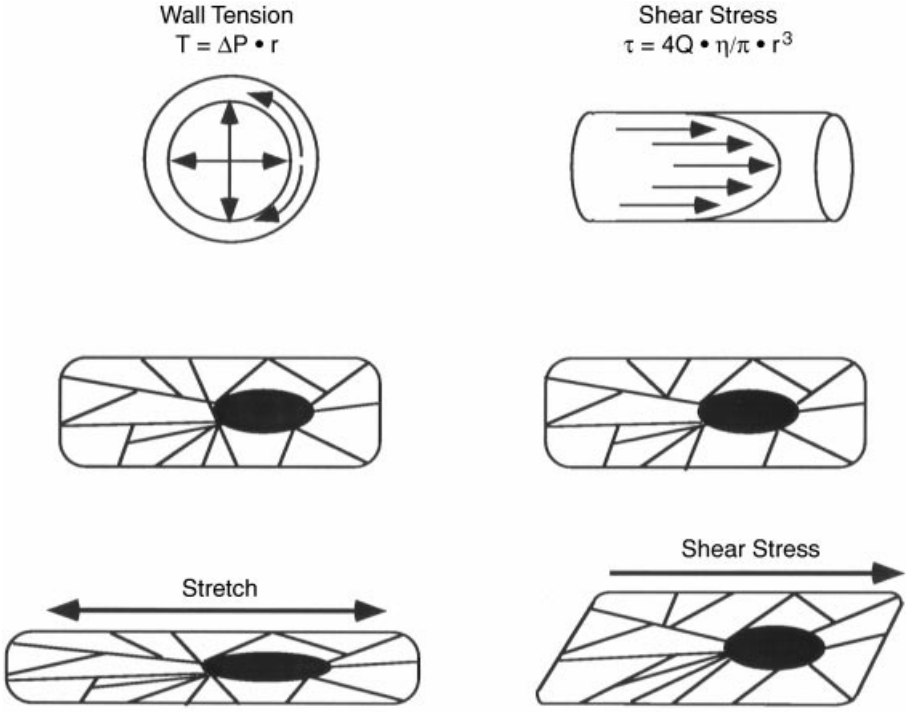

Fig. 1. Schematic representation of the effects of vessel wall tension and shear stress on endothelial cells. Wall tension develops in response to transmural pressure gradients and causes cell stretch, with cell deformation in all directions. Shear stress is the fluid frictional force acting at the apical surface of endothelial cells. Shear stress results in unidirectional cell deformation.

produced by transmural pressure gradients (Fig. 1). In the purest sense, therefore, shear stress and transmural pressure gradients are unrelated forces, each of which cause EC strain. In both cases, there is deformation of cytoskeletal elements, cell membranes, and sites of cell-cell and cell-matrix adhesion with consequent tension development. However, in the case of shear stress, the force on a given cell acts in one direction, whereas stretch due to transmural pressure gradients occurs in all directions. It is also of note that only the ECs, but not vascular smooth muscle cells or pericytes, are exposed to shear stress, whereas all vessel wall components are deformed by transmural pressure gradients.

In vivo shear stress

Biological systems depart from the defined conditions mentioned earlier here, as blood is non-Newtonian; that is, blood viscosity tends to decrease with increasing velocity, and vessels are nonuniform, variably distensible containers [1]. At very low blood velocities, aggregation of cellular elements tends to raise blood viscosity markedly, whereas its viscosity at high velocities is only about fourfold greater than that of water [2]. Although blood flow tends to be laminar in vessels > 0.5 mm in diameter, it departs somewhat from this behavior in very small vessels such as glomerular capillaries. In addition, shear stress is influenced by transmural pressure gradients because vessel distention tends to increase vessel diameter, tending to lower shear stress. This effect of vessel distention on shear stress can be significant, as shear stress is inversely proportional to the third power of the vessel radius. Variations in vessel wall folds and cell structure additionally create variations of shear stress at different points within the same vessel [3] and even on the same cell [4]. Estimates of wall shear stress at various locations in the circulation have been made [5–7]; some have taken circulating cellular elements and vessel structure and distensibility into account. Mean shear stress is lowest in the large veins where it often is $<$ 1 dyne/cm^2. It tends to be highest in small arterioles, where it can reach 60 to 80 dynes/cm^2. Of note, mean shear stress in small venules also tends to be high (20 to 40 dyne/cm^2) given the high flow rates and small diameters of these vessels [8]. Studies of the carotid bifurcation in humans show that the curvature and configuration of the vessel can dramatically affect wall shear stress, with levels ranging from $<$ 1 dyne/cm^2 to $>$ 600 dyne/cm^2 at different sites within the same vessel [9]. In glomerular capillaries, shear stress has been estimated using a computer modeling approach and ranges from approximately 1 to about 95 dyne/cm^2, with mean values in most loops of 5 to 20 dyne/cm^2 [10].

MECHANICAL-CHEMICAL COUPLING IN ENDOTHELIAL CELLS

The precise mechanisms of mechanical-chemical coupling in ECs exposed to shear stress are not fully understood. Ingber [11] has reasoned that physical forces are transmitted from the environment to the cell at points where extracellular and intracellular structural elements are interconnected and that mechanical-chemical coupling

therefore reflects adaptation to forces that alter tension in cell structural components. Indeed, he has shown that application of stress to integrins, known to be connected to the cell cytoskeleton, results in cell deformation (strain) with secondary tension development, whereas the same stress applied to cell-surface receptors not connected to the cytoskeleton does not alter cytoskeletal tension [12]. Because many signaling molecules are associated with the cytoskeleton [13], it seems plausible that strain and tension development *per se* could couple mechanical stimuli to chemical responses in cells. Focal adhesions [13], cell-cell adhesions, points of contact between subplasma membrane cytoskeleton and stretch-activated receptors, and any other structural elements capable of eliciting chemical signals within cells could participate in such responses. As is discussed later here, there is considerable evidence that signaling via integrins, which anchor cells to their substratum, and focal adhesion-associated proteins regulates a number of cell signaling cascades. Signaling via integrins may also play a role in the acute shear stress response. In addition, stretch-sensitive Ca^{2+} channels are activated by shear stress, probably as a result of their deformation by transmitted tension from the plasma membrane and subplasma membrane scaffolding proteins to the channels. The possibility that ECs might express specific mechanosensors at their apical cell surface [14] distinct from known cytoskeletal proteins, though attractive, is not supported by experimental data at this time. On the other hand, the fact that EC responses to cyclical strain and shear stress differ [15–18] suggests that the shear stress response cannot be due solely to nonspecific cytoskeletal tension development.

SHEAR STRESS RESPONSES IN ENDOTHELIAL CELLS

Effects of acute and chronic shear stress on ECs have been studied extensively *in vitro*. Acute shear stress refers to conditions in which ECs not previously accustomed to shear stress are suddenly exposed to this force with a time frame measured in seconds to hours [14]. Chronic shear stress refers to conditions in which ECs are cultured for several days to weeks under the influence of shear stress, with or without superimposed acute alterations in the level of shear stress. The chronic shear stress model more closely approximates conditions *in vivo*, where ECs are continuously exposed to shear stress with variations in the level of shear stress due to alterations in blood flow rate and vessel wall diameter. In contrast, acute shear stress conditions are only observed *in vivo* when flow is first initiated through newly formed blood vessels or when blood flow in an established vessel is reinitiated after temporary occlusion. Acute shear stress responses may also mimic, at least in part, *in vivo* responses of injured or activated endothelium to shear stress.

Ion channel activation

The most rapid responses of ECs to shear stress involve activation of at least two distinct apical ion channels. Olesen et al have reported activation of an inwardly rectifying K^+ channel within milliseconds after initiation of shear stress [19]. This K^+ channel is Ca^{2+} activated [20, 21] and also is regulated transcriptionally by acute shear stress [22]. Activation of channel activity by shear stress appears to be EC specific [19]. Acute shear stress also elicits a biphasic rise in cytosolic [Ca^{2+}]. Rapid mobilization of Ca^{2+} from intracellular stores [23] ensues upon initiation of shear stress and is then followed by a sustained increase dependent on Ca^{2+} influx via shear stress-activated channels [24–26]. With continued shear stress, Ca^{2+} oscillations [27] at discrete points within cells and not associated with a generalized increase in cytosolic [Ca^{2+}] have also been observed. These probably relate to localized Ca^{2+} channel activation. Evidence obtained so far is in keeping with the hypothesis that cell strain directly activates stretch-responsive Ca^{2+} channels, with secondary activation of the inward rectifying K^+ channel, and consequent cell hyperpolarization [28].

Intracellular [Ca^{2+}] mobilization

The initiation of shear stress also leads to rapid generation of inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) from membrane phosphatidylinositol [29, 30]. Currently, the mechanism stimulating phospholipase C (PLC) and the isoform of PLC activated in response to shear stress are unclear. Nevertheless, rapid generation of IP3 in response to shear stress initiation is linked to intracellular [Ca^{2+}] mobilization [23], and DAG production is linked to protein kinase C (PKC) activation [31]. In addition, phospholipase A2 (PLA2) is activated in response to shear stress, with the release of arachidonic acid from membrane phospholipids [30]. To investigate the potential role of intracellular [Ca^{2+}] mobilization in shear stress-mediated signaling, the effects of chemical agonists and shear stress on nitric oxide (NO) and prostacyclin production have been compared. Ca^{2+}-mobilizing agonists stimulate PLC and PLA2 activity in ECs, with consequent IP3, DAG, and arachidonic acid release, and production of the two most well-described endothelium-derived vasodilators: NO and prostacyclin. Like these chemical agonists, shear stress stimulates acute NO [32] and prostacyclin [33, 34] secretion by ECs. However, thapsigargin, an inhibitor of Ca^{2+} adenosine triphosphatase, which depletes intracellular [Ca^{2+}] stores, profoundly inhibits agonist-, but not shear stress-stimulated NO and prostacyclin secretion [35]. Hence, even though chemical agonists and shear stress similarly mobilize intracellular [Ca^{2+}] and stimulate NO and prostacyclin release, such findings strongly suggest that acute NO and prostacyclin release in response to shear stress are not due

to mobilization of Ca^{2+} from intracellular stores. Nevertheless, because shear stress-induced mediator release is inhibited by chelators of extracellular [Ca^{2+}] [26, 36, 37], it seems that NO and prostacyclin release are dependent, at least in part, on Ca^{2+} influx. In addition, a role for tyrosine phosphorylation of EC NO synthase and a consequent increase in NO synthesis has been described [38, 39].

Reorganization of the actin cytoskeleton

Upon acute shear stress exposure, rapid reorganization of the EC cytoskeleton takes place so that the cells align in the direction of flow [40, 41]. There also is a change in integrin distribution [42] with a consequent increase in focal adhesion contacts at the upstream ends of cells [43]. The distribution of shear stress itself varies at different points of the cell surface [4], with the greatest force upstream of the nuclear prominence. It has been suggested that redistribution of integrins and focal contacts serves to strengthen EC adhesion at the points of greatest stress [40]. Reorganization of cytoskeletal components requires initial actin depolymerization and then repolymerization, events observed immediately after application of shear stress [43, 44]. Actin depolymerization and cytoskeletal reorganization in response to shear stress are inhibited by chelators of extracellular and intracellular [Ca^{2+}] and by tyrosine kinase inhibitors, suggesting a role for intracellular [Ca^{2+}] and integrin-mediated signaling in this process [45, 46]. Of interest, actin depolymerization by shear stress has been linked causally to the initial increase in endothelin (ET)-1 gene transcription observed upon initiation of acute shear stress [44], suggesting that the depolymerization event itself can also initiate signaling responses. However, the precise mechanism whereby actin depolymerization might activate ET-1 promoter activity remains elusive.

Integrin-mediated signaling and mitogen-activated protein kinase (MAPK) and stress-activated protein kinase (SAPK) activation

The possibility that shear stress signals are transmitted via the integrin-focal adhesion kinase (FAK) signaling cascade has been examined recently. The central role of FAK in transmitting responses to integrin clustering by extracellular ligand or cell attachment is well supported by experimental data [47]. Integrin engagement leads to rapid autophosphorylation of FAK [48] and phosphorylation of the FAK-associated protein paxillin. Recruitment and activation of Src (and Src family members) to focal adhesions, with secondary Src-mediated hyperphosphorylation of FAK on several tyrosine residues follows. Consequent association of the adaptor GRB2 with FAK provides a potential link to the MAPK cascade via $p21^{ras}$ activation [47, 49], although integrins may also signal MAPK activation independently of FAK [50]. Two other proteins, paxillin and Cas, become phosphorylated by association with FAK/Src [51, 52]. Both bind Crk-family adaptor proteins, which in turn regulate cytoskeletal integrity through monomeric G proteins such as Rap1, RhoA, CDC42, and Graf [53] and phosphatidyl-inositol-3-kinase and -4-phosphate-5-OH kinase [47, 54]. Rho and CDC42 also provide a link to N-terminal Jun kinase (JNK) and c-*jun* activation, referred to as the SAPK cascade [55].

Exposure of ECs to acute shear stress activates the downstream effectors of the MAPK family cascade ERK1 and ERK2 [56, 57]. Activation of the MAPK cascade by acute shear stress is dependent on PKC, but not on mobilization of intracellular [Ca^{2+}] [58], and involves heterotrimeric G proteins [57]. Furthermore, the findings that acute shear stress stimulates β1-integrin—dependent FAK phosphorylation and MAPK activity in ECs [59] are in keeping with a role for the focal adhesion signaling complex in initiating the shear stress response. In addition to enhanced ERK1/2 activity, JNK activity also is stimulated by acute shear stress, although with a different time course. ERK activation is rapid and transient, whereas JNK activation is more sustained [57]. These findings are consistent with activation of both MAPK and SAPK cascades in response to acute shear stress. The ERK1/2 activation in response to acute shear stress appears to be sensitive to inhibition of both PKC and tyrosine kinase, but it is only partially dependent on intact actin filaments [56]. Furthermore, whereas the time-course of shear-stress stimulated c-Src and ERK1/2 activation in response to acute shear stress is similar, FAK phosphorylation in shear stress-treated cells is much more sustained [56, 60]. Taken together with previous reports that MAPK activation by integrin engagement may be independent of FAK, these findings suggest that c-Src/MAPK activation by shear stress is a rapid and transient response that might not require FAK. Clearly, more work is required to unravel the mechanisms whereby acute shear stress activates integrin-dependent tyrosine phosphorylation cascades in ECs.

Activation of nuclear factor kappa B (NF-κB)

Acute shear stress in the venous range activates NF-κB-IκB signal system with consequent translocation of NF-κB to the nucleus and transcriptional activation of some, but not all, NF-κB-responsive promoters. The mechanism whereby acute shear stress leads to NF-κB activation has not been elucidated. Of note, NF-κB activation is greatest and most sustained in aortic cells exposed to venous levels of shear stress, whereas NF-κB activation is only briefly stimulated in the same cells exposed to arterial levels of shear stress [61]. A six-nucleotide promoter element, which has been named the shear stress response element and is required for activation of the platelet-derived growth factor (PDGF)-B gene by acute shear stress, binds NF-κB [62, 63]. The shear stress response element has been identified also in the transforming growth factor (TGF)-β1 and t-PA genes [62], but the role of this and NF-κB in regulating transcription of these genes has not been established. The

switch from no flow to shear stress activates transcription of a number of other genes, among them cyclooxygenase-II, endothelial nitric oxide synthase (eNOS), and manganese superoxide dismutase [36, 64], tissue factor [65], C-type natriuretic peptide [66, 67] as well as TGF-β1 [68]. Induction of TGF-β1 expression is dependent on K^+ channel activation [68]. Acute shear stress also stimulates expression of several early response genes in ECs, including c-*fos* and c-*jun* [69, 70]. As ERKs are upstream regulators of c-*fos*, JNK phosphorylates c-*jun* and c-*fos*/c-*jun* complexes activate AP-1 promoter sites, it is evident that shear stress activates transcription of some genes, including MCP-1 [71] and probably also eNOS [64] through this mechanism. However, whether cytoskeletal strain and signaling via integrins are central to the activation of these cascades remains to be firmly established.

EFFECTS OF CHRONIC SHEAR STRESS

The rapid activation of ion channels, cytoskeletal remodeling, and gene transcription in response to acute shear stress is followed by chronic adaptation of ECs to shear stress. Cytoskeletal remodeling of ECs to chronic shear stress leads to alignment in the direction of flow and rearrangement of actin microfilaments so that stress fibers are observed principally in subplasma membrane locations at the periphery of cells [41, 72, 73]. Furthermore, cells become much flatter than those in static culture, such that the maximal shear stress force, usually at the nuclear prominence, is minimized [74]. Cell realignment and flattening are sustained with prolonged shear stress and mimic the EC response to chronic shear stress *in vivo* [75]. Thus, just as aerodynamic design reduces wind resistance for human-made vehicles, ECs appear to reorganize structurally with time to minimize the force of shear stress.

With time under shear stress, ECs also display ultrastructural features of increased differentiation compared with proliferating cells in culture [76]. In response to chronic shear stress, cells hypertrophy, the density of actin microfilaments and focal adhesions increases, and the abundance of focal adhesion-associated proteins is greater than in cells not exposed to shear stress [60, 76]. In renal microvascular ECs exposed to chronic shear stress, there also is a dramatic increase in the density of clathrin-coated pits and vesicles as well as endocytic vesicles and possibly calveoli, in keeping with the ultrastructural appearance of microvascular ECs *in vivo* [76, 77]. Finally, in aortic ECs, a nearly 40-fold greater density of Weibel-Palade bodies, the EC-specific, electron-dense granules storing von Willebrand factor and P-selectin [78], occurs in cells exposed to chronic arterial shear stress, compared with those in conventional static culture [76]. The ultrastructural changes in aortic and renal microvascular ECs cultured in the presence of chronic shear stress suggest that the cells achieve a greater degree of differentiation with chronic shear stress exposure than that observed in cells cultured without shear stress [76] and raise the intriguing possibility that shear stress is one of the stimuli promoting differentiation of ECs *in vivo*.

In addition to structural reorganization, shear stress also stimulates cell adhesion to the substratum. ECs cultured for nine days under arterial levels of shear stress (15 dyne/cm^2) adhere much more firmly than cells cultured for the same length of time and under otherwise identical conditions, with 1 dyne/cm^2 shear stress [60]. Enhanced cell adhesion is associated with greater tyrosine phosphorylation of both FAK and paxillin and is reversed by tyrosine kinase inhibitors. Interestingly, acute changes in the level of shear stress are not associated with alterations in FAK and paxillin phosphorylation in cells adapted to chronic shear stress. These findings are in keeping with sustained (24-hour) FAK phosphorylation observed by Takahashi and Berk [56] with arterial shear stress exposure and argue that FAK and paxillin phosphorylation play a role in maintaining EC adhesive strength upon adaptation to shear stress, but not in cellular signaling responses to acute changes in the level of shear stress once adaptation to chronic shear stress has taken place [60].

Chronic shear stress exposure also alters expression of EC mediators, but the pattern of mediator expression and release differs from that observed in response to acute shear stress. Although acute shear stress can activate ET-1 gene transcription [44], a process related to acute cytoskeletal remodeling, sustained shear stress results in a profound reduction of ET-1 gene expression [79, 80]. Induction of ET-1 transcription by ECs is also observed in response to cyclical strain [81], an *in vitro* model of vessel distention, thus mimicking the acute, but not the chronic shear stress response. The effect of chronic shear stress on ET-1 down-regulation is not explained by generation of cAMP, a second messenger induced by shear stress, but seems to require a regulatory site in the 5′ flanking region of the ET-1 gene, some 2.5- to 2.9-kb upstream from the transcription initiation site [79]. Transcriptional regulation of ET-1 by NO has been described [82], and shear stress-induced transcriptional repression is relieved by NO synthase inhibitors in ECs, suggesting that enhanced NO generation stimulated by shear stress may reduce ET-1 expression in an autoregulatory fashion [83]. A potential link between NO synthesis and repressor function of the far upstream 5′ regulatory element in the ET-1 gene has, however, not been established. It is of note that differentiated, quiescent ECs *in vivo* similarly repress ET-1 transcription.

Chronic adaptation to shear stress also results in profound down-regulation of PDGF-B message levels [84], whereas PDGF-B transcription is up-regulated by acute shear stress [62]. However, unlike ET-1 expression, PDGF-B transcription is repressed further in cells accustomed to chronic shear stress at 1 dyne/cm^2 when the level of shear stress is raised to 15 dyne/cm^2 [84]. Whether this process is related to reduced NF-κB activation in cells

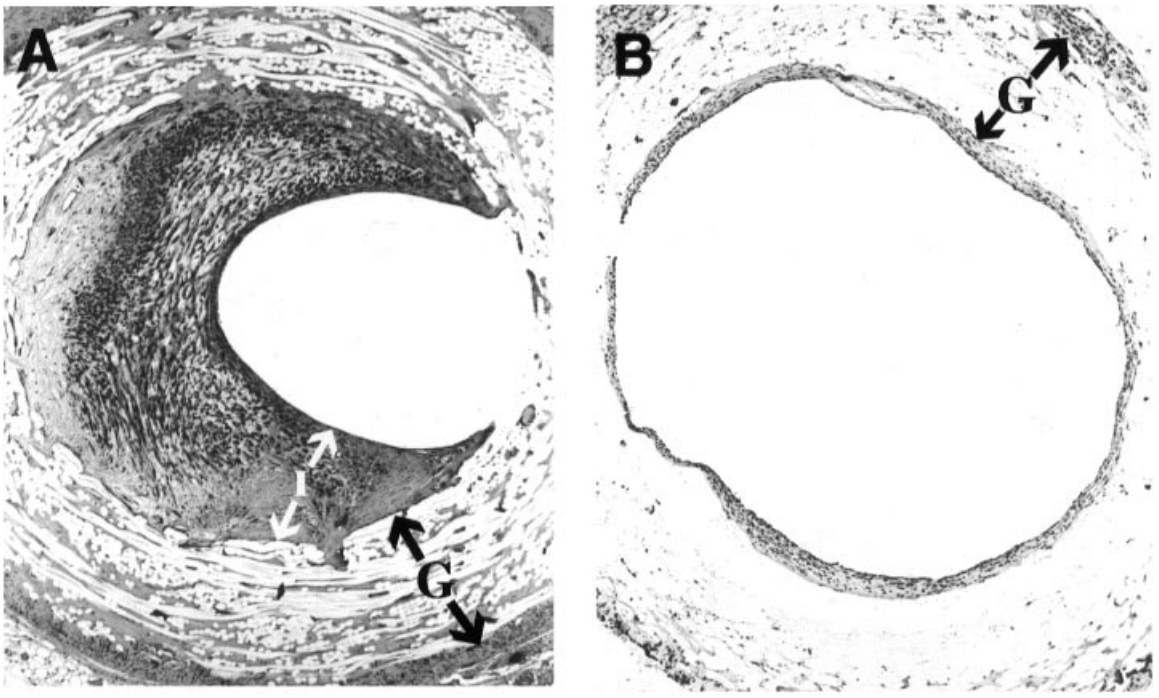

Fig. 2. Inhibition of neointimal formation on prosthetic vascular grafts by endothelial cells. Polyurethane vascular grafts (kindly provided by Corvita Co.) without endothelial cells (*A*) or with shear stress-pretreated, tightly adherent endothelial cells (*B*) were implanted in rats. The degree of neointimal hyperplasia was examined three months later in graft cross-sections. Polyurethane graft material is indicated by (G). Intima is indicated by (I). Grafts bearing shear stress-pretreated endothelial cell monolayers displayed much less graft neointimal hyperplasia at three months than grafts without endothelial cells.

accustomed to shear stress or other as yet poorly understood mechanisms remains to be determined. A possible explanation might be that the force acting on ECs already accustomed to shear stress is less than that acting on cells before remodeling and flattening has occurred [74]. *In vivo*, PDGF-B expression by ECs is normally repressed and is stimulated when there is only mechanical injury or activation of endothelium by inflammatory cytokines [85, 86]. Thus, findings in ECs exposed to acute shear stress cannot be extrapolated simply to chronic shear stress conditions, and cells accustomed to chronic shear stress may respond differently to acute alterations in the level of this force than cells not adapted to shear stress. Differences in these responses may help to unravel the mechanisms whereby shear stress elicits effects in ECs. It is also possible that acute shear stress responses in conventional culture may resemble *in vivo* responses of activated, proliferating endothelium, whereas responses observed in cells adapted to chronic shear stress may more closely reflect those of quiescent, differentiated endothelium.

FULLY ENDOTHELIALIZED VASCULAR GRAFTS ESTABLISHED BY SHEAR STRESS PRETREATMENT

The effect of chronic shear stress to enhance EC adhesive strength has been exploited to generate EC-lined vascular grafts for implantation *in vivo* [60, 87, 88]. To line intravascular devices with ECs has long been an objective in vascular surgery, because ECs serve anticoagulant and antiproliferative functions *in vivo* [89, 90]. Endothelialization, therefore, should reduce thrombosis and neointima formation on prosthetic vascular devices. However, this goal has been elusive because ECs implanted on seeded prosthetic grafts tend to detach rapidly when exposed to the circulation *in vivo* [91]. We reasoned that shear stress pretreatment, which stimulates cell adhesion, might prevent EC detachment *in vivo*. When woven polyurethane vascular grafts seeded with ECs are exposed to chronic (6-day) shear stress at arterial levels, cells were retained on exposure to a much larger shear force *in vitro*, whereas cells not pretreated with shear stress detached [88]. The grafts on which ECs were retained because of prior shear stress exposure also inhibited fibrin clot formation significantly better than grafts not pretreated with shear stress. Such EC-seeded, shear stress-pretreated, polyurethane vascular grafts have been implanted recently *in vivo* and show better than 98% cell retention if the seeded monolayers are treated with arterial (25 dyne/cm^2) shear stress for six days *in vitro* prior to implantation. In contrast, only about 50% cell retention is found when cells are pretreated for the same time with only 1 dyne/cm^2 shear stress [60]. Thus, the level of *in vitro* shear stress to which ECs are exposed prior to implantation strongly influences cell retention on the grafts *in vivo*. Grafts without ECs developed a thick intimal

layer composed of smooth muscle cells and luminal endothelium. This intima was, on average, 3.6-fold thicker in nonendothelialized grafts than that in grafts on which adhesive EC monolayers had been established by shear stress pretreatment (Fig. 2). Hence, the importance of ECs in inhibiting formation of intimal smooth muscle on vascular grafts has been established. The use of shear stress to endothelialize vascular grafts for *in vivo* use potentially could be applied to small-vessel bypass procedures and to prolong survival of dialysis access grafts in hemodialysis patients. Endothelialized grafts also express the *LacZ* gene *in vivo* after transduction of shear stress-pretreated ECs with an adenovirus-LacZ vector *in vitro*, suggesting that grafts with ECs made adherent by shear stress may be useful as a tool for *in vivo* gene delivery [60].

CONCLUSION

Much experimental information suggests that EC responses to acute shear stress resemble those observed during EC injury or immune activation *in vivo*. The precise mechanism of mechanical-chemical coupling has not been established, though experimental data indicate that transmission of force at points at which the cell cytoskeleton and extracellular structures interconnect is necessary to elicit shear stress responses. However, because the effects of cyclical stretch and shear stress differ in ECs, a specific shear stress sensor is still being sought. The effects of chronic shear stress on ECs and differences in responses by ECs from distinct vascular beds have received attention only recently. This area of investigation should prove of great interest, as adaptation of ECs to chronic shear stress appears to be accompanied by EC differentiation and reduced expression of injury-associated genes.

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APPENDIX

Abbreviations used in this article are: DAG, diacylglycerol; ECs, endothelial cells; eNOS, endothelial nitric oxide synthase; ET, endothelin; FAK, focal adhesion kinase; IP3, inositol 1,4,5-trisphosphate; JNK, N-terminal Jun kinase; MAPK, mitogen-activated protein kinase; NFκB, nuclear factor κ B; NO, nitric oxide; PDGF-B, platelet-derived growth factor-B; PKC, protein kinase C; PLA2, phospholipase A2; PLC, phospholipase C; SAPK, stress-activated protein kinase; TGF, transforming growth factor.

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