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REVIEW ARTICLE

Spatial and temporal dynamics of the endothelium

W. C. AIRD

Division of Molecular and Vascular Medicine, Department of Medicine, and Center for Vascular Biology Research, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

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Summary. The endothelium is a highly metabolically active organ that is involved in many physiological processes, including the control of vasomotor tone, barrier function, leukocyte adhesion and trafficking, inflammation, and hemostasis. Endothelial cell phenotypes are differentially regulated in space and time. Endothelial cell heterogeneity has important implications for developing strategies in basic research, diagnostics and therapeutics. The goals of this review are to: (i) consider mechanisms of endothelial cell heterogeneity; (ii) discuss the bench-to-bedside gap in endothelial biomedicine; (iii) revisit definitions for endothelial cell activation and dysfunction; and (iv) propose new goals in diagnosis and therapy. Finally, these themes will be applied to an understanding of vascular bed-specific hemostasis.

Introduction

The endothelium, which forms the inner lining of the blood vessels, is a truly expansive cell layer, weighing approximately 1 kg in an average-sized human and covering a total surface area of 4000–7000 m^2 [1]. Endothelial cells from a single human, when lined end-to-end, would wrap more than four times around the circumference of the earth. The endothelium is not an inert cell layer, but rather is highly metabolically active, participating in many homeostatic processes, including the control of vasomotor tone, the trafficking of cells and nutrients, the maintenance of blood fluidity, the regulation of permeability, and the formation of new blood vessels [2]. An important feature of the endothelium is that its properties vary between different sites of the vasculature and from one moment in time to the next [3]. These differences reflect the capacity of the endothelium to respond to the unique needs of the underlying tissue. As an important corollary, the endothelium is heterogeneous in its response to pathophysiological stimuli, thus contributing to the focal nature of vasculopathic disease states.

The goal of this review is to provide an overview of the spatial and temporal dynamics of the endothelium with a particular emphasis on: (i) the mechanisms of phenotypic heterogeneity; (ii) the bench-to-bedside gap in endothelial biomedicine; (iii) endothelial cell activation and dysfunction; and (iv) the need for new diagnostic and therapeutic approaches in endothelial-based diseases.

Correspondence: William C. Aird, Beth Israel Deaconess Medical Center, RW-663, Boston MA 02215, USA.
Tel.: +1 617 667 1031; fax: +1 617 667 1035; e-mail: waird@bidmc.harvard.edu

Scales of investigation

The term 'vascular' refers to blood vessels, the elaborate series of blood-filled hollow tubes that deliver oxygen and nutrients to all tissues of the human body. The vascular system comprises both blood vessels and lymphatic vessels. For purposes of this review, we will focus on the former. For more information about lymphangiogenesis and lymphatic endothelium, the reader is referred to several excellent reviews [4–7].

Arteries and veins

Most of our knowledge about the vasculature is restricted to conduit vessels, namely the large arteries and veins (Table 1). Arteries deliver well-oxygenated (red colored) blood from the heart to the various tissues of the body, whereas veins collect and return deoxygenated blood (blue in color) back to the heart. Arteries have a thick muscular wall; veins have a thin, distensible wall. Arteries pulsate, whereas veins do not. Large arteries are located deep within tissues, and are thus protected from traumatic injury. Veins lie both deep and superficial. So distinct are these properties that until the discovery of the capillaries in the 17th century, arteries and veins were considered to be completely separate systems.

From a clinical standpoint, physicians and surgeons are far more informed about disorders of conduit vessels, than they are of the microcirculation. The most commonly recognized disease of arteries is atherosclerosis. Indeed, acute myocardial infarction and stroke account for a full 40% of fatalities in the United States in people older than 40 years. Arterial lesions that occur outside the coronary and carotid circulation – for e.g. in the renal artery or iliac/femoral artery – are more likely to cause chronic symptoms and signs. The most common disorders of veins are varicosities and deep venous thrombosis. In contrast to arteries, venous obstruction results in impaired

Table 1 Properties of arteries, veins and capillaries

	Arteries	Veins	Capillaries
Discovery	Recognized and characterized by Hippocrates and Galen	Recognized and characterized by Hippocrates and Galen	Existence surmised by William Harvey in 17th century, first observed by Marcello Malpighi in 17th century
Surface area	+	+	+ + + + + +
Blood Volume	450 mL	2800 mL	250 mL
Length			60 000–100 000 miles
Pressure	+ + + + *	+	+ +
Velocity	+ + + + †	+ + +	+
Valves	Absent	Present	Absent
VSMC	Present	Present	Absent
Wall components	Endothelium, I/M/A	Endothelium, I/M/A	Endothelium/pericytes/ECM
Wall thickness	+ + + +	+ +	+
Diameter	0.02–10 mm	0.03–12.5 mm	5–8 μM
pO_2 (blood)	+ + + + *	+	+ +
pCO_2 (blood)	+	+ + + +	+ +
Function	Conduit, blood pressure control	Conduit, reservoir, temperature regulation, leukocyte emigration	Exchange (e.g. gases, nutrients)
Involvement in disease (examples)	Atherosclerosis, Buerger's disease, Kawasaki's Disease, aneurisms, embolus	Varicose veins, telangiectasias, DVT	Most disease states

VSMC, vascular smooth muscle cells; I, intima; M, media; A, adventitia; ECM, extracellular matrix; DVT, deep venous thrombosis.
*Pulmonary artery is exception.
†Difference in flow rate in arterial tree (aorta to precapillary arterioles) eight orders of magnitude, whereas shear stress varies by a factor of only two [56].

drainage of blood, secondary swelling, and increased risk for pulmonary embolus.

Capillaries

Capillaries are the macroscopically invisible blood vessels that connect arterioles (arterial side) to venules (venous side), thus creating a closed circulation. If the arteries and veins are considered the major conduits of the vascular tree, capillaries represent the 'business end' of the circulation, carrying out the bulk of exchange (of gases and nutrients) with underlying tissue. In keeping with Fick's law of diffusion, capillaries comprise the vast majority of the surface area of the circulation (Fig. 1). Moreover, they have an extremely thin wall, consisting of a single layer of endothelial cells surrounded to a variable degree by occasional pericytes and extracellular matrix. Indeed, capillaries are so densely packed as to be in intimate contact with virtually every cell of the body.

A helpful way to place arteries, veins and capillaries into perspective is to consider the analogy of a roadmap of the United States. The large interstate highways are analogous to the arteries and veins of the body – major routes of transportation *into* which or *from* which local routes flow. At this scale, the equivalent of the capillaries is not visible. To recognize the capillaries, one must zoom in on the cities and towns, where they are represented by the neighborhood streets, roads, and back alleys. Just as each neighborhood has a unique architecture and is adapted to the local needs of population, each capillary bed – whether in the brain, lung, kidney or other organ – is topologically distinct. City neighborhoods and capillary beds are both vulnerable to dysfunction through mechanisms that are highly site-specific.

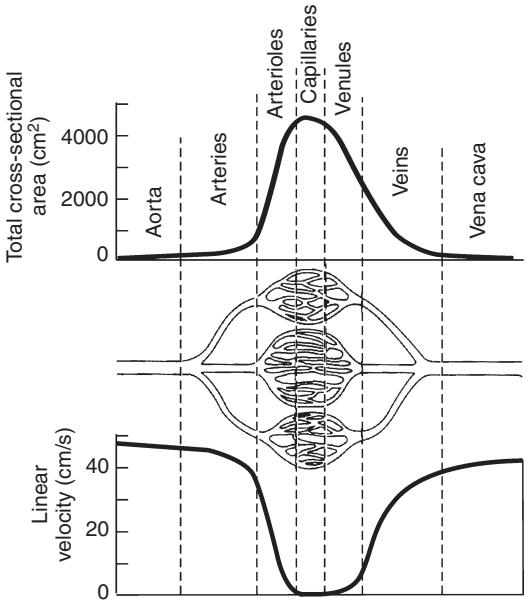

Fig. 1. Surface area of the human vasculature. Shown is the estimated total cross-sectional area of the vascular tree in humans (top) and the linear velocity (bottom). The cross-sectional area of the more clinically recognized blood vessels (coronary, carotid and peripheral arteries) represents a tiny fraction of the total. Reprinted from *Physiology and Biophysics*, Vol. 2, 20th edn, E.O. Feigl, pp. 10–22, with permission from Elsevier.

It is noteworthy that the fields of vascular biology, vascular medicine and vascular surgery have focused primarily on just a few (albeit critical) inches of the vascular tree – namely the large arteries of the heart and brain, and the deep veins of the leg – an

infinitesimally narrow focal point relative to the tens of thousands of miles of uncharted territory in the microvascular beds of every organ in the human body. This hidden, underexplored circulation holds important clues about the pathophysiology of most, if not all, human disease states.

Endothelium

The endothelium represents the cellular interface between circulating blood and underlying tissue. To return to the map analogy, if the arteries and veins of the body represent the interstate highways, and the capillaries the neighborhood streets, the endothelium may be thought of as the city sidewalk, brimming with life, commerce, and activity. Ary Goldberger has argued that health is associated with organized (fractal chaotic-like) complexity, and disease with loss of variability [8]. Like the bustling urban sidewalk, the healthy endothelium is highly active and adaptive (we will return to this concept later). Loss of organized complexity, whether in deserted downtown districts, isolated suburban towns, or the endothelium, is characteristic of disease. Indeed, Goldberger's theory of decomplexification supports the counter-intuitive notion that it is the *dysfunctional* endothelium that approaches quiescence [9].

While 20 years ago, one was hard pressed to name more that a small number of diseases in which the endothelium played a primary role (namely atherosclerosis), today it may be argued that the endothelium plays a part in most if not all diseases, either as a primary determinant of pathophysiology or a 'victim of collateral damage' (Table 2).

Table 2 Examples of endothelial involvement in human disease

Disease	Selected references
Hematology–oncology	
Cancer	[57,58]
Hemoglobinopathies	
SSD	[59–62]
Thalassemia	[63,64]
Hemachromatosis	[65]
Myeloproliferative diseases	[66,67]
Bone marrow transplantation	[68,69]
Transfusion medicine	[70–73]
TTP/HUS	[74,75]
Coagulation	[33,34]
Infectious disease	
Infection	[76–78]
Sepsis	[79,80]
Cardiology	
Atherosclerosis	[81–86]
Congestive heart failure	[87–90]
Valvular heart disease	[91,92]
Pulmonary	
Asthma	[93,94]
COPD	[95,96]
Pulmonary hypertension	[97–99]
ARDS	[3,100]
Nephrology	
Acute renal failure	[101–103]
Chronic renal failure	[104–106]
Gastroenterology	
Peptic ulcer disease	[107,108]
Inflammatory bowel disease	[109,110]
Hepatitis	[111,112]
Cirrhosis	[113,114]
Pancreatitis	[115,116]
Rheumatology	
Rheumatoid arthritis	[117–119]
Scleroderma	[120–122]
Endocrinology	
Diabetes	[123,124]
Neurology	
Stroke	[125–127]
Multiple sclerosis	[128,129]
Other	
Pre-eclampsia	[130,131]

SSD, sickle cell disease; TTP, thrombotic thrombocytopenic purpura; HUS, hemolytic uremic syndrome; COPD, chronic obstructive pulmonary disease; ARDS, acute respiratory distress syndrome.

Bench-to-bedside gap in endothelial biomedicine

A recent search for the key term 'endothelium' on PubMed yielded no fewer than 92 000 articles. These numbers reflect untold hours of 'sweat and toil' on the part of investigators, not to mention billions of dollars of research funding. In clinical practice, however, the endothelium is largely ignored. Physicians are poorly attuned to the health of this cell layer. The indices of clinical textbooks contain few if any entries for the 'endothelium' or 'endothelial cells'. Medical school curriculum are generally lacking in courses on endothelium or the vascular tree. The above discordance amounts to one of the most striking examples of a bench-to-bedside gap in biomedicine today.

There are several possible explanations for this chasm (Table 3). First, medicine was fragmented into myriad subspecialties well before there was an appreciation for the important functional of for the endothelium in health and disease. Thus, endothelial cell specialists tend to work with colleagues in their organ-specific discipline, rather than with like-minded 'endotheliogists' from other subspecialties. A second reason is that the endothelium is hidden from view and is poorly accessible in the patient. The endothelium does not lend itself to inspection, palpation, percussion or auscultation. Although certain other organs such as the pancreas and kidney are also difficult to examine at the bedside, they are spatially confined in space and are thus amenable to diagnostic imaging. Moreover, whereas disease of these latter organs is associated with changes in blood chemistry (e.g. amylase, blood urea nitrogen, and creatinine), endothelial dysfunction occurs in the absence of reliable circulating markers. Thirdly, the endothelium – like other organs in the body – is highly complex and displays emergent properties such that the whole is far greater than the sum of its parts. Most endothelial cell biologists (this author included) study specific

Table 3 Explaining the bench-to-bedside gap in endothelial biomedicine

1. Historical legacies*
2. Out-of-sigh-out-of-mind†
3. Phenomenon of emergence‡
4. Adaptiveness§

*Medicine was divided into subspecialties long before the endothelium was recognized as a functional entity.
†Endothelium is not readily accessible to the practicing clinician, thus may be overlooked.
‡Endothelium, like every biological system displays emergent properties in which the whole is greater than the sum of the parts. In other words, its behavior cannot be fully predicted by studying the parts (endothelial cells) in isolation (cell culture). Studying endothelial cells *in vitro* is analogous to studying an ant or a fish outside the context of its colony or school, respectively.
§The endothelium, perhaps more than most other organs, is highly adaptive and flexible. When removed from its native environment and cultured *in vitro* the endothelial cell is like a fish out of water, behaving in ways that run counter to its *in vivo* counterpart.

aspects of endothelial cell function in tissue culture and in doing so tend to overlook critical levels of organization that are essential to a full understanding go the system. Just as one could never predict the behavior of an ant colony by studying an individual ant in isolation, one cannot rely solely on isolated endothelial cells to fully understand the endothelium in health and disease. Finally, the endothelium, more so than most tissues in the human body, is extraordinarily adaptive and flexible. It is like a chameleon, 'marching to the tune' of the local microenvironment. Indeed, so tightly coupled is the endothelium to the extracellular milieu that when it is removed from its native environment and grown in tissue culture, it undergoes phenotypic drift. Therefore, any results from *in vitro* studies must be interpreted with caution and ultimately validated *in vivo*.

In summary, the bench-to-bedside gap arises from a convergence of circumstances, ranging from the drift in cellular phenotype *in vitro*, the inability to predict higher order behavior from studies of isolated cells, the out-of-sight-out-of-mind nature of the cell layer in clinical practice, and the orphan status – from a subspecialty standpoint – of the endothelium as a clinically relevant organ.

No two endothelial cells are identical

Each of our 60 trillion endothelial cells is analogous to a miniature adaptive input–output device (Fig. 2). Input arises from the extracellular environment and may include biomechanical and biochemical forces (Table 4). The nature of the output depends on the level of organization and scale of investigation. For example, single endothelial cells may undergo a change in cell shape or alteration in protein or mRNA expression, or they may proliferate, migrate or undergo apoptosis. Monolayers of endothelial cells express barrier properties and may be assayed for adhesion and transmigration of white blood cells. Other properties of the endothelium emerge only in the context of the blood vessel, whole organ or organism, such as endothelial-dependent regulation of vasomotor tone, angiogenesis, and redistribution of blood flow.

The input–output device is of course not a black box, but rather contains a highly elaborate non-linear array of dynamic signal transduction pathways that couple signals at the membrane surface to cellular response or phenotype.

The input from the microenvironment differs in space and time. For example, the blood–brain barrier endothelium is exposed to astroglial-derived factors, whereas capillary endothelium in the heart is subject to paracrine signals

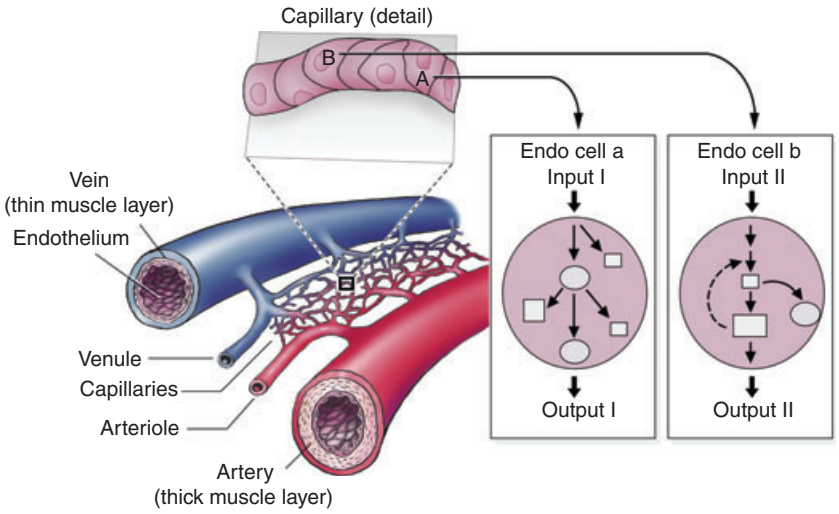

Fig. 2. Endothelial cell as an input–output device. There are approximately 60 trillion endothelial cells in the human body. Each of these endothelial cells (two are shown in detail) integrates input from the extracellular environment and uses this information to generate output. As input changes in time and space (e.g. between different vascular beds, different segments of the vascular loop, and between neighboring endothelial cells), so too does output. This gives rise to phenotypic heterogeneity. Examples of spatially and temporally regulated input are thrombin, tumor necrosis factor-α and lipopolysaccharide. Examples of output are hemostatic balance, vasomotor tone, leukocyte adhesion and transmigration. Each input signal is coupled to transcriptional and post-transcriptional events through overlapping yet distinct signaling pathways (shown schematically by shapes and arrows). Endo, endothelial. Illustrated by Steve Moskowitz.

Table 4 Examples of endothelial input and output*

Input
Biomechanical
Circumferential stress
Circumferential strain
Longitudinal stress
Longitudinal strain
Cyclic strain
Shear stress
Biochemical
Hypoxia
Glucose
Pathogens
Growth factors
Extracellular matrix
Sex hormones
Serine proteases
Chemokines
Cytokines
Nucleosides
Sphingolipids
Lipoproteins
Contact system
Heparan
Nitric oxide
Cell–cell interactions
Hyperthermia/hypothermia
Acid base balance
Output
Level of single cell
Cell shape
Calcium flux
Protein translation
Post-translational modification
Gene expression
Proliferation
Migration
Apoptosis
Level of cellular monolayer
Barrier function
Leukocyte adhesion
Level of blood vessel/organ/organism
Vasomotor tone
Angiogenesis
Antigen presentation
Inflammation
Activation of coagulation with fibrin deposition

*Not shown are the myriad intracellular signal transduction pathways

Table 5 Examples of Phenotypic Heterogeneity of Endothelium

Gene/protein	Distribution*
vWF	V [50,132]
TFPI	C [47]
EPCR	V/A
t-PA	A
TM	V/A/C except in brain [23]
Heparan	EC AT-binding sites vary between tissues and within organs [133]; HS differentially distributed in vessels [134]
eNOS	A
TF	Undetectable in normal endothelium
CD39 (NTPDase-1)	A/V; variable in C; absent in normal fenestrated EC
CD39L1 (NTPDase-2)	Abluminal surface of splanchnic endothelium; undetectable in cardiac endothelium
RPTPμ	A; non-fenestrated capillary endothelium [135,136]
ESM-1 (endocan)	Adult lung, kidney and GI endothelium [137,138]; tumor endothelium
MECA-79	HEV [139]
GlcNAc6ST	HEV [139]
EphrinB2	A [140,141]
EphB4	V [142]
Notch 4	A [143]
Gridlock	A [144]
Sox-13	A [145]
Activin receptor-like kinase 1	A [146]
EPAS-1	A [147]
HRT1-3	A [148]
Neuropilin-1	V [149]
Neuropilin-2	A [150]
EG-VEGF signaling	Steroidogenic glands [151]
Egr-1	Brain and heart endothelium [152]; induced in endothelium by VEGF, EGF, atherosclerosis [152,153]
NF-κB	Preferential expression at sites of atherosclerosis [154]

A, arteries; V, veins; C, capillaries; HEV, high endothelial venules; EC, endothelial cells; vWF, von Willebrand factor; TFPI, tissue factor pathway inhibitor; EPCR, endothelial protein C receptor; t-PA, tissue-type plasminogen activator; TM, thrombomodulin; PAR, protease-activated receptor; eNOS, endothelial nitric oxide synthase; TF, tissue factor; ESM-1, endothelial specific molecule-1, AT, antithrombin; HS, heparan sulfate.
*Vessel type in which the gene (mRNA and/or protein) is predominantly expressed.

from neighboring cardiomyocytes. Input also varies within the same organ. For example, in the heart, endothelial cells that line the epicardial arteries and endocardium are exposed to vastly different wall stresses. Endothelial cells in arteries and veins are exposed to distinct hemodynamic forces, pO_2 and pH. Finally, input varies even on a micro-scale. For example, Davies *et al.* [10] have demonstrated differences in hemodynamic forces between neighboring endothelial cells.

As input is coupled to output, variation in the extracellular environment across space and time leads to phenotypic heterogeneity (Table 5). Indeed, if the phenotype of a given endothelial cell could somehow be color coded, e.g. by mapping its transcriptome, proteome and/or function, then at any given point in time, the normal endothelium would display a rich color palette (Fig. 3). If one were to 'roll the film' and observe the endothelium in real time, the colors would fade and brighten, and perhaps even flicker on and off like lights on a Christmas tree. To return to the theory of decomplexification, diseased or dysfunctional endothelium would be associated with a restricted color palette, and/or a reduction in the degree and frequency of flickering.

If all endothelial cells in the vasculature were intrinsically identical, the link between environmental input and cellular output would be sufficient to fully explain the phenomenon of

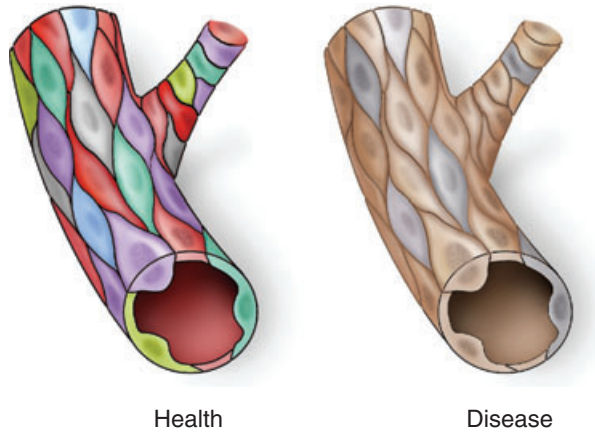

Fig. 3. The endothelial color palette. In this hypothetical scheme, endothelial cell phenotype is represented by a color shade. Left, At any given point in time, the endothelium would display a rich color palette. These colors likely change from one moment to the next. Right, According to a model of decomplexification, the dysfunctional endothelium may display a more restricted range of colors. In addition (or alternatively), decomplexification may be associated with a loss of variability over time (e.g. less fluctuation in color shades). Illustrated by Steve Moskowitz.

endothelial heterogeneity (Fig. 4). However, in all likelihood endothelial cells are *not* intrinsically identical. Rather, the local extracellular environment may induce epigenetic changes within the endothelium. As a result, certain site-specific properties of endothelial cells are mitotically heritable and are thus 'locked in'. While epigenetic changes are thought to occur primarily during embryonic development, it is tempting to speculate that they may also arise in the postnatal period as a consequence of disease or aging.

The relative contribution of environment and epigenetics in mediating phenotypic heterogeneity has important therapeutic implications. For example, the more the phenotypes are epigenetically fixed, the less plastic is the endothelium (Fig. 5). Site-specific phenotypes that are non-heritable will be more responsive to therapeutic manipulation of the surrounding microenvironment, compared with their epigenetic counterparts.

Endothelial cell activation and dysfunction

When considering the role of the endothelium in disease, the two most common terms that are used are endothelial cell activation and endothelial cell dysfunction. Each of these terms is discussed below and qualified based on recent advances in the field.

The concept of endothelial cell activation first arose from *in vitro* studies demonstrating the ability of well defined stimuli [e.g. lectin phytohemagglutinin, endotoxin, tumor necrosis factor (TNF)-α and interleukin (IL)-1] to induce the expression of so-called 'activation antigens' on the surface of endothelial cells (Ia-like antigen; ELAM-1, later designated E-selectin). These changes were in turn correlated with the expression of pro-adhesive, antigen-presenting and procoagulant activities [11–20]. The term 'activation' was considered to reflect the capacity of endothelial cells to perform new functions without evidence of cell injury or cell division [21].

Although not intended as such, the initial observations regarding endothelial cell activation have given way over the years to the notion of the endothelium as a toggle switch. According to this view, quiescent endothelial cells express an anticoagulant, antiadhesive and vasodilatory phenotype,

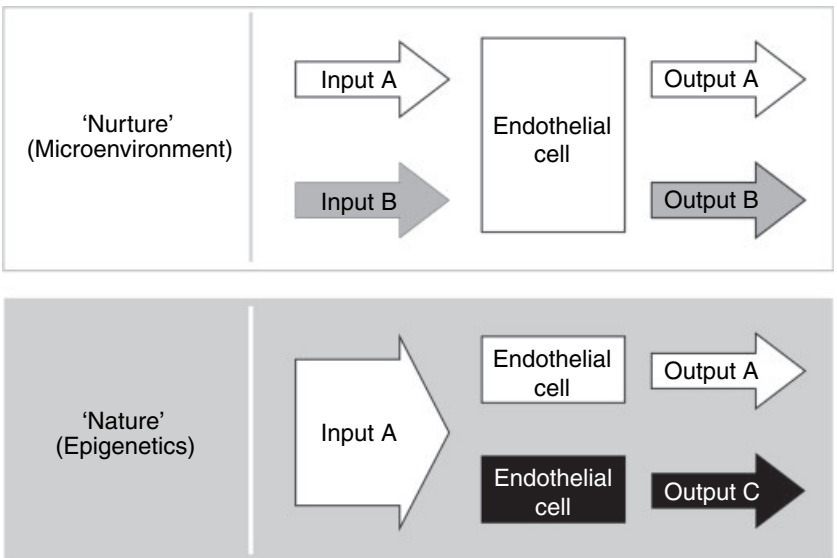

Fig. 4. Mechanisms of endothelial cell heterogeneity. If endothelial cells are intrinsically identical (top), then spatial and temporal differences in input signals (e.g. input A, B) will result in spatial and temporal differences in output (output A, B), resulting in heterogeneity. If endothelial cells are epigenetically modified (bottom), then they may display heterogeneous phenotypes (output A, C) at rest and/or in response an identical input (input A). Both mechanisms are operative in the intact organism and contribute to generation and maintenance of endothelial cell heterogeneity. Reprinted from *Endothelial Cells in Health and Disease*, 1st edn, W.C. Aird, with permission from Taylor & Francis.

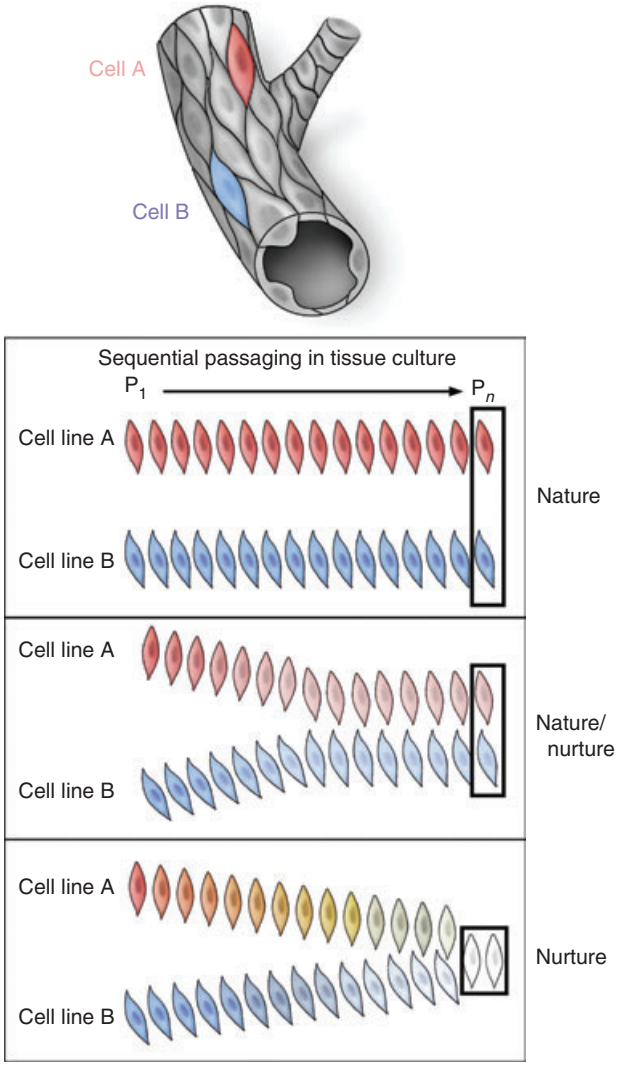

Fig. 5. Boundaries of plasticity. In this conceptual scheme, endothelial cells are removed from two different sites of the vascular tree (for simplicity, neighboring endothelial cells are shown) and propagated *in vitro* under identical culture conditions indefinitely from passage (P) one to passage 'n'. Three hypothetical scenarios are shown. Top, If all site-specific properties are mitotically heritable (epigenetic), then the cellular phenotype (represented by color) will be impervious to subsequent changes in the extracellular environment and remain constant over multiple passages. Middle, If all site-specific properties are non-heritable and tightly (and reversibly) coupled to the immediate extracellular milieu, then the cellular phenotype of the two cells will ultimately reach identity. Bottom, If site-specific properties are controlled by a combination of epigenetics and microenvironment (as is actually the case), the cellular phenotype will approach but never reach equivalence. The more these phenotypes approximate each other over sequential passages, the more plastic they are. The less they approach each other in phenotype, the less plastic or more fixed they are. These boundaries of plasticity may change with disease or aging (the height of the rectangle is inversely correlated with plasticity). Illustrated by Steve Moskowitz.

whereas activated endothelial cells express procoagulant, pro-adhesive and vasoconstricting properties. However, the notion that endothelial cell activation is an all-or-none phenomenon is an over-simplification.

For one, endothelial cells follow a spectrum of response (one only has to look at dose–response studies to appreciate this point). Thus, the endothelium is more analog in its behavior than it is digital.

Secondly, what constitutes activation for one cell type at a particular snapshot in time may not meet the definition of activation at another site or another moment in time. For example, while P-selectin is considered a marker for endothelial cell activation, it is constitutively expressed in dermal microvessels of uninflamed skin [22]. As another example, inflammation is associated with reduced thrombomodulin (TM) expression in endothelial cells. However, the brain expresses little or no TM to begin with [23]. The toggle hypothesis would suggest that the blood–brain barrier is in a chronic state of activation. This of course is not the case. Rather, the brain relies on other anticoagulants to balance local hemostasis. The important message, for purposes of this discussion, is that endothelial cell activation must be adjudicated in an appropriate temporal and spatial context.

Thirdly, not all inflammatory mediators or endothelial cell activators are created equal [24]. Commonly studied mediators such as TNF-α, thrombin and lipopolysaccharide exert overlapping, yet distinct effects on endothelial cell phenotypes [25].

Finally, the terms 'activation' and 'activity' are not synonymous. Normal endothelium is by its very nature highly active – constantly sensing and responding to alterations in the local extracellular environment, as might occur in the setting of transient bacteremia, minor trauma and other common daily stresses, most of which we are not consciously aware.

In summary, endothelial cell activation is not an all-or-nothing response, nor is it necessarily linked to disease. Instead, endothelial cell activation represents a spectrum of response and occurs under both physiological and pathophysiological conditions.

Early descriptions of endothelial cell dysfunction focused on structural changes or loss of anatomical integrity, particularly in the context of atherosclerosis [26]. Following Ross' response-to-injury hypothesis [27], there was a growing appreciation that the *intact* endothelium may actively contribute to disease initiation and/or progression [28]. The term endothelial cell dysfunction was first coined in 1980 to describe hyper-adhesiveness of the endothelium to platelets [29]. In 1986, acetylcholine was shown to induce paradoxical vasoconstriction of coronary arteries in early and advanced human atherosclerosis, suggesting that abnormal vascular response to acetylcholine may represent a defect in endothelial vasodilator function [30]. Subsequently, a role for endothelial-leukocyte adhesion molecules was implicated in the pathogenesis of atherosclerosis [31,32].

Given that basic and clinical research in vascular biology has focused predominantly on coronary (and to a lesser extent carotid) arteries, it is not surprising that the term endothelial cell dysfunction is most often used to describe changes associated with atherosclerosis. However, endothelial cell dysfunction is not restricted anatomically to large arteries, nor is it limited in disease scope to atherosclerosis. Indeed, the term endothelial cell dysfunction may be broadly applied to

states in which the endothelial cell phenotype – whether or not it meets the definition of activation – poses a net liability to the host, as occurs locally in coronary artery disease or systemically in sepsis. Assigning liability scores is of course a subjective exercise. An evolutionary biologist might argue that endothelial cell dysfunction is most relevant in its effect on an individual's reproductive capacity. A physician would surely expand the meaning of dysfunction to include a far broader spectrum of morbidity. An investigator interested in applying evolutionary principles to an understanding of endothelium in health and disease would point out that the endothelium evolved to a state of maximal fitness in the early ancestral environment, and is not adapted to withstand the rigors of high fat diet, epidemics associated with high density populations, sedentary lifestyle or old age.

To summarize, endothelial cell activation represents a *predefined phenotype* of the endothelium. Although highly specific to location and time, the activated phenotype generally consists of some combination of increased cell adhesiveness, shift in hemostatic balance to the procoagulant side, secretion of inflammatory mediators and change in cell survival/proliferation. In contrast, endothelial cell dysfunction represents the *cost of the endothelial cell phenotype* to the host, and as such represents a more subjective (and in many ways, *post hoc*) determination.

Diagnostic and therapeutic implications

An important goal in vascular biology is to develop novel tools for interrogating the endothelium in humans. Several diagnostic assays hold promise for the future. These include the use of proteomics to measure panels of circulating biomarkers, systematic analyses of pathology specimens from different vascular beds, quantitation and phenotyping of circulating endothelial cells and microparticles, the development of vascular bed-specific catheters, and the application of molecular imaging techniques. A full discussion of these methods is beyond the scope of the current review. However, it should be emphasized that the successful pursuit of diagnostic strategies is a prerequisite for major advances in endothelial-based therapy.

The endothelium has enormous, though largely untapped potential as a therapeutic target. The endothelium is strategically located between the blood and tissue and is therefore rapidly and preferentially exposed to systemically administered agents. The endothelium is highly plastic and thus amenable to therapeutic modulation. Finally, in establishing a dialogue with the underlying tissue, the endothelium provides the pharmacotherapist with a direct line of communication with every organ in the body.

When applying concepts of endothelial cell activation and endothelial cell dysfunction to a consideration of therapeutics, it is important to recognize that endothelial cells may be activated – for e.g. they may express a phenotype that is characteristic of an inflammatory response – without being dysfunctional. Indeed, there are many instances in which endothelial cell activation is a welcome response, whether in wound healing, physiological angiogenesis, local defense against pathogens and foreign bodies. Therapy is perhaps best reserved for cases in which the phenotype of the endothelium (whether or not it meets the definition of activation) represents a net liability to the host. The notion that endothelial cells resemble input–output devices and that their behavior is not binary, but continuous, has important therapeutic implications. The goal in treating the endothelium is not to reset the switch, but rather to fine-tune and recalibrate the cell, nudging it back to its ideal state. An important challenge is to learn how to determine the nature of that ideal state. Endothelial cell dysfunction usually arises from otherwise adaptive responses (or at least ones that were adaptive in the ancestral environment) that are now excessive, sustained, or spatially and/or temporally misplaced. The transition between endothelial cell function and dysfunction is not always clear. As more effective treatments become available for attenuating dysfunctional endothelium, it will be important to avoid overshooting the desired effect or 'lobotomizing' the cells. Finally, given that endothelial cell phenotypes vary according to time and location in the vascular tree – in both health and disease – it will be essential to target therapy to specific vascular beds.

Applying principles of endothelial cell biology to an understanding of hemostasis

Hemostasis represents a finely tuned balance between anticoagulant and procoagulant forces. A tip in the hemostatic scale is associated with one of two clinical phenotypes, bleeding or clotting. An interesting feature of the hypercoagulable states is the propensity to develop thrombotic lesions in discrete and characteristic sites of the vascular tree [33,34]. For example, patients with congenital deficiency of protein C, protein S or antithrombin III (ATIII) have a propensity to form clots in deep veins but not arteries. On the contrary, those with nephrotic syndrome and acquired ATIII deficiency are at particularly high risk for renal vein thrombosis. Warfarin-induced skin necrosis, which is associated with acute depletion of functional protein C relative to the other vitamin K-dependent serine proteases, confers a high risk for thrombosis in postcapillary venules of the dermis [35–37].

The idea that hemostasis is regulated by vascular bed-specific mechanisms is supported by genetic mouse models. For example, mice carrying the factor V Leiden mutation have increased fibrin levels in the lung, heart, spleen kidney and brain [38]. A functional deficiency of TM results in vascular bed-specific fibrin deposition in mixed genetic backgrounds, but not in a C57BL strain [39]. Compared with wild-type mice, over-expression of fibrinogen resulted in increased fibrin deposition in the spleen, but not in the heart, liver, lung, brain or kidney [40]. Remarkably, when the hyper-fibrinogenemia mice were crossed with C57BL/TM−/− animals, fibrin deposition in the spleen was abrogated, while fibrin levels increased further in the liver [40]. Lineage-specific deletion of TM in the endothelium of mice resulted in severe thrombosis and lethal consumptive coagulopathy [41]. Fibrin deposition was

increased in all tissues examined except the brain [41]. On the opposite side of the hemostatic scale, deficiency of procoagulant proteins is associated with organ-specific hemorrhage. Tissue factor (TF)−/− mice that have been genetically engineered to express low levels of human TF have develop spontaneous bleeding in the adult heart, whereas mice that are null for factor IX have no such defect [42].

An important question is how a systemic imbalance in clotting factors results in local thrombosis. A common response is to invoke Virchow's triad and conclude that the systemic imbalance is channeled locally by stasis of flow and/or loss of vascular integrity [43–45]. For example, the propensity to develop deep venous thrombosis in congenital hypercoagulable states may be explained by relative stasis of blood in the lower extremities. Likewise, the increased risk for patients with heparin-induced thrombocytopenia (HIT) to develop clot at sites of invasive procedures or vascular lines may be attributed to disruption of the vascular wall [46].

However, the use of Virchow's triad, as it was originally proposed, to explain the systemic hypercoagulability-local thrombosis paradox does not fit all occasions. For example, patients with warfarin-induced necrosis do not have detectable loss of vascular integrity. The same is true for patients with myeloproliferative disorders and hepatic vein thrombosis, or post-bone-marrow-transplantation patients with veno-occlusive disease of the liver. Yet in each case, a systemic imbalance in hemostatic factors is translated into a local thrombotic phenotype.

The paradox is best explained by considering the biology of the endothelium. Endothelial cells are mini-factories for hemostatic factors. On the anticoagulant side, endothelial cells synthesize tissue factor pathway inhibitor (TFPI), heparan, ecto-ADPase, TM, endothelial protein C receptor (EPCR), nitric oxide, tissue-type plasminogen activator (t-PA), and cyclo-oxygenase. On the procoagulant side, the endothelium produces plasminogen activator inhibitor (PAI)-1, thromboxane, von Willebrand factor (vWF), protease-activated receptors, and possibly TF. In keeping with the theme of heterogeneity, the expression of these hemostatic factors varies in space and time. For example, on the spatial axis, TFPI is expressed predominantly in the microvascular endothelium [47], EPCR in the conduit vessels [48], TM in blood vessels of every caliber in all organs except the brain [23], t-PA in the pulmonary artery and pia mater of the brain [49], vWF on the venous side of the circulation [50], and endothelial nitric oxide synthase on the arterial side [51,52]. Expression of each of these products also varies over time. For example, in animal models of sepsis, endotoxin induces expression of vWF, TF and PAI-1 in ways that differ between vascular beds [50,53]. Based on the these observations, it seems likely that under normal conditions, endothelial cells from different sites of the vascular tree rely on different 'formulas' of anticoagulants and procoagulants to balance local hemostasis (Fig. 6).

These observations provide a foundation for a new perspective of hemostasis (Fig. 7). According to this model, the healthy

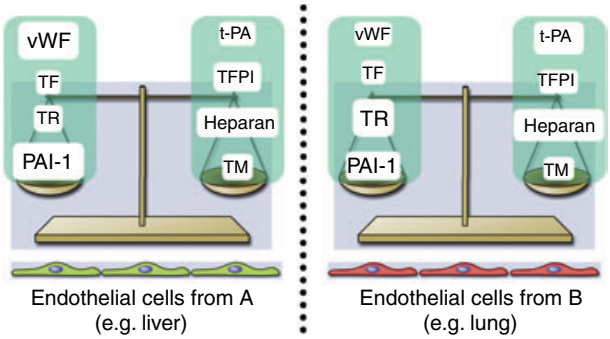

Fig. 6. Site-specific hemostatic formulas. Each endothelial cell contributes to the hemostatic balance by expressing and/or secreting surface receptors and soluble mediators. Receptors include the protease-activated receptors (or TR, thrombin receptor), thrombomodulin, tissue factor (TF), ecto-ADPase (not shown). Soluble mediators include von Willebrand factor (vWF), plasminogen activator inhibitor-1 (PAI-1), tissue-type plasminogen activator (t-PA), tissue factor pathway inhibitor (TFPI), and heparan. Each of these factors is differentially expressed from one site of the vascular tree to another. Thus at any point in time, the hemostatic balance is regulated by vascular bed-specific 'formulas'. Shown is a hypothetical example, in which an endothelial cell from a liver capillary relies more on vWF, PAI-1 and TFPI to balance hemostasis, whereas an endothelial cell from a lung capillary expresses more thrombin receptor, t-PA and heparan. Adapted with permission from W.C. Aird, *Crit Care Med.* 2001 July; 29(7 Suppl.): S28–34.

liver produces a relatively constant supply of serine proteases, cofactors (factors V and VIII, and protein S), fibrinogen, and ATIII. The bone marrow releases a constant number of monocytes and platelets each day, cells which are capable of expressing TF and/or providing cell surface membrane for assembly of clotting factor reaction complexes. The liver-derived proteins and the bone marrow-derived blood cells are systemically distributed to the various tissues of the body where they are integrated into the unique hemostatic balance of each and every vascular bed.

When there is a change in the systemic balance of proteins and/or cells – for e.g. congenital deficiency of protein C, activated monocytes in sepsis, or activated platelets in HIT – the systemic imbalance will interact with local endothelial-derived balances in ways that differ from one site to the next, resulting in vascular bed-specific thrombin generation and fibrin deposition. As one example, the observation that warfarin-induced skin necrosis, homozygous protein C deficiency and meningococcemia/purpura fulminans are each associated with a deficiency in functional protein C and dermal microvascular thrombosis, suggests that the local hemostatic balance of dermal postcapillary endothelial cells is disproportionately sensitive to systemic changes in protein C.

According to the above model, normal variation in endothelial properties is sufficient to explain the systemic imbalance-local thrombosis paradox. However, local changes in one or another vascular bed may also contribute to (or accentuate) the thrombotic phenotype. For example, subsets of endothelial cells may become activated and/or dysfunctional, leading to a local shift in hemostatic balance. Alternatively, stasis of flow –

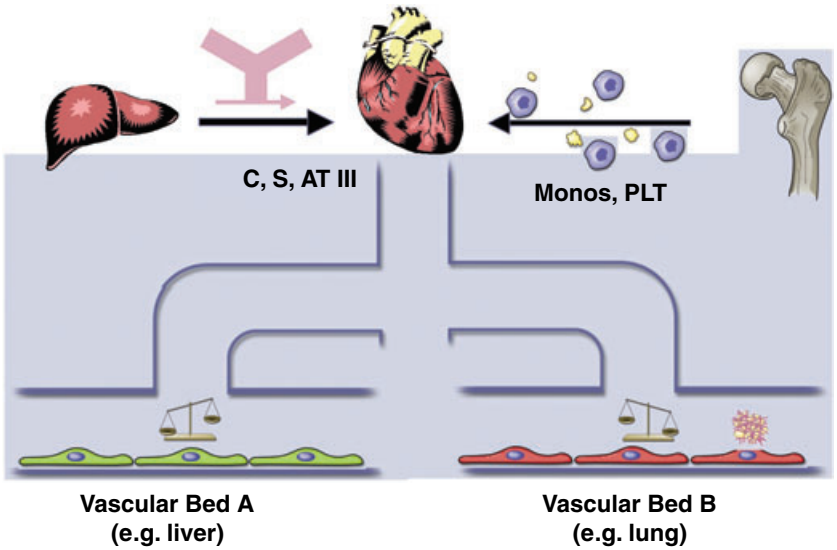

Fig. 7. Integrated model of hemostasis. The liver (left) produces the serine proteases, cofactors fibrinogen of the clotting cascade (shown as Y-shape); and many of the circulating natural anticoagulants [shown are protein C (C), protein S (S), antithrombin III (ATIII)]. The bone marrow (right) releases monocytes and platelets, which are capable of expressing tissue factor and/or an activated cell surface. The liver and bone marrow-derived proteins and cells are systemically distributed and integrated into the unique hemostatic balance of each vascular bed (shown are balances in two hypothetical vascular beds). Monos, monocytes; PLT, platelets. Adapted with permission from W.C. Aird, *Crit Care Med.* 2001 July; 29(7 Suppl.): S28–34.

arising from immobilization contractions, external compression, increased hydrostatic pressure, accumulation of leukocytes, and/or vasoconstriction – may contribute to the local imbalance. Reduced blood flow not only impairs clearance of activated serine proteases and cells, but also results in hypoxia and reduced laminar shear stress, both of which may promote a procoagulant endothelial phenotype. Finally, a disruption or denudation of the endothelium (e.g. as occurs in trauma or surgery) may result in the exposure of blood to TF in the subendothelial vessel wall.

In addition to providing a useful conceptual framework for understanding the local nature of thrombotic diathesis, this revised scheme recognizes the involvement of four functionally linked organ systems in mediating coagulation, namely the liver, the bone marrow, the cardiovascular system and the endothelium. In this way, one is reminded of the importance of the hepatocyte in synthesizing the serine proteases, the two co-factors, fibrinogen as well as the natural anticoagulants, ATIII, protein C and protein S; the critical role of the TF-expressing monocyte in initiating coagulation; the participation of the platelet in localizing and perpetuating the coagulation response; and the importance of the endothelial cell as an important manufacturer of hemostatic factors and regulator of the hemostatic balance. Moreover, the scheme incorporates both primary and secondary hemostasis. All too often, the cellular and soluble phases of coagulation are perceived as separate and independent entities that operate in series, when in fact they are highly integrated, parallel processes.

Given the mosaic-like nature of the hemostatic balance, an important goal will be to decode the equations (anticoagulants and procoagulants) that govern vascular bed-specific hemostasis. This may be accomplished, in part, through continued proteomic and genomic mapping of the intact endothelium. Future efforts to model site-specific hemostasis will need to take into consideration not only the mix of classic endothelial-derived procoagulants and anticoagulants, but also the relative contribution of other local hemostatic forces, including regional flow, monocyte and platelet interactions, and microparticle release.

Another strategy that may yield important information about local balances is the use catheters to sample blood from different sites of the vascular tree. The current practice of assaying pooled venous blood from the brachial vein risks overlooking 'hot spots' in the vascular tree by virtue of a dilution effect. It is not out of the question that circulating endothelial cells and microparticles may carry information about the hemostatic balance from their sites of origin and that such a signature may ultimately be leveraged for diagnostic and/or therapeutic gain. Finally, the field of molecular imaging, while in its infancy, will likely revolutionize our capacity to interrogate the endothelium and to assay for activation peptides and fibrin deposition in real time.

By correlating the pattern of expression of hemostatic genes with the phenotype of genetic mouse models of hyper- and hypo-coagulability, we should gain additional insight into site-specific balances. As one example, the finding that endothelial cell-specific deletion of TM increases fibrin deposition in the many tissues except the brain is consistent with the observation that TM is expressed at low levels in the brain to begin with [23,41]. When taken together, these data suggest that the vasculature of the brain relies on anticoagulant mechanisms other than TM to balance local hemostasis. Countering these findings, however, is the observation that mice null for protein C develop extensive thrombosis in the brain during embryogenesis and in the neonatal period [54]. Clearly, much work

needs to be done to unravel the complexities of the *in vivo* models.

With few exceptions, anticoagulation therapy is administered systemically, either orally, or by the subcutaneous or intravenous route. Most if not all agents inhibit coagulation throughout the vasculature, and not just locally where they are needed. For this reason, the therapeutic window of these agents is exceedingly narrow and the risk for bleeding disproportionately high. Over the past years, there has been a concerted effort to develop new classes of anticoagulants with improved safety profiles.

With continued advances in endothelial cell biology, it will ultimately be possible to tailor anticoagulant strategies to specific vascular beds. To some extent, such an effort has already begun – although the methods are relatively crude and the results anecdotal. Examples include the local administration of thrombolytic agents to venous or arterial clots, and the use of the protein C zymogen, which might be predicted to undergo maximal activation at sites of demand. A more detailed understanding of site-specific balances should provide a powerful foundation of site-specific therapy.

Conclusions

The first successful isolation of human endothelial cells in 1973 has been said to mark the beginning of 'modern vascular biology' [55]. There is no question that the cell culture system provided the research community with a powerful new tool for dissecting endothelial cell biology and paved the way for breathtaking advances in the field. Indeed, most of our present-day knowledge about the endothelium – from cell surface receptors, to signaling pathways, transcriptional networks, cytoskeleton, and cellular function – is directly attributable to our capacity to study endothelial cells in culture. It may be argued, however, that cell culture studies – *in and of themselves* – are beginning to yield diminishing returns. Simply put, the *in vitro* system fails to fully capture the spatial and temporal dynamics of the endothelium. An important challenge for the future is to learn how best to leverage the advantages of *in vitro* and *in vivo* approaches to further advance the field and narrow the chasm between bench and bedside (Fig. 8).

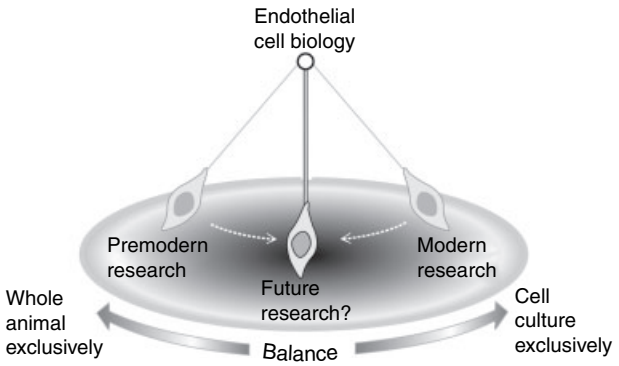

Fig. 8. The scientific model in endothelial cell biology. Protocols for reliably culturing endothelial cells *in vitro* did not exist before the 1970s. Thus, investigators focused on the endothelium in the context of the intact blood vessel or whole animal. These studies – which revolved largely around ultrastructure and physiology – engendered an integrated (or holistic) approach to vascular biology ('pre-modern' research). The discovery of endothelial cell culture in 1973 has been said to mark the beginning of modern vascular biology. As powerful and important as the cell culture system may be, it cannot – when used alone – reliably address properties of emergence. There is increasing appreciation for the importance of an integrated approach, in which *in vitro* and *in vivo* strategies are employed together. Illustrated by Steve Moskowitz.

Acknowledgements

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