Tissue Engineering

Effects of Fluid Flow on Cells

Cecil D. Murray, who was a Professor of Biology at Bryn Mawr College, proposed in 1926 that the geometry of the vascular tree should be such that it minimizes the energy cost of pumping the fluid and the volume of the total system. Under conditions of laminar flow, this will be achieved if at any branch point the radius of the parent vessel cubed equals the sum of the cubes of the radii of the daughter vessels. Tests of Murray's law have been carried out in various tissues and in various species, with surprisingly little deviation from the expected exponent value of 3.

A consequence of Murray's law is that the average wall shear stress (the drag force of the fluid on the walls of the vessel) is nearly constant throughout the vascular tree. Moreover, blood vessels exposed to surgically induced chronic increases in blood flow will increase in diameter, maintaining the same shear stress. Acute increases in blood flow also cause vessel dilation, which is reversible upon return to normal flow.

Increasing the viscosity of the fluid moving at a constant flow rate through arterial segments in vitro also causes vessel dilation, suggesting that it is the wall shear stress and not the wall shear rate (the velocity gradient at the surface) to which the vessel responds. It has also been found that an intact vascular endothelium is necessary in order to observe both the acute and chronic flow-induced changes in vessel size.

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How flow causes a physiological response is under active investigation. Endothelial cells in culture can be subjected to fluid shear stress under well-defined conditions and in the absence of other mechanical and biological confounding factors such as stretch or local soluble mediators that may affect the cellular response.

Experimental systems used for this purpose generally consist of an endothelial monolayer cultured to confluence on a surface inside a narrow channel or gap through which a simple physiological fluid is moving. Because wall shear stress is difficult to measure directly, the geometry of the flow channel and the properties of the fluid are well characterized so that wall shear stress can be predicted mathematically.

The response of endothelial cells to fluid shear is akin to that triggered by hormones. Within seconds after the onset of flow, GTP-binding proteins and ion channels are activated and intracellular second messengers such as calcium ions and phospholipid metabolites are released.

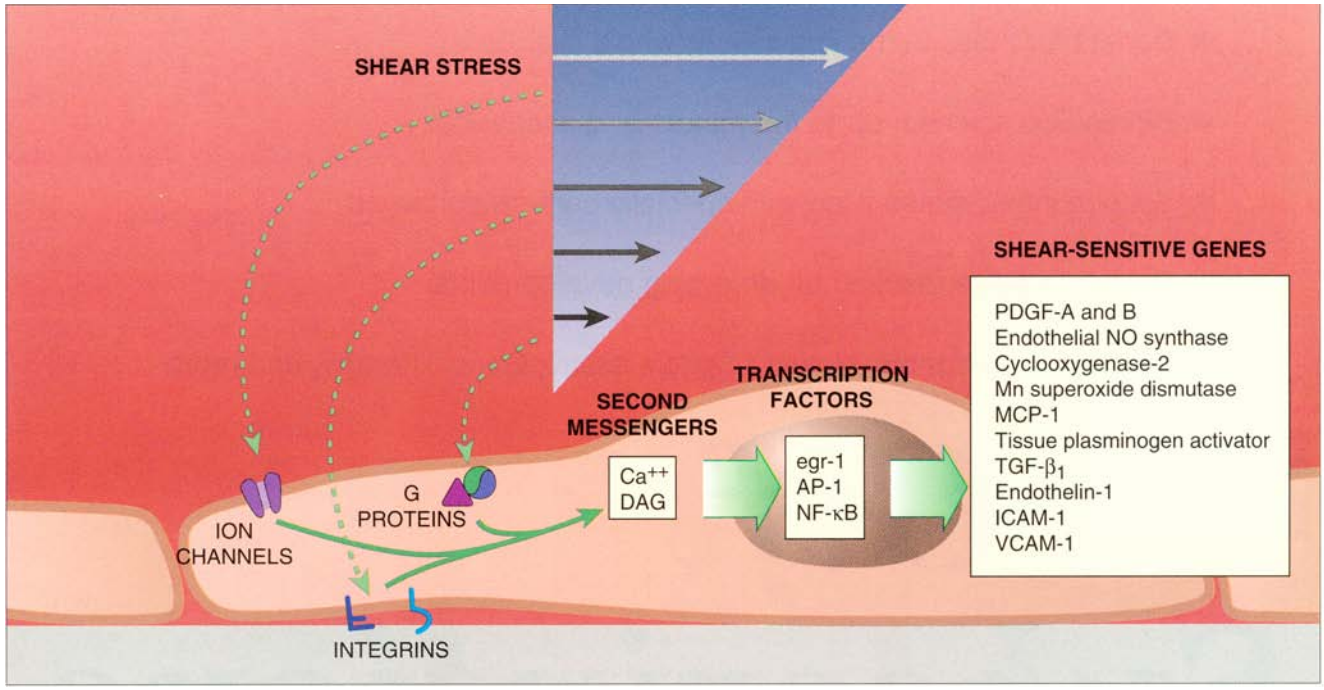
The "mechano-chemical" transduction mechanism that converts the flow signal into a biochemical signal inside the cell is unknown. It has been postulated that flow could induce changes in the conformation of membrane-associated proteins, either directly or indirectly via flow-induced alterations in plasma membrane properties (an increase in membrane fluidity, for example). A second possibility, which does not exclude the first, is that there is a force-sensing mechanism within the focal adhesions and integrins that bind the cells to their underlying extracellular matrix.

Through pathways that have not been entirely elucidated, but which likely involve extracellular signal-regulated kinase (ERK) and stress-activated protein kinase (SAPK), these early events turn on various transcription factors. Among these factors, NF-κB has been shown to bind to a shear stress response element and mediate the transcriptional activation of the gene for platelet-derived growth factor B (PDGF-B) in response to flow. Several other genes possess the same functional shear stress response element, including tissue plasminogen activator, TGF-β_1, monocyte chemoattractant protein 1 (MCP-1), endothelin-1, endothelial nitric oxide synthase, and intercellular adhesion molecules.

Fluid flow in the circulation is not steady because of the pulsatile pumping action of the heart and because complex flow patterns with flow reversal emerge at vessel branching points. Studies dating back to the 19th century have observed that atherosclerotic plaques are preferentially located in regions of relatively low and unsteady wall shear stress, which is to say mostly around arterial bifurcations.

Recent in vitro studies suggest that the response to steady shear stress is indeed different from the response to fluctuations in shear stress. For example, steady levels of shear stress inhibit endothelial apoptosis and up-regulate expression of genes that are largely vasoprotective, such as endothelial nitric oxide synthase, cyclooxygenase-2, and manganese superoxide dismutase. These enzymes promote the formation of agents that prevent platelet adhesion (nitric oxide, prostacyclin) or scavenge free radicals.

In contrast, rapid flow increases induce PDGF-A and MCP-1. A greater response is observed when sudden, as opposed to gradual,



increases occur in shear stress. PDGF-A is a potent mitogen for smooth muscle cells, and MCP-1 is a potent monocyte attractant. Both smooth muscle cell overproliferation and monocyte recruitment have been implicated in intimal hyperplasia, a well-known pathological change in atherosclerosis. A paradigm emerging from these findings is that steady shear stress is antithrombogenic, while unsteady shear stress is pro-thrombogenic.

The effect of fluid flow on vascular endothelial cell function is an important consideration in the design of tissue-engineered blood vessels for use as grafts. Cultured under pulsatile flow conditions, such vessels develop wall thickness, collagen content, mechanical strength, and cellular density that resemble features of natural arteries more closely than similar vassels cultured under nonpulsatile flow conditions. The biological mechanisms underlying this phenomenon have not been investigated, but it is likely that they involve some of the responses to fluid shear stress already mentioned.

Along with flow, there is a growing interest in the effects of other mechanical effects such as stretch on cellular metabolism. Relevant to vascular tissue engineering, reports indicate that stretch regulates the differentiation and growth of smooth muscle cells.

Flow effects are not limited to vascular cells. They play an important part in the development and maintenance of other mammalian cell types in vivo, especially in load-bearing tissues such as bone and cartilage. Mechanical deformation of these tissues, which resemble fluid-filled sponges, causes the interstitial fluid to move, subjecting cells embedded in the tissue matrix to fluid shear stress.

Several pieces of evidence suggest that the flow induced by mechanical deformation is important in bone remodeling. First, loading a bone in bending, which creates regions of high fluid flow, triggers much more cellular activity than loading the same bone in torsion, which causes little fluid flow. Second, sites of maximal bone growth correlate better with areas experiencing the highest strain gradients than with areas of maximal deformation or strain, and high strain gradients induce high fluid flows. Third, a correlation has been established between reduced blood flow and increased bone loss in the lower extremities.

In vitro studies have also shown that fluid shear stress stimulates osteoblasts and osteoclasts, further supporting the notion that fluid flow plays a role in bone remodeling. Flow also potentiates cartilage development in vitro, as cyclical mechanical loading increases the deposition of extracellular matrix by chondrocytes cultured in three-dimensional matrices and improves the mechanical stiffness of the resulting cartilage.

In conclusion, flow is an important mediator of cellular function in vivo and in vitro. A better understanding of the regulation of cell function by fluid flow will be invaluable in optimizing culture conditions for tissue engineering. Furthermore, because the effective transport limit by molecular diffusion in tissues is in the range of 0.1 to 1 mm, incorporation of some type of convective transport system with flow in the tissue will be essential as tissue engineers build more complex tissues with larger numbers of cells.

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