HIGHLIGHTED TOPIC | Biomechanics and Mechanotransduction in Cells and Tissues

Mechanical, biochemical, and extracellular matrix effects on vascular smooth muscle cell phenotype

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Stegemann, Jan P, Helen Hong, and Robert M. Nerem. Mechanical, biochemical, and extracellular matrix effects on vascular smooth muscle cell phenotype. J Appl Physiol 98: 2321–2327, 2005; doi:10.1152/japplphysiol.01114.2004.—The vascular smooth muscle cell (VSMC) is surrounded by a complex extracellular matrix that provides and modulates a variety of biochemical and mechanical cues that guide cell function. Conventional two-dimensional monolayer culture systems recreate only a portion of the cellular environment, and therefore there is increasing interest in developing more physiologically relevant three-dimensional culture systems. This review brings together recent studies on how mechanical, biochemical, and extracellular matrix stimulation can be applied to study VSMC function and how the combination of these factors leads to changes in phenotype. Particular emphasis is placed on in vitro experimental studies in which multiple stimuli are combined, especially in three-dimensional culture systems and in vascular tissue engineering applications. These studies have provided new insight into how VSMC phenotype is controlled, and they have underscored the interdependence of biochemical and mechanical signaling. Future improvements in creating more complex in vitro culture environments will lead to a better understanding of VSMC biology, new treatments for vascular disease, as well as improved blood vessel substitutes.

VASCULAR SMOOTH MUSCLE CELLS (VSMC) can respond to a variety of environmental cues in the dynamic environment of the blood vessel wall. These cues can be biochemical or mechanical in nature and can lead to changes in cell function and phenotype, both under normal physiological conditions and in pathological states. In the native vessel, VSMC are surrounded by a complex, highly structured extracellular matrix (ECM) consisting largely of collagens type I and III, elastin, and proteoglycans. These matrix molecules are important in maintaining tissue structure but also play key roles in guiding cell function. Cells bind to the ECM via specific integrin receptors, and this binding can directly affect cell function. Furthermore, other signals that a cell receives from its environment are transmitted through and modulated by the ECM. Biochemical signals (e.g., ions, small proteins, or growth factors) must pass through the ECM and in some cases are sequestered and released by the matrix. Mechanical signals (e.g., tensile, compressive forces, or shear forces) are also transmitted by the ECM to the cell via integrin receptors that link the external environment to the cytoplasm and cytoskeleton.

Blood vessels are typically thought of as having a three-layered structure: an intimal lining of an endothelial cell monolayer, surrounded by a medial layer containing ECM and smooth muscle cells, and an outer adventitial layer consisting of more matrix and fibroblasts. During blood vessel formation, the phenotype of the smooth muscle cells in the medial layer of the wall changes such that secretion of ECM proteins is reduced and the formation of intracellular myofilaments is increased. This transition, from a synthetic to a contractile state, is required for the smooth muscle cell to perform its primary function: contraction and dilation of the blood vessel wall to regulate blood pressure and flow. Under pathological conditions, cells in mature vessels can undergo a reverse phenotypic shift from the normal contractile state to proliferative, synthetic cells that can migrate from the media and into the intimal region. A similar shift in cell phenotype is observed when VSMC are removed from their native environment and placed in cell culture, presumably because of the absence of the normal physiological signals that maintain and regulate VSMC phenotype in the vessel wall.

It should be noted that the phenotype of VSMC is a continuum, as opposed to a discrete set of phenotypic states. The terms “contractile” and “synthetic” are used to refer to relative positions along this continuum, indicating cell functions and expression of markers that are associated with either a contractile or a synthetic function. Therefore, there are no rigorous guidelines as to when a cell can be labeled contractile or synthetic, and most experimental studies focus on shifts in phenotypic markers or compare separate cell populations in terms of these markers. In addition, it has been established that...
there is phenotypic heterogeneity in VSMC in the native vessel wall (4, 20). This variability in the state of VSMC can further complicate the interpretation of experimental results, because not all studies make a distinction between VSMC from different parts of the vessel wall. It has been suggested that these heterogeneous cell populations are differentially involved in the pathogenesis of vascular disease (19); however, it is clear that essentially all VSMC have the ability to shift phenotypes in response to environmental cues.

Cell function is regulated by the entirety of the cellular environment, including cell-cell interactions, ECM components, humoral factors, local chemical conditions, and mechanical forces. In vivo studies of VSMC function have the advantage that they maintain this complex environment, but the large number of variables that are difficult to control makes it challenging to isolate specific effects in experimental studies. In vitro studies have the advantage that treatment variables can be controlled; however, it can be difficult in a cell culture system to recreate the large number of environmental cues necessary for VSMC phenotype maintenance and modulation. The large majority of in vitro studies of VSMC function have been performed under standard culture conditions, in which cells are adhered to two-dimensional (2D) surfaces in monolayer culture. The richness of the extracellular environment is lost in these studies, and there thus has long been an interest in developing more complex, yet still well controlled, in vitro model systems to study vascular biology.

This review will describe recent advances in understanding how VSMC function and phenotype are affected by their mechanical environment, as well as by the ECM and biochemical stimulation. In particular, we will highlight in vitro studies that have combined multiple elements of the cellular environment in an effort to gain a more complete understanding of how VSMC function is controlled. We first summarize studies related to the effects of ECM and soluble biochemical stimulation on VSMC, followed by a description of recent studies directed at understanding mechanical effects on VSMC, and finally we examine recent efforts at combining these stimuli to better understand vascular biology and pathology. In each section, we begin by reviewing work done in 2D monolayer culture and subsequently address what is known about similar stimuli applied in three-dimensional (3D) culture systems. The application of knowledge of VSMC phenotype to the area of vascular tissue engineering is then discussed, with particular emphasis on how control of cell phenotype can be used to create improved vascular tissue substitutes.

EFFECT OF ECM AND EXOGENOUS BIOCHEMICAL FACTORS

It is clear that VSMC phenotype can be modulated by ECM components. Binding of specific matrix molecules can have a biochemical effect of its own through integrin-mediated signaling, and other biochemicals in the cellular milieu will also affect cell function. In addition, it is known that receptors for soluble ligands (e.g., growth factor receptors) can interact with ECM-bound integrins and that this causes cross talk between signaling pathways and a resulting modulation in response (48).

In 2D monolayer studies, various ECM components have been shown to cause changes in VSMC phenotype. Substrates of fibronectin and collagen type I induce shifts toward the synthetic state (21, 74), whereas laminin tends to induce the opposite effect (45). The MAPK family of signaling pathways, and in particular the ERK pathway, is of interest in understanding these effects because it is functional in VSMC (50) and is closely associated with cell growth and differentiation (26). It has been shown that the ERK pathway plays an important role in phenotypic modulation in VSMC (49), regulating both contractile (51) and synthetic markers (41). In addition, a number of studies have been performed to examine the effects of biochemical stimulation in combination with an ECM substrate. In the presence of fibronectin, supplementation with hepatoocyte growth factor triggers cell migration (68) and PDGF inhibits cell adhesion (6). The combination of a 2D collagen matrix and stimulation with PDGF has been shown to stimulate VSMC migration (66), whereas PDGF stimulation of cells on laminin causes increased cell migration and decreased cell adhesion (33, 34).

It is not only the composition of the ECM that affects VSMC function, because the physical structure of the matrix also has been shown to be important. In 3D collagen type I gels, cell proliferation is greatly reduced, relative to 2D cultures, and the expression of the contractile protein smooth muscle alpha-actin (SMA) is downregulated (63). Although these results suggest a contradiction, because in conventional 2D cultures decreased cell growth is generally associated with the contractile phenotype whereas decreased SMA suggests a shift away from the contractile state, they also serve to point out that cell behavior can be markedly different in 3D matrices, compared with 2D. The presence of a 3D ECM also has been shown to modulate the cellular response to growth factor stimulation. The effects of transforming growth factor-beta (TGF) and PDGF on SMA expression are attenuated in 3D collagen gels, and a study of VSMC gene expression in 3D collagen gels suggests that collagen synthesis is increased and phosphorylation of focal adhesion kinase decreased, compared with monolayer cultures (37). In 3D fibrin gels, biochemical stimulation with TGF and insulin has been used to increase the gel’s mechanical properties, through the increased production of collagen by embedded VSMC (17).

EFFECTS OF MECHANICAL FACTORS

Smooth muscle cells in the arterial system are exposed primarily to cyclic tensile stresses as a result of the pulsatile pressure produced by the heart that causes cyclic distention of the vessel wall. The magnitude of these forces can be altered by changes in the arterial wall and by the alteration of forces associated with vascular diseases such as hypertension and atherosclerosis. In severe cases of hypertension, as well as during surgical intervention such as balloon angioplasty or implantation of a stent, compressive forces also may be exerted on the vessel wall. Interventional procedures also can denude the vessel lumen, leaving VSMC exposed to shear forces. It is now recognized that these mechanical forces play an important role in governing cell function and phenotype; however, the investigation of how these forces exert their effects on cells, and in particular how mechanical forces interact with ECM and biochemical signals, is still in the early stages.
CYCLIC TENSIILE STRESS

Cyclic strains are commonly applied to cultured cells by growing them as 2D monolayers on silicone substrates that can be distended in a controlled fashion using a vacuum or solid actuator. By use of this 2D system, a variety of responses of VSMC to cyclic strain have been observed, including altered cell proliferation, alignment, and protein expression. Biochemical markers upregulated by cyclic strain include growth factors [e.g., PDGF AA and BB (73), VEGF (59), IGF (61), and FGF (67)], ECM molecules [e.g., fibronectin (69), collagen type I (13), and type IV (30)], contractile proteins (7, 46), as well as osteogenic markers (58). Many of the responses to cyclic strain have been shown to be mediated by the p38 MAPK pathway, including alignment (10), apoptosis (71), and SMA degradation (15). Both increased (9, 32) and decreased (37, 52) cell proliferation have been reported in response to strain in 2D systems, and it has been suggested that the proliferative response of VSMC will depend on their initial phenotype, such that contractile cells proliferate in the presence of mechanical strain, whereas synthetic cells do not (8). This concept is interesting because it supports the idea that not only can the mechanical environment affect cell phenotype but also that the mechanosensitive response itself is phenotype dependent.

Efforts to create more physiologically relevant environments to study VSMC function have led to an increased focus on true 3D ECM environments. For example, a number of studies have been performed to evaluate the effect of cyclic mechanical strain applied to tubular 3D matrices in which VSMC are embedded. Studies using a pulsatile bioreactor system have shown that cyclic strain can improve the mechanical strength and histological organization of VSMC-seeded tubular constructs made of both collagen (53) and synthetic degradable polymers (42). In addition, mechanical stimulation increases expression of enzymes involved in tissue remodeling (55), while decreasing collagen mRNA levels and increasing elastin mRNA levels (54). Mechanical stimulation has been shown to be necessary to prevent a phenotypic shift toward the osteoblastic phenotype (43). The frequency and the duration of the applied stimulation also can be important. Whereas both adult (90 beats/min) and fetal (165 beats/min) pulse rates cause increased matrix metalloproteinase-1 activation and collagen deposition, only a fetal pulse rate results in higher tissue inhibitor of matrix metalloproteinase-1 expression (60). Longer term (5 wk) cyclic distention has been shown to increase elastin production by VSMC in 3D collagen gels, although no increase in collagen expression was noted (29).

A wide variety of effects have been observed in VSMC in response to mechanical strain. It is interesting that some of these (increased matrix synthesis, increased proliferation) are more indicative of a synthetic phenotype, whereas others (increased contractile protein content) are suggestive of the contractile phenotype. It should be noted that currently there are no standard systems or protocols for applying mechanical strains to VSMC. Many strain studies are currently done on flexible 2D membranes (e.g., Flexercell units), whereas others have been done in 3D engineered tissues. The varied observations are probably related to the differences between 2D and 3D culture, as well as to differences in the strain system or protocol (amplitude, frequency, duration) and experimental specifics (cell type, passage, culture conditions, etc.). It also has been proposed that there may be a graded response of VSMC to mechanical forces. This theory holds that a zero (or very low) strain represents a nonphysiological situation and therefore leads to a loss of the contractile phenotype. Optimal, physiological strains mimic the in vivo environment and therefore promote a quiescent, contractile phenotype. Large strains are associated with pathological conditions and lead to hypertrophy, hyperplasia, and increased matrix synthesis, hallmarks of the synthetic phenotype (72).

SHEAR STRESS

The effects of shear stress in the vascular system are most commonly associated with the endothelial cells that line the vessel wall. In contrast to VSMC embedded in the vascular wall, endothelial cells exist in vivo as a monolayer, and therefore in vitro studies that examine their function as a monolayer on a 2D surface are relevant to the native geometry. The addition of fluid flow above the cells, to simulate shear forces caused by flowing blood, adds to the physiological relevance, and indeed this is now a commonly used system to study endothelial function. There has thus developed a large body of literature on endothelial responses to shear, but VSMC response to shear has not been as fully characterized. In monolayer cultures, shear stress has been shown to decrease cell proliferation (65) while inducing FGF secretion (47), cell contraction (1, 11), cytoplasmic calcium (56), cell alignment (35, 39), and apoptosis (2). Less is known about the effects of shear stress on VSMC in 3D matrices. In VSMC-seeded synthetic polymer matrices, it was observed that shear increases ECM production, contractile phenotype markers, and DNA synthesis (44). In a 3D collagen gel system, embedded VSMC increased PG12 and PGE2 secretion (70). Studies of shear-induced effects on cell function are becoming more advanced, for example with the use of pulsatile and oscillatory flow regimes (3) instead of the simple steady laminar flow that most studies to date have employed. In the vascular arena, the effects of shear are now beginning to be studied using coculture models that combine an endothelial cell monolayer and VSMC embedded in 3D matrices (28).

EFFECTS OF COMBINED STIMULATION

The need for more physiologically relevant models with which to study vascular biology has led to studies in which specific biochemical factors, mechanical forces, and ECM materials are combined to recreate a more complex environment. These types of studies are also important in the field of tissue engineering and regenerative medicine, where the ability to understand and reconstitute the physiological environment is a key to developing biologically based vascular tissue substitutes.

Static 2D monolayer studies have demonstrated that binding of VSMC to the ECM is mediated by specific integrin molecules, which are clustered in signaling complexes that can trigger a variety of intracellular signaling pathways. For example, the ERK signaling pathway is known to be activated by integrins, growth factor receptors, and mechanical forces, and it has been further shown that mechanical activation of the ERK pathway in SMC is mediated through specific integrins (16). Studies combining 2D ECM substrates and cyclic me-
Mechanical strain have shown that at low strain frequencies collagen type I, laminin, and pronectin reduce cell proliferation (23). At higher frequencies, cyclic strain and type I collagen increase DNA synthesis through oxidative stress pathways (24), while also increasing apoptosis through integrin-mediated pathways (71). These varied responses to cyclic strain may be explained by the finding that ERK phosphorylation depends on matrix alignment and strain rate (40). The combination of soluble biochemical stimulation and mechanical strain in 2D systems also has been investigated, and studies have suggested that biochemically induced proliferation is inhibited by the application of cyclic mechanical strain, for example through effects on cell cycle proteins (9) or early response genes (52). Similarly, it has been shown that the hormone estrogen mitigates strain-induced proliferation on fibronectin (38).

Combined biochemical and mechanical stimulation in 3D cultures has not yet been investigated extensively. In 3D collagen gel systems, it has been reported that mechanical strain increases gel compaction and cell proliferation relative to static controls. PDGF increases cell proliferation and decreases SMA expression but negates the effects of mechanical stimulation on cell proliferation and results in a more open matrix structure. TGF inhibits cell proliferation and increases SMA expression and produces a histologically denser matrix, and this effect is further amplified in the presence of cyclic mechanical strain (64).

IMPLICATIONS FOR VASCULAR TISSUE ENGINEERING

An improved understanding of cell phenotype in vascular tissue engineering is important to direct the development of engineered vascular tissues in vitro toward constructing with improved mechanical and functional properties. In addition, the phenotype of the cells in an implanted vascular tissue must be appropriate, to avoid unwanted remodeling that could lead to restenosis, to provide a suitable substrate for normal endothelial cell function, and to promote normal physiological function of the vessel as a whole. The challenge for the tissue engineer is to harness the tools of cellular and molecular biology and to use the rapidly growing understanding of vascular biology to induce the cells that make up a vascular construct to behave as they would in vivo. This does not imply, however, that the goal is simply to drive the smooth muscle cells of the vessel wall toward their in vivo phenotype. However, the ability to cause a deliberate shift toward the synthetic phenotype in such a model would also be a useful tool in simulating certain vascular diseases in vitro.

Several approaches to the development of an engineered blood vessel have shown promise in producing blood vessel constructs that resemble native tissue. Degradeable synthetic scaffolds seeded with isolated VSMC have been used by a number of groups (42, 57). In these models, a high cell proliferation rate is required to initially populate the polymer scaffold and replace the degrading polymer with new cellular and matrix material. Other groups have used naturally derived protein matrices such as collagen (5, 14) and fibrin (12, 18) to create tubular tissue constructs with VSMC embedded directly in the matrix. This approach depends on the active compaction and remodeling of the scaffold by VSMC to produce a suitably dense and mechanically robust tissue. The synthetic capabilities of VSMC have also been used to create blood vessel substitutes from cell/matrix sheets that are rolled into a tubular configuration (36), a technique that depends exclusively on matrix synthesis by the cellular component. Acellular approaches that use a decellularized native tissue as a scaffold, which is intended to be repopulated by VSMC (27, 31), also require cells to be migratory to populate and remodel the matrix.

Phenotype shifts can certainly be used to the tissue engineer’s advantage; however, the shifts must be controllable to obtain the desired result. In general, the shift toward a synthetic phenotype that occurs when VSMC are removed from their in vivo environment is beneficial because the accompanying increase in proliferation rate allows cells to be expanded rapidly in culture. This phenotype is also useful in the fabrication of vascular tissue, because synthetically active cells are more able to populate, produce, and remodel the matrix in which they reside. By the same token, an engineered vascular tissue would presumably need to be in a contractile or at least quiescent state to be implanted. Many of the undesired outcomes of vascular interventions are related to VSMC that are in a synthetic state. This includes intimal hyperplasia and restenosis after bypass grafting or angioplasty. Therefore, implanting a blood vessel substitute that contains cells in a proliferative, migratory state could lead to some of the same complications as these pathologies. In addition, to be a useful model with which to study vascular biology in vitro, an engineered blood vessel would have to function as the native vessel functions, thus requiring a contractile VSMC phenotype. However, the ability to cause a deliberate shift toward the synthetic phenotype in such a model would also be a useful tool in simulating certain vascular diseases in vitro.

SUMMARY AND FUTURE DIRECTIONS

Our knowledge of how VSMC are affected by their environment is the result of decades of experimental research using both in vivo and in vitro systems. These studies have given the vascular biologist insight into how cell behavior changes in both normal physiological and pathological conditions, but our understanding of how different environmental cues combine to produce these changes in cell function is still incomplete. In vitro experimental systems are a valuable tool in isolating specific treatments and observing their effects; however, 2D culture systems do not fully reflect the environment to which cells in native tissues are exposed. Current 3D in vitro culture systems are also simplified versions of the in vivo environment, but they recreate a very important element of tissue structure, namely the fact that the ECM surrounds the cell, such that integrin and growth factor receptors are distributed over the entire cell surface. This change in the spatial organization of signal receptors may have important consequences in the initiation of cell signaling and in the consequent alterations in cellular phenotype and function. This is particularly true in the case of combined biochemical and mechanical signal induction, owing to the known cross talk between these pathways.

The development of more complex, physiologically relevant in vitro culture systems is necessary to further our understanding of VSMC phenotype shifts. These systems must provide combined biochemical and mechanical stimulation in a 3D environment to more closely mimic conditions in vivo. VSMC in the vascular wall are embedded in an ECM that surrounds...
the cells and modulates the signals they receive. Therefore, these cells must be studied in systems that recreate the 3D nature of the matrix, as well as the biochemical and mechanical environment. The recreation of complex 3D environments is also of central importance to the fields of tissue engineering and regenerative medicine, the goals of which are to build living tissues by controlling cell function. A better understanding of the mechanisms by which a cell’s environment leads to changes in cell function offers the possibility of creating environments that direct cell function to produce improved tissue substitutes.

Improved systems for studying VSMC biology must also incorporate relevant biochemical and mechanical stimuli. It is becoming increasingly clear that studying individual aspects of the cellular environment in isolation will not fully illuminate how cell function is controlled, because biochemical and mechanical cues often act in concert and the ECM modulates both sets of signals. As we learn more about the relevant signaling pathways and how they are linked and interdependent, it becomes even clearer that combined stimulation is a key to understanding and potentially controlling VSMC phenotype and function. Creation of such complex in vitro culture systems is a practical challenge, but the information obtained promises to be of greater relevance to the in vivo situation. Future culture systems also will increasingly include cocultures of cells [e.g., endothelial cells with VSMC (22)] to create a more physiologically relevant environment. Genetic modification of VSMC to express phenotype-regulating proteins is another area that is still in its infancy (62) but that holds promise for more directly controlling cell function. The past two decades have produced a broad base of research regarding the phenotype of VSMC, as well as an advanced set of tools for studying the cellular- and molecular-level markers of VSMC phenotype. This knowledge and these tools must now be applied in more relevant 3D culture systems to realize the next generation of advances in vascular biology, ones that will enable the development of improved treatments for vascular disease, as well as the ability to rationally design and build living substitute vascular tissues.

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