Published in final edited form as:

Microcirculation. 2010 April ; 17(3): 159–163. doi:10.1111/j.1549-8719.2010.00028.x.

Frontiers in Microcirculation: Control Processes and Clinical Applications

Steven S. Segal

Department of Medical Pharmacology and Physiology, University of Missouri, Columbia, MO 65212, USA & Dalton Cardiovascular Research Center, Columbia, MO 65211, USA

Abstract

This edition of *Microcirculation* presents five current and emerging perspectives of the microcirculation in development, health and disease. The onset of blood flow and pressure are central to cardiovascular development. These hemodynamic forces are explored in light of underlying molecular signaling pathways that affect vascular and cardiac cell shape and proliferation. Shear-induced strain exerted on the plasma membrane and cytoskeleton is transmitted to cell nuclei and thereby affects gene activation through mechanotransduction. Altered stiffness or disturbed surfaces of aberrant vascular cells may affect an array of vasculopathies through altered gene expression. The endothelium is both a site and source for inflammatory responses triggered by cardiovascular risk factors often mediated through reactive oxygen species and angiotensin II. Tumor microenvironments are disturbed with abnormal growth and remodeling of blood and lymphatic vessels. More effective targeting strategies for delivering anti-angiogenic and cytotoxic agents are being developed through advances in intravital imaging. Blood flow control requires both vasodilation and vasoconstriction to be coordinated along and among arterioles and feed arteries. Evolving insights into signaling pathways between smooth muscle cells and endothelial cells illuminate how such processes can be affected in vasculopathies. These timely reviews provide a novel reference for advancing research frontiers in microcirculation.

Keywords

Microcirculation; Plasticity; Inflammation; Cell signaling; Intercellular communication

This edition of *Microcirculation* presents five state-of-the-art reviews prepared by keynote speakers of the fall 2009 meeting of the Microcirculatory Society, Inc., which was held at the University of Missouri – Columbia on October 16-17, 2009. The purpose of the meeting was: “To explore and advance the frontiers of research in microcirculation through identifying and integrating fundamental regulatory processes that underlie microvascular function with the purpose of advancing clinical practice and patient care”. As exemplified in these invited reviews, a key goal of the meeting was to identify evolving research directions, particularly for new investigators interested in studying the microcirculation. To address this goal, research topics spanning molecular, cellular, and systems levels of organization explore how plasticity, inflammation, cell signaling and intercellular communication contribute to the (dis)regulation of tissue homeostasis, development of the cardiovascular system, cardiovascular disease, and cancer. For brevity, this preface cites only the invited reviews which respectively contain primary references for each of the topics addressed.

Molecular signaling initiated through mechanical forces is integral to microcirculatory structure and function. Culver and Dickinson (1) discuss cell signaling events elicited by blood flow that are required for normal blood vessel and cardiac development in mammalian embryos. Basic concepts and molecular signaling pathways are developed by which hemodynamic forces exerted by blood pressure (circumferential wall stress) and flow (luminal shear stress) on the vascular wall lead to changes in cell shape and proliferation. These physical forces stimulate the endothelium to release paracrine substances that influence the structure and function of adjacent cells and tissues, including renewal and differentiation of progenitor stem cells at key stages of development. In pointing out the complexity of interactions that contribute to development of the heart and vasculature the authors argue that such interactions need to be accounted for *in vivo* because they cannot be answered using *in vitro* model systems. During pregnancy and embryonic development, initiation of the heartbeat and the onset of blood flow are critical events in remodeling the primitive capillary plexus of the yolk sac and delivering blood flow to the embryo. Paracrine signaling by the endothelium is integral to blood vessel stabilization and maturation through recruitment of mural cells. Hemodynamic forces promote the underlying signaling events along with differentiation of developing blood vessels into arteries or veins. The role of blood flow and pressure in cardiac development is discussed in light of how disruption of normal hemodynamic stimuli is deleterious to formation of cardiac chambers and valves. Morphogenesis of the aortic arch is presented in the context of shear stress-induced remodeling and its interplay with an array of genetic factors. The authors extend their discussion to consider the role of hemodynamic forces exerted by maternal blood flow in promoting implantation of the embryo and development of the placenta as well as how disturbance of these events may give rise to preeclampsia. Shear stress exerted by the flowing blood promotes the differentiation of embryonic stem cells towards an endothelial cell lineage and contribute to determining specialized microenvironments (“niches”) harboring distinct populations of stem cells. A prime example is the recruitment of neural progenitor cells that populate the developing brain, providing a framework for investigating the role of hemodynamic forces in regulating neurogenesis. Such insight gained from studying the microcirculation during development may foster new therapeutical approaches for recovery from stroke and treatment of mental illness.

In looking deeper into cellular responses to fluid shear stress, Dahl et al. (2) focus on the role of nuclear deformation. Mechanotransduction is presented in the context of gene activation through mechanical forces exerted on the cell nucleus, mediated by signaling events induced by strain exerted on the plasma membrane and cytoskeleton. With the nucleus identified as the largest and stiffest organelle, the morphological changes that endothelial cells undergo in response to shear stress effectively minimize the shape of the nucleus to reduce drag by aligning with the direction of shear. Though cytoskeletal remodeling appears to be linked to gene expression, key signaling events remain to be defined. An increase in nuclear stiffness is associated with upregulation of lamins, proteins that provide structural support of the nucleus. The ability of lamins to interact with chromatin and affect gene expression supports the hypothesis that mechanical force regulates gene expression. Remarkably, abnormalities in nuclear lamina and stiffness are associated with pathological states. Hutchinson-Gilford Progeria Syndrome (HGPS) is a form of premature aging associated with accumulation of lamins in the nuclei of endothelial cells and vascular smooth muscle cells. The reduced deformability of nuclei in affected cells may well contribute to altered gene expression associated with this disease. Whereas cardiovascular function is adversely affected in HGPS, it remains to be determined how this disease affects the microcirculation. The authors caution that the large forces and deformations exerted under experimental conditions may not represent those more typically encountered by cells and their nuclei *in vivo*. To circumvent such limitations, *in situ* particle tracking is presented as an alternative optically-based method for observing mechanical

properties of nuclei in their native environment. Such an approach complements imaging techniques being pioneered in the Dickinson laboratory (1) to understand hemodynamic forces exerted during development and how they shape the heart and vasculature. Computational fluid dynamics coupled with *in vitro* modeling is presented as an evolving approach to provide quantitative insight into the mechanical forces exerted by the non-Newtonian nature of blood flow within the microcirculation. This emerging insight into mechanobiology suggests that greater stiffness or disturbed surfaces of vascular cells presented to the flow stream may contribute to vasculopathies associated with inflammation, vascular disease and cancer.

Microvascular responses to cardiovascular risk factors are discussed by Granger et al. (4) in light of underlying inflammatory mechanisms and their pathophysiological consequences. Risk factors for cardiovascular disease and vascular pathology share the common denominator of systemic inflammation. That the endothelium is a primary target is documented by endothelium-dependent vascular dysfunction throughout the body, with the microcirculation acting both as a site of and a source for inflammatory responses triggered by risk factors. The role of oxidative stress is presented as integral to the induction of a pro-inflammatory phenotype in the vasculature. The cellular origins, actions, reversal and prevention of reactive oxygen species (ROS) production are considered in the pathological settings of hypertension, diabetes, obesity and hypercholesterolemia. Animal models focused on dietary and genetic manipulations complemented by adoptive transfer procedures provide critical insight into elucidating the roles of endothelial cells, leukocytes and T-lymphocytes as sources of ROS. The participation of mitochondria in generating ROS is highlighted in the context of obesity, hyperglycemia and diabetes along with commensurate reduction of antioxidant defenses. Mechanisms underlying endothelial cell activation and enhanced leukocyte-endothelial cell adhesion during metabolic disturbances are considered with particular attention to the roles of angiotensin II and adipokines. Impairment of the endothelium's function as a barrier is a further manifestation of inflammation, resulting in such maladies as cerebral edema, retinopathy and nephropathy often associated with the diabetic state. Concomitant changes in microvessel density include rarefaction in hypertension while chronic disturbances in blood glucose and cholesterol are associated with both impaired and unregulated angiogenesis. Further contributing to vascular disturbances are abnormalities in platelet function with enhanced risk of thrombosis, leading to further disruption of tissue integrity, which is particularly apparent during reperfusion following ischemia. Insight provided through studying the interplay between the microcirculation and molecular signaling events during development (1) and cancer (3) in light of hemodynamic forces acting on gene expression (2) promise to shed new light on developing novel therapies for patient care.

Beyond supplying oxygen and nutrients, Fukumura et al. (3) present abnormal growth and remodeling of blood and lymphatic vessels as contributing to abnormalities in the microenvironment of tumors. Advances in intravital microscopy have enabled greater optical penetration to observe events deeper into intact tissues and thereby minimize physical disruption of the prevailing state. The network architecture, hemodynamics and barrier functions of the tumor vasculature are disorganized and compromised relative to those in healthy tissue. The molecular signals underlying such abnormalities are under vigorous study and future research in this area is likely to benefit from perspectives afforded by those studying development of the microcirculation (1). In turn, abnormal vasculature compromises effective delivery of therapeutic agents while fostering an abnormal microenvironment within the tumor. Optical probes confirm hypoxia and acidosis within affected regions, which promote growth factor release and angiogenesis. Imaging fluorescent reporter proteins provides insight into how and where gene expression is altered within affected tissue. Whether abnormalities in cellular and nuclear stiffness are manifest

within tumor cells and their aberrant vasculature (2) remain to be defined. Remarkably, functional lymphatic vessels are absent within solid tumors, attributable to mechanical compression from tumor cell proliferation. Nevertheless, lymphatic vessels surrounding the perimeter of tumors provide an alternative path to the bloodstream for collecting fluid and cells released from the tumor and thereby provide paracrine signals and an alternative route to the bloodstream for metastases. Observing tumors originating from the same cell line grown in different tissues provides insight into the nature of organ-specific microenvironments affecting angiogenesis and vascular permeability. Understanding gained from these studies provides new strategies for the detection and treatment of cancer, for example how and where to most effectively target anti-angiogenic or cytotoxic agents in an effort to restore a more normal vasculature and microenvironment.

Structural and functional adaptations of the microcirculation during development, cardiovascular disease and cancer are studied over time courses spanning hours, days and even weeks. A more acute dynamic of cell signaling and intercellular communication entails the moment-to-moment regulation of tissue blood flow. In contrast to the long history of studying how the local control of blood flow is governed by metabolic demand, increasing attention is directed at understanding the nature of signaling among smooth muscle and endothelial cells underlying vasomotor control. Arterioles and their feed arteries serve as the principal sites of peripheral vascular resistance. Understanding how vasodilation and vasoconstriction are coordinated along and among respective segments of resistance networks is central to promoting the delivery of oxygen and nutrients to parenchymal cells. In turn, such insight is critical to developing new therapeutical approaches for promoting tissue perfusion through defining how intercellular signaling events may be altered during vascular disease.

When an arteriole or feed artery is stimulated at a discrete site, the vasomotor response can travel along the vessel wall via intercellular communication. The ability of vasoconstriction [or responses initiated on smooth muscle cells (SMC)] to travel along the vessel is less effective than that of vasodilation (or responses initiated via endothelial cells (EC)). Tran and Welsh (5) present the conduction of vasomotor responses in light of distinct hypotheses concerned with how electrical signals are initiated, modulated and transmitted among cells of the vessel wall. In brief, the *syncytial hypothesis* presents SMC and EC as a functional unit of blood flow control, effectively signaling each other by charge movement and Ca^{2+} flux through myoendothelial gap junctions in concert with the activation of ion channels within and along respective cell layers. Local modulation through such interactions is a key determinant of whether the concerted vasomotor response entails limiting the spread of constriction or the conduction of dilation along the vessel wall. The concept of “nullification” is introduced to describe negative feedback (e.g., from endothelium to smooth muscle) purported to attenuate the spread of vasoconstriction. Citing apparent inconsistencies in the syncytial hypothesis, the authors develop an alternative explanation to explain the difference in behavior of events initiated on smooth muscle versus those initiated via the endothelium. Their *differential hypothesis* is based upon distinct differences in the structural and functional organization between SMC and EC of the resistance vasculature complemented by computational modeling according to the biophysical properties of respective cell layers. In brief, the circumferential orientation of SMC together with a paucity of gap junction channels between adjacent SMC effectively restricts the spread of signals (e.g., depolarization) along the media. In contrast, electrical signals originating in the endothelium (e.g., hyperpolarization) are readily transmitted along axially oriented EC, which are complemented by robust gap junctions coupling adjacent EC. The ability to evoke SMC relaxation over considerable distances through myoendothelial gap junctions is attributed to the high input resistance of SMC, producing a concerted vasomotor response with negligible charge dissipation along the endothelium. In turn, computational modeling

illustrates how local vasospasm can be explained in regions of endothelial cell damage, which compromises the ability of the endothelium to source hyperpolarizing current into SMC for relaxation. As intended by the authors, presenting alternative perspectives on how vasomotor responses travel along the vessel wall is provocative and should stimulate further work in this active area of investigation. Whether (and if so, how) such signaling events are manifested in the lymphatic microcirculation, how they are affected during development (1), with cardiovascular disease (4) and in cancerous tissues (3) are questions that are ripe for new and future investigators.

These cross-disciplinary perspectives into the workings of the microcirculation provide new insight for developing novel approaches to clinical practice and patient care. For example, an emerging perspective incorporates understanding physical and chemical interactions between blood and lymphatic microvessels in developing targeted therapeutic strategies for treating inflammation and tumor cell metastases. These timely reviews provides a unique reference for both new and established investigators to refine their thinking and define new avenues of investigation to advance the frontiers of knowledge for microcirculation in health and disease.

Acknowledgments

The scientific conference from which these articles originate was supported by grant R13-HL097542 and S.S. Segal is supported by grant R37-HL041026. Respective awards are from the National Heart, Lung and Blood Institute of the National Institutes of Health, United States Public Health Service.

References

1. Culver JC, Dickinson ME. The effects of hemodynamic force on embryonic development. *Microcirculation*. 2009; 17(3):xx-yy.
2. Dahl KN, Kalinowskia A, Pekkan K. Mechanobiology and the microcirculation: Cellular, nuclear and fluid mechanics. *Microcirculation*. 2009; 17(3):xx-yy.
3. Fukumura D, Jain RK. Tumor microvasculature and microenvironment: novel insight through intravital imaging. *Microcirculation*. 2009; 17(3):xx-yy.
4. Granger DN, Rodrigues SF, Yildirim A, Senchenkova EY. Microvascular responses to cardiovascular risk factors. *Microcirculation*. 2009; 17(3):xx-yy.
5. Tran CHT, Welsh DG. The Differential Hypothesis: A provocative rationalization of the conducted vasomotor response. *Microcirculation*. 2009; 17(3):xx-yy.