MECHANICAL STRESS AND CELL GROWTH

The ability of unicellular organisms, such as amoeba, to respond to physical stimuli is well established and is an integral mechanism for their function and survival. Animals have evolved to become multicellular and more complex organisms, the response to direct physical stimuli has been overshadowed by the response of cell receptors to internal chemical signals. It is not surprising then that the role of physical stimulation and the cellular responses to such a stimulus have been essentially neglected in the modern study of cell biology. However, although the cells' response to physical stimuli may no longer be dominant to the normal functioning of the cells, the ability of the cells to respond to changes in their environment has not been lost. The dynamic environment continues to play a significant role not only in the development of the cardiovascular system during embryology but in the face of diseases such as hypertension. For cells that normally exist in a pulsatile environment in vivo, such as the cellular components of the cardiovascular system, a dynamic environment may be necessary for the maintenance of the cell phenotype in culture.

The vessel wall is subjected not only to the constantly changing composition of blood across its surface but to pulsatile flow. There are two major hemodynamic forces acting on the cells of the vessel wall. First, the flow of the viscous fluid across the luminal surface of the blood vessel produces a tractive force, shear stress, whose magnitude is dependent on the blood velocity and viscosity. Values of shear stress in large arteries are around 2 to 20 dynes/cm², whereas at the branches they average between 30 to 100 dynes/cm². Second, the oscillating cycles of systole and diastole create a pressure waveform, which produces a periodic variation in the vessel circumference, which is translated to a cyclic strain on the cells of the vessel wall. Electronic caliper measurements of human thoracic aorta indicate a circumference change from diastole to systole of 17% to 25%. Furthermore, turbulent flow and boundary layer separations occur at bifurcations and branches and lead to secondary local hemodynamic forces that will impact on cells in the vessel wall in these areas.

The importance of fluid shear stress and pressure-induced elongation in modulating the function of endothelial cells (ECs) and smooth muscle cells (SMCs) in culture is becoming increasingly recognized. Endothelial cells have been subjected to varying degrees of shear stress by placing them in a cone and plate rotational viscometer or in closed circulation flow loops. Pulsed endothelial cells have been shown to respond to the changes in shear stress by transiently increasing fluid endocytosis, by increasing prostacyclin production, by aligning in the direction of flow, and by reorienting their actinomyosin filaments in the direction of fluid flow. However, no alterations in the kinetics or density-dependent growth behavior of the stressed ECs have been noted. Endothelial cells and SMCs have also been subjected to pulsatile stress in culture. A number of ingenious devices have been developed in an attempt to mimic the physiologic situation. They have ranged from deformation of cells grown on plastic culture dishes by weights or orthodontic expansion screws, to axial stretching of cells grown on silicone rubber membranes, to repetitive plunger agitation of an elastin membrane to which cells are adherent. The main drawbacks of these units are the difficulty in maintaining a sterile culture environment, which impairs the capability to perform large numbers of experiments, and the challenge of providing deformation regimens that have physiologic relevance. However, early studies with these devices have proved, that vascular cells will alter their behavior in culture when exposed to repetitive mechanical strain regimens. Smooth muscle cells have been shown to have an enhanced synthesis of collagen and other matrix proteins, maintain their morphologic characteristics in culture, and orient perpendicular to the direction of stretch when subjected to cyclic strain. Smooth muscle cell proliferation has been found to be unchanged in some studies and decreased in others. Endothelial cells have also been subjected to cyclic stretching, and whereas no apparent change in growth kinetics has been noted, their conditioned medium has been noted to inhibit SMC growth.

Our laboratory has developed the instrumentation and technology to mechanically stretch attached monolayers of cultured cells with different deformation regimens by means of a vacuum-operated stress unit to deform flexible membrane culture dishes. We are capable of performing a large number of experiments at a given time and are able to change the frequency, degree of elongation (strain), and duration of the applied force regimen. Subjecting ECs and SMCs to a regimen consisting of 10 seconds at 24% elongation followed by 10 seconds of relaxation (3 cycle/min) resulted in an alteration in their phenotype. Fluorescent staining with rhodamine phallolidin, an F-actin probe, shows that ECs subjected to cyclic strain for 5 days had polygonal shapes, pseudopods, and actin stress fibers, whereas the control static cells appeared smaller and rounded and had a diffuse distribution of actin. In addition, SDS-PAGE of 35S-methionine-labeled EC after 5 days of the cyclic regimen had both the appearance of new proteins in the stressed ECs and a concomitant decrease in or repression of other proteins in the same stressed cells. Production of secretory macromolecules and extracellular matrix proteins was also altered in cells undergoing the cyclic stress regimen. The amount of prostacyclin produced by ECs during stretch was higher than that seen with the static cells. Moreover, when the ECs were stimulated with exogenous arachidonic acid, there was almost a 1000-fold increase in prostaglandin synthesis in the stretched cells compared to the static cells. In contrast, the rate of von Willebrand factor synthesis of EC did not appear to be significantly altered, whereas collagen produced by ECs subjected to pulsatile stretching environments was significantly depressed. On the other hand, the rate of synthesis of collagen and noncollagen proteins was significantly increased in SMCs subjected to the stretch regimens.

The 3 cycle/min regimen also resulted in an increase
in EC proliferation and DNA synthesis compared to the stationary control. In contrast, the growth curve for the SMC showed a slower rate of proliferation and DNA synthesis during the same strain regimen. Further studies also indicate that the enhanced proliferation of ECs and the inhibitory effect on SMC proliferation of cyclic deformation could also be noted when the cells were stretched at frequencies of 60 and 100 cycle/min. Studies are underway exploring the effect of various deformation regimens on cell proliferation. However, the initial experiments indicate that different cells from the same tissue can react differently to the same stress regimen and may imply control at the level of cell division. In addition, our recent data suggest that the conditioned media from stretched SMCs will inhibit proliferation of target quiescent SMCs, whereas conditioned media from stretched ECs will stimulate proliferation of quiescent target ECs. We are currently investigating this phenomenon and the production of growth factors and inhibitory substances by vascular cells during cyclic stretching.

What is the transducing signal by which external mechanical forces affect the cell phenotype? Numerous potential pathways exist, but it seems likely that control is at the level of the cell membrane or cytoskeleton or both. At the cell membrane level, cell surface receptors, chemical second messengers such as cyclic adenosine monophosphate or the phosphoinositide metabolites, or the actual deformation of the membrane activating distinct ion channels may be important. The cellular microfilament system might be involved as the extension of the cells, and the generation of tension within them may depend on the microfilament system.

Other important issues need to be addressed in the future. Is the increase in EC growth a result of entry of quiescent cells into the cell cycle or a general speeding up of the cell cycle? Does mechanical deformation alter the composition of the extracellular matrix, and does this in turn modulate cell function? Are autocrine and paracrine substances released by physical stress forces? What is the mechanism for cell alignment with shear and pulsatile stress, and what is the significance of this alignment in terms of cell function? What is the effect of these forces on cell-to-cell interaction?

In summary, it is now evident that the stationary tissue culture condition may be suboptimal and inappropriate for the study of the biology of cells that reside in a dynamic environment in vivo. Further studies detailing the precise contribution of external forces to cell function and adaptation are needed to extrapolate studies done in vitro models with the living dynamic state.

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REFERENCES

IS INTIMAL HYPERPLASIA AN ADAPTIVE RESPONSE OR A PATHOLOGIC PROCESS?—OBSERVATIONS ON THE NATURE OF NONATHEROSCLEROTIC INTIMAL THICKENING

The artery wall is a living tissue subserving a mechanical function. Adjustments to imposed deviations from normal or "ideal" conditions should, within fairly well defined limits, be expected to be self-limiting and to terminate when the ideal baseline conditions are reestablished. For the blood-endothelial interface, this condition is related to an apparent ideal wall shear stress level of about 15 dyne/cm². The media consist of bundles of commonly oriented smooth muscle cells in close association with an encompassing, similarly oriented array of branched elastic fibers. These musculoelastic fascicles, the putative units of structure of the media, are aligned with the direction of the mural tensile forces at any given location, and their size is closely related to vessel curvature. Ideal tensile stress levels are likely to depend on distance from the heart, artery diameter, and pressure. Thus hemologenous arteries in mammals normally tend to have similar wall structure and similar levels of tensile stress. For the aorta, this level is apparently 2000 dyne/cm² for each structural layer. For the pulmonary artery and major muscular arteries the level is about half that value. In general, a tensile stress of about