

Integration of cardiovascular regulation by the blood/endothelium cell-free layer

C. Makena Hightower,¹ Beatriz Y. Salazar Vázquez,^{1,2}
Sung Woo Park,³ Krishna Sriram,³ Judith Martini,⁴ Ozlem Yalcin,¹
Amy G. Tsai,¹ Pedro Cabrales,¹ Daniel M. Tartakovsky,³
Paul C. Johnson¹ and Marcos Intaglietta^{1*}

The cell-free layer (CFL) width separating red blood cells in flowing blood from the endothelial cell membrane is shown to be a regulator of the balance between nitric oxide (NO) production by the endothelium and NO scavenging by blood hemoglobin. The CFL width is determined by hematocrit (Hct) and the vessel wall flow velocity gradient. These factors and blood and plasma viscosity determine vessel wall shear stress which regulates the production of NO in the vascular wall. Mathematical modeling and experimental findings show that vessel wall NO concentration is a strong nonlinear function of Hct and that small Hct variations have comparatively large effects on blood pressure regulation. Furthermore, NO concentration is a regulator of inflammation and oxygen metabolism. Therefore, small, sustained perturbations of Hct may have long-term effects that can promote pro-hypertensive and pro-inflammatory conditions. In this context, Hct and its variability are directly related to vascular tone, peripheral vascular resistance, oxygen transport and delivery, and inflammation. These effects are relevant to the analysis and understanding of blood pressure regulation, as NO bioavailability regulates the contractile state of blood vessels. Furthermore, regulation of the CFL is a direct function of blood composition therefore understanding of its physiology relates to the design and management of fluid resuscitation fluids. From a medical perspective, these studies propose that it should be of clinical interest to note small variations in patient's Hct levels given their importance in modulating the CFL width and therefore NO bioavailability. © 2011 John Wiley & Sons, Inc. *WIREs Syst Biol Med* 2011 3 458–470 DOI: 10.1002/wsbm.150

INTRODUCTION

The cell-free layer (CFL) is a thin zone between the column of blood and the endothelial membrane. It is from time to time intruded by red blood cells (RBCs) and activated leukocytes, and contains a gel-like glycocalyx composed of different sugars attached to the endothelial membrane surface. The CFL is similar to the lubricating layer between moving parts in mechanics, being a viscous medium interposed between blood and the vessel wall (Figure 1). Similar to mechanical systems it is in part generated in dynamic conditions, while blood is flowing, as cells and formed elements drift away from the vessel wall due to the flow velocity gradient and the effect of shear on the glycocalyx.^{1–4} Different from mechanical

*Correspondence to: mintagli@ucsd.edu

¹Department of Bioengineering, University of California, San Diego, La Jolla, CA, USA

²Facultad de Medicina, Universidad Juárez del Estado de Durango, Durango, Durango, México

³Department of Mechanical and Aerospace Engineering, University of California, San Diego, La Jolla, CA, USA

⁴Department of Anesthesia and Intensive Care Medicine, Innsbruck Medical University, Innsbruck, Austria

Based on a presentation to the 8th International Conference on Pathways, Networks and Systems Medicine. www.aegeanconferences.org



DOI: 10.1002/wsbm.150

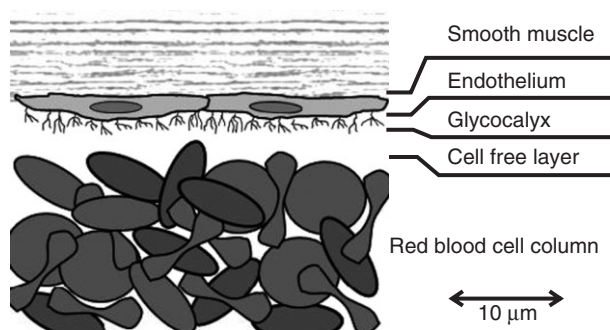


FIGURE 1 | Diagrammatic representation of the compartments at the blood tissue interface drawn approximately to scale during flow.

systems, it separates a comparatively viscous fluid, blood, from a protein surface, the endothelial cell membrane.

The discovery that shear stress at the vessel wall is a principal mechanism for causing the endothelium to produce a number of mediators such as nitric oxide (NO), prostaglandin, and endothelin has focused attention on the biology and biophysics of the endothelium as a principal cardiovascular regulator.^{5–7} Nonetheless, the endothelium and the tissue respond to events regulated in the CFL. In particular, the width of the CFL determines the bioavailability of NO as it separates its source, namely the tissue and the endothelium, from a major NO sink, the hemoglobin (Hb) contained in RBCs that actively scavenge NO diffusing from the vessel wall into blood.^{8,9} Therefore, the bioavailability of NO is determined by the balance between the rate of production of NO by the tissue and the endothelium by biochemical mechano-transduction and the rate of consumption of NO by Hb and the tissue. This balance is strongly influenced by the width of the CFL.¹⁰

While the endothelium is a producer of NO, the width of the CFL is the regulator of its bioavailability. Furthermore, the width of the CFL also regulates the resistance to oxygen diffusion to and from the column of blood and how shear stress is transmitted from the flowing blood to the endothelial surface. The bioavailability of NO is a critical factor in regulating the oxygen metabolic demand of the tissues^{11,12} and controlling inflammation.¹³ Therefore, the CFL is directly involved in the regulation of several principal homeostatic mechanisms.

STRUCTURE OF THE CELL-FREE LAYER

The CFL is a region bound on the luminal side by a stochastic surface made by the outer boundary of the RBC core and on the abluminal side by the

endothelia cell membrane. This region is occupied by blood plasma, the fluid in which RBCs are suspended and which extends and permeates into the gel-like structure of the glycocalyx, next to the endothelial cell membrane.

The mechanism by which RBCs under flow conditions present a surface separated from the wall by a fluid layer is complex.¹⁴ The concentration of RBCs near the vessel wall is not uniform since as a consequence of their particulate nature of RBC can be positioned nearer to the wall than the radial distance to the RBCs center. Therefore, given the discoidal shape and random orientation of RBCs in flow there is an annulus at the tube wall with a volume concentration of cells that is lower than in the central blood vessel core. Flow superposes to this geometrical constraint hydrodynamic effects that cause particles to migrate toward an annulus according to the Segre–Silberberg effect² which is opposed by a dilation effect due to the tendency of closely packed suspensions subjected to shear to expand at right angle to the direction of shear,¹⁵ an effect that underlies the physics of shear induced viscosity augmentation. These phenomena are also in part dependent on vessel radius, particularly for the smaller microcirculatory vessels.

The high volumetric concentration of RBCs, their flexibility, their discoidal shape, and the nature of the plasma layer boundaries significantly complicates the rigorous analysis of mechanisms that determine the width of the plasma layer. On the luminal side relative motion of RBCs due to flow at the edge of the RBC core causes the interface between blood and the plasma layer to have a spatial and temporal stochastic configuration (see Section on *Stochastic Surfaces*). At the abluminal side the space between the endothelial cell membrane and the free flowing plasma transitions from a gel-like structure of carbohydrates attached to the endothelial cell membrane into a forest-like configuration where branches and tree tops are bent by the wind-like action of the flowing plasma.

The CFL is described in terms of its thickness, defined by the separation between the endothelial cell membrane and the stochastic boundary that limits the RBC core. This parameter is primarily determined by the extent of the glycocalyx, the hematocrit (Hct), the velocity gradient at the wall, and the endothelial membrane surface.

THE GLYCOCALYX

The vascular endothelial glycocalyx is a dynamic carbohydrate network cover connected to the endothelium by proteoglycans and glycoproteins. Soluble plasma components are incorporated at

its luminal side in dynamic equilibrium with the components in the flowing blood, which determines composition and thickness of the glycocalyx. Shear stress and enzymatic action also influence the glycocalyx extent as they affect its composition. The glycocalyx presents a net negatively charged surface to the bloodstream.

The glycocalyx is as an RBC 'exclusion zone' or 'gap' between the flowing RBCs and the endothelium. However, no exclusion zone has been found for rolling white blood cells, suggesting a low mechanical stiffness for the glycocalyx.³

The glycocalyx influences blood cell/vessel wall interactions limiting access to the endothelial cell membrane. It repulses RBCs from the endothelium. In the microcirculation, an RBC exclusion zone adjacent to the endothelium seen *in vivo* is decreased upon breakdown of the glycocalyx.¹⁶ Platelets seldom interact with the endothelium in control conditions, while partial glycocalyx removal increases platelet/vessel wall interactions.¹⁷ In terms of leukocyte/vessel wall interactions the glycocalyx contains adhesion molecules (P-selectin, ICAM-1, and VCAM-1) and attenuates adhesion of leukocytes to these molecules via steric hindrance.

The endothelial glycocalyx is a determinant of vascular fluid permeability, as evidenced by the partial removal by enzymes causing myocardial edema.^{18,19} According to the classical Starling model of fluid exchange,²⁰ fluid filtration across the capillary endothelium results from the balance of hydraulic and colloid osmotic pressures across a system of hydraulic pores at endothelial cell junctions that confer to the endothelium the characteristics of an oncotic membrane. The existence of the endothelial glycocalyx and its influence on edema formation led Weinbaum²¹ to propose that fluid exchange is mostly determined by the endothelial glycocalyx.

In healthy vessels, the endothelial glycocalyx mediates shear stress sensing, as shown in the treatment with heparinase which impairs the response to changes of shear stress variations and NO production.²² In healthy volunteers the systemic glycocalyx volume decreases by half within 6 h after induction of acute hyperglycemia¹⁸ and is reduced by high-fat and high-cholesterol diet.¹⁸ It is reduced by hyperglycemia,²³ in type 1 diabetic patients, and is further reduced in diabetes with microalbuminuria.²⁴ Microvascular dysfunction²⁵ the common component of many pathologic process is manifested in endothelial cell swelling, leukocytes adhesion and transmigration, inflammation and vascular permeability increases, all phenomena mediated by the extent and composition of the glycocalyx.^{18,26,27}

DIMENSIONS OF THE GLYCOCALYX

Measurement of the glycocalyx thickness in the microcirculation using intravital microscopy is approximate due to the limits of resolution of the optical microscope. Furthermore, the endothelial glycocalyx of larger vessels cannot be visualized by the methods of intravital microscopy. A frequently used method consists in visualizing and measuring the diameter of the RBC + plasma column of flowing blood and subtracting from this the anatomical internal diameter of the microvessels in the range of 100–15 μm yields dimensions of the order 0.4–0.5 μm . Direct visualization of the glycocalyx can be obtained by selectively binding fluorescent markers to its different molecular components and using confocal laser scanning microscopy. This and similar methodologies were reviewed by Reitsma et al.²⁶ and yield estimates of glycocalyx layer thickness as large as $2.5 \pm 0.5 \mu\text{m}$.²⁸ Conversely, Potter et al.,²⁹ using fluorescent microparticle image velocimetry,³⁰ found that for venules the mean glycocalyx thickness is $0.52 \pm 0.27 \mu\text{m}$.

A different approach consist in using two dies (or markers),³¹ one n that does not dissolve in the glycocalyx layer, and a second m that dissolves. Upon introduction into the circulation the concentration of the non-soluble die c_n is determined by the intravascular volume of the circulation V_{vasc} minus the volume of the plasma layer $V_{\text{pl-layer}}$, and the concentration of the soluble die c_m is determined by V_{vasc} . Therefore:

$$c_n = \frac{n}{V_{\text{vasc}} - V_{\text{pl-layer}}}; c_m = \frac{m}{V_{\text{vasc}}}$$

$$\therefore V_{\text{pl-layer}} = \frac{m}{c_m} - \frac{n}{c_n}$$

Application of this technique yields estimates of the glycocalyx volume as a large as 25% of the total vascular volume. However, this result is problematic because the partition coefficient of the tracers between plasma and the fluid within the glycocalyx and the total area of the circulation is not known with the necessary precision.³²

PLASMA LAYER AND CELL-FREE LAYER

Information on the cell-free layer width *in vivo* has been limited and mostly available in terms of mean width and its standard deviation. Methods for measuring the width of the CFL primarily used bright field microscopy, and reported measurements usually include the thickness of the glycocalyx. Kim

et al.³³ developed a methodology for measuring the CFL width based on high-speed framing rate microscopic recordings with a 0.4- μm resolution. This methodology shows that the mean width of the CFL is dependent on vessel diameter. In the diameter range 10–40 μm the CFL layer is in the range of 1.1–2.1 μm and for a glycocalyx thickness of 0.4–0.5 μm the free flowing plasma layer would be in the range of 0.7–1.6 μm .

The CFL changes dimensions as a function of time in a non-Gaussian way since variations in the luminal side can extend into the RBC core, while those in the abluminal side are limited by the endothelial surface.

VARIATION OF THE CELL-FREE LAYER WITH Hct

The CFL thickness varies with Hct as the concentration of RBCs and shear stress dilate the RBC column. Variation of CFL thickness also affects shear stress in the CFL, which influences the glycocalyx thickness and therefore the extent of the CFL. The combination of these effects has been found to affect blood viscosity,¹⁴ which in combination with shear stress in the CFL determines the production of shear stress-dependent mediators by the endothelium.

In terms of vascular regulation Hct in the healthy population varies as function of age, gender, environmental factors, genetics, nutrition, level of hydration, time in the daily cycle, and seasonal variations,³⁴ yielding a dispersion of values which may be of the order of $\pm 10\%$ of the average for the population stratified by age and gender. Therefore, the variability of Hct implies the variability of CFL thickness.

Changes in CFL width also influence the degree of scavenging of NO released from the endothelium by the RBC core.^{8,35} Furthermore, the rate of NO production by the endothelium is a function of the shears stress imparted by the flowing blood to the endothelial membrane, an effect transmitted by the CFL.

MEASUREMENT OF THE CELL-FREE LAYER WIDTH

Data on the separation between the vessel wall and the RBC column was until recently primarily available from visual microscopic estimates from video images.³⁶

This methodology based on visual observation of the microcirculation provided an estimate of average and statistical variation of the CFL, but did not report detailed information on temporal or spatial

variation of the CFL. Kim et al. developed a specific methodology for spatially and temporally quantifying the boundaries of the CFL using the position of the outermost RBC surface from the blood vessel centerline as the interface between the RBC column and the plasma layer.³³

The dimensional properties of microvessels, microscopic optical resolution, the dynamics of microvascular flow, and the pixel resolution of television high frame rate television cameras determined that a field size of $200 \times 200 \mu\text{m}$ could be recorded with $\sim 0.4 \mu\text{m}$ spatial resolution in the axial flow direction, for a maximal edge velocity of the RBC column of 9 mm/s typical for large arterioles requiring a framing rate in the range of 4500/s to detect all RBCs.

This methodology determined the position of the vessel wall by measuring the light intensity transition from dark to light pixels along the direction of an analysis line perpendicular to near the vessel wall.^{37,38} The width of the RBC column is found by converting its light intensity image into a binary image by means of an automatic thresholding method³⁹ that maximizes the variance between pixels presumed to belong to the RBC column, and those outside of this column.

The study of Kim et al.⁴⁰ provided the principal temporal and spatial information on the CFL characteristics in the arterioles of the rat cremaster. It was found that CFL width is directly related to the vessel diameter, increasing from 0.8 μm in 10 μm arterioles to 2.9 μm in 50 μm arterioles. Temporal variability was a direct function of vessel diameter and was not normally distributed, being greater on the blood side that vessel wall direction. The persistence of the temporal variations of the CFL downstream from a point of observation declined exponentially with a correlation length of the order of 30 μm that was independent of vessel diameter. There was no temporal variability on this time scale at vessel side and spatial variability was significantly smaller. Therefore, the effective boundaries of the CFL have statistical features that must be considered when data on the width is used to analyze its physiological effects.

THE STOCHASTIC SURFACES BOUNDING THE CELL-FREE LAYER

A mathematical model that includes the stochastic (randomness) nature of the CFL boundaries is constructed by defining its two radii, that of the RBC column R_{RBC} and R_{e} , which denote the inner radius of the endothelial surface according to Figure 2. Both radii vary along the length of a blood vessel, i.e., depend on the spatial coordinates (ϕ, z) ; the RBC

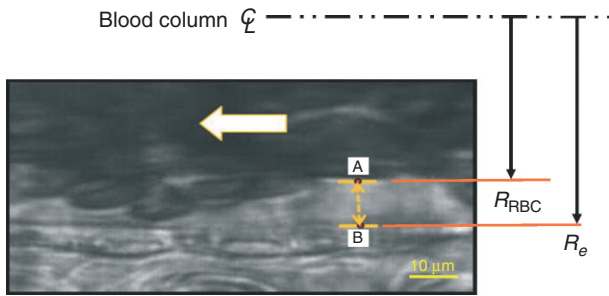


FIGURE 2 | Cell-free plasma layer (CFL) *in vivo*. Image of an arteriolar wall showing CFL width, the distance between the RBC column (black), and the endothelium lining the vessel wall. This image shows a few red blood cells (RBCs) positioned closer to the endothelium. CFL width is the distance between A and B, or the difference between the radius of the inner endothelial surface R_e and radius of the RBC column R_{RBC} . The RBCs are $7\ \mu\text{m}$ in diameter. Blood flow is from right to left (arrow).

column radius R_{RBC} also varies with time t , reflecting changes in the CFL width due to intruding RBCs. In other words, $R_e = R_e(\varphi, z)$ and $R_{RBC} = R_{RBC}(\varphi, z, t)$. The spatiotemporal variability of the radii bounding the CFL, combined with the practical impossibility to measure their values at every point (φ, z) along a blood vessel and every point in time, renders estimates of the radii R_{RBC} and R_e uncertain. This uncertainty propagates through the modeling process, affecting predictions of key physiological quantities as wall shear stress (WSS), effective blood viscosity, and definition of the barriers to oxygen and NO transport.

The uncertainty is quantified by treating the radii R_{RBC} and R_e as random fields, which vary not only in physical space (φ, z) and time t , but also in the probability space $\omega \in \Omega$ (i.e., the collection of all possible realizations of the random radii R_{RBC} and R_e) so that $R_e = R_e(\varphi, z, \omega)$ and $R_{RBC} = R_{RBC}(\varphi, z, t, \omega)$.

The randomness (uncertainty) of R_{RBC} and R_e must be introduced into the modeling process that defined flow and transport processes in the CFL, requiring that flow (e.g., Stokes) and transport (advection–dispersion) equations be defined on the random domain bounded by $R_e = R_e(\varphi, z, \omega)$ and $R_{RBC} = R_{RBC}(\varphi, z, t, \omega)$. Analysis of this problem is facilitated by the use of stochastic mappings, an approach that has been promulgated in a series of recent papers.^{41–43} The basic idea of this approach is to map a random domain onto its deterministic counterpart. In the present context, this is accomplished by transforming the (r, φ, z) coordinate system associated with the random boundaries of the CFL into the (ξ_1, ξ_2, ξ_3) coordinate system, such that $\xi_1 = (r - R_{RBC})/(R_e - R_{RBC})$, $\xi_2 = \varphi$, and $\xi_3 = z$. While the radius r varies between the

two random limits, $R_{RBC}(\varphi, z, t, \omega) \leq r \leq R_e(\varphi, z, \omega)$, the transformed variable ξ_1 is defined on the deterministic interval $0 \leq \xi_1 \leq 1$.

As the transformation Jacobian $J(\xi_1, \xi_2, \xi_3, \omega) \equiv \partial(r, \varphi, z)/\partial(\xi_1, \xi_2, \xi_3)$ is random, the transformed flow and/or transport equations become stochastic. Because the theory of stochastic differential equations defined on deterministic domains is relatively mature, one can use a variety of well-established techniques, including perturbation expansions,⁴¹ polynomial chaos expansions,⁴² and stochastic collocation methods.⁴⁴

Random roughness of the CFL boundaries can significantly affect physiological phenomena taking place in the CFL. For example, a classical treatment of blood flow in arteries whose walls are modeled as smooth surfaces of radii R relies on the Poiseuille law to relate volumetric flow rate Q to pressure gradient dp/dz (pressure drop across a blood vessel divided by a vessel's length),

$$Q = -\frac{\pi R^4}{8\mu} \frac{dp}{dz},$$

where μ is the dynamic blood viscosity of blood. For flow in vessels with rough walls, this expression has to be modified as:

$$Q = -\frac{\pi R^4 C_L}{8\mu} \frac{dp}{dz}$$

where $C_L \leq 1$ is the Lomize roughness coefficient.⁴⁵ Depending on the degree of wall roughness, the reliance on the standard Poiseuille law can yield estimates of the blood viscosity μ that are as much as 20% lower.

As the Lomize roughness coefficient C_L is largely phenomenological, its values for different vessel radii cannot be ascertained with certainty and measurements of the CFL topography (i.e., observations of R_{RBC} and R_e) cannot be readily incorporated to improve estimates of C_L . The stochastic framework overcomes these limitations by both providing a theoretical foundation for the concept of the Lomize roughness coefficient and facilitating data processing using data to estimate statistical properties of the random fields R_{RBC} and R_e , including their means, variances, and space-time correlation functions.

EFFECTS ON OXYGEN DELIVERY

Oxygen exits the blood vessel driven by radial concentration gradients. The diffusional properties of

the medium through which oxygen traverses from the blood core center to the vessel wall are complex and became highly nonuniform in the CFL with potentially large consequences for the diffusion process.

Oxygen diffuses in blood by direct diffusion in the plasma space, and facilitated diffusion within RBCs.⁴⁶ The relative effect of these processes in blood was first analyzed by Hellums⁴⁷ and subsequently extended to blood vessels.⁴⁸ These studies indicate the presence of a substantial barrier to diffusion in the plasma layer. The presence of this barrier remains a controversial issue, as shown in the studies and analysis of Tsai et al.⁴⁹ who measured the presence of substantial oxygen concentration gradients near the vessel wall of arterioles. These large gradients can be explained by the proposed large barrier function of the CFL; however, the study of Tsai et al. determined by performing mass balance analysis that oxygen exits the blood vessels at a rate that was incompatible with the presence of a significant barrier, and concluded that the large vessels wall oxygen gradients were due to the large oxygen consumption of the vessel wall, rather than a high diffusional resistance in plasma.

Analysis of the dispersion of the RBC trajectory as they flow in arterioles in conditions of normal Hct and in hemodilution shows a variability (standard deviation) in the trajectory over its path of $1.8 \pm 1.1 \mu\text{m}$. Within the RBC core oxygen diffuses by a combination of facilitated and conventional diffusion, and in the CFL the process is one of conventional diffusion, and therefore likely to have a greater diffusional hindrance. This effect appears to be in part mitigated by convective effects created by the variability of the outer surface of the RBC core.⁵⁰ Therefore, the previously discussed stochastic methods of analysis may resolve some of these existing controversies⁵¹ on how oxygen is managed as it transits from the blood to the tissue.

TRANSMISSION OF SHEAR STRESS TO THE ENDOTHELIUM IS A FUNCTION OF PLASMA LAYER VISCOSITY AND THICKNESS

No exclusion zone was found for rolling white blood cells, suggesting that they have the ability to compress the glycocalyx in these vessels, which complies with the estimated low stiffness of the glycocalyx.^{52–54}

Tarbell and Pahakis reviewed the current concepts on mechano-transduction by the (membrane-bound) glycocalyx.^{55,56} They conclude that the glycocalyx core proteins are responsible for the transmission of shear stress signals into specific cell signaling processes, e.g., NO production and cytoskeletal

reorganization. At the same time, shear stress is transmitted to other regions of the endothelial cell as well, such as intercellular junctions and basal adhesion plaques, which are responsible for additional shear sensing even in the absence of a glycocalyx. It should be noted that the actual shear stress developed is also a function of the stochastic nature of the RBC column interface and the geometry of the vessel wall surface.⁵⁷

MODULATION OF NO VESSEL WALL BIOAVAILABILITY BY PLASMA LAYER THICKNESS

Hct is one of the principal factors affecting CFL width as well as NO bioavailability given its multiple roles as a determinant of blood viscosity and NO scavenging; therefore, it becomes important to determine the sensitivity of NO concentration in the vascular to changes in Hct, which varies due physiological and pathophysiological effects.

Blood flow is also a critical factor in CFL formation, as shown in the study of Liao et al., in 60- μm isolated arterioles.⁵⁸ In these experiments blood flow caused almost no NO depletion in the arteriolar wall, whereas flow stasis caused a large effect, a difference attributable to the formation of the CFL by flow.

Experimental studies where NO concentration in the vascular wall is measured have mostly focused on the direct determination of perivascular NO by means of polarographic microelectrodes during comparatively large changes in Hct due to hemodilution with different plasma expanders.^{59,60} These studies confirm the tenets of prior theoretical modeling concerning the effects of NO scavenging and shear stress, but did not test the sensitivity of vessel wall NO concentration to small changes of Hct. This problem was analyzed by Sriram et al.³⁵ by a mathematical model where changes in Hct within the range of variability found in the normal population^{61,62} change apparent blood viscosity,⁶³ WSS,⁶⁴ the rate of NO production by the endothelium,^{7,65–68} the rate of NO scavenging by RBCs⁶⁹ and the interactions between NO and oxygen transport.^{9,70–72}

The development of the CFL and its dependence on Hct, flow and the rheological properties of blood is described in terms of a rigorously derived blood velocity profile that represents arteriolar hemodynamics as two-phase flow with the RBC rich core and the RBC free plasma layer,⁶⁴ and experimental data^{33,73} is used to characterize the CFL dependence on Hct.

This model demonstrates that relatively small changes in systemic Hct significantly change the concentration of NO in the vascular wall, a result that is strongly dependent on the relationship between NO production by the endothelium and shear stress. The relationship between WSS and NO production is reported in previous studies^{7,65,71,74}; however, the *in vivo* functional relationship between these quantities is still not well defined and a present an S-shaped curve appears to be the most probable functional relationship between the parameters.⁷⁴

BLOOD PRESSURE REGULATION, Hct, AND CFL WIDTH

The bioavailability of NO in the vessel wall is a major determinant of peripheral vascular resistance (PVR) as it governs constriction and relaxation of arterioles. A direct consequence is that Hct is critical in blood pressure (BP) regulation. This regulation is counterintuitive as in conditions of normal endothelium the increase of Hct and, therefore, blood viscosity and WSS lowers BP, an effect experimentally verified in acute interventions. Conversely, lowered endothelial shears stress sensitivity, endothelial dysfunction, injury, as well as abnormalities diminishing WSS lower NO bioavailability promoting vasoconstriction and a pro-inflammatory environment, leading to and preserving a hypertensive state. Notably, elevated Hct levels were previously related to the incidence of hypertension and cardiovascular diseases. The proposed significant involvement of the CFL in the regulation of NO bioavailability suggests a mechanism by which elevation of Hct (and therefore blood viscosity) can be of cardiovascular benefit.

The human population presents a small range of individual Hct variability whose physiological significance has not been studied until recently, as most experimental investigations of anemia and polycythemia typically deal with changes in Hct > 20%.^{75–77} In fact, it is generally assumed that Hct changes associated with the normal variability of this parameter in the population are too small to significantly affect blood flow regulation and oxygen transport.

Directly measuring CFL is experimentally challenging, requiring the availability of tissue where blood vessels can be visualized with maximal resolution and clarity. A more practical approach is to use changes of Hct as a surrogate for changes in CFL, and to investigate the corresponding changes in BP. Martini et al.⁷⁸ acutely varied Hct in unanesthetized normal hamsters by performing isovolemic exchange transfusion with packed RBCs finding that Hct

increases between 7 and 13% of baseline caused BP to drop by about 10 mmHg from baseline. As expected, when Hct was increased further BP returned to baseline values and when Hct > 30% pressure rose above the baseline.⁷⁹ A similar effect was reported by Richardson and Guyton⁷⁷ who found that injection of packed RBCs in anesthetized dogs caused BP change from 111 to 97 mmHg.

Changes in Hct of the order of 5% are a weak stimulus; however, it cannot be excluded that cardiovascular regulation may be extraordinarily sensitive in matching oxygen demand and delivery. This kind of regulation would presumably lower cardiac output as Hct is increased, thus maintaining oxygen delivery constant. Remarkably, it was found that increasing Hct was associated with significant increased (up to 25% of baseline) cardiac output, showing that small increases in Hct do not lower BP to regulate oxygen delivery.⁷⁸ The divergent changes in BP and cardiac output support the alternative explanation that these effects are due to a significant decrease in PVR due to vasodilatation, far exceeding the effect due to the small increase of vascular resistance as a result of increased blood viscosity. These effects in combination with the changes in CFL found over the range of Hct variations related to this study are shown in Figure 3.

The role of oxygen carrying capacity in regulating BP when Hct increases was investigated by elevating Hct with non-oxygen carrying RBCs whose Hb was converted to met-Hb.⁸³ In this experiment, small increases in Hct caused a similar reduction of BP as in the case of normal RBCs, and cardiac output also increased but not to the same extent probably due to the lowered intrinsic oxygen carrying capacity of blood. In these experiments, the effects took place over a greater range of Hct increase which could be due to the decreased plasma layer content of NO scavenging Hb.

The direct involvement of NO regulation in the effects due to small changes in Hct was demonstrated by performing an Hct augmentation experiment after administration of pharmacological dosage of *N*-nitro-L-arginine methyl ester (L-NAME) in hamsters. Inhibition of NO synthesis by L-NAME caused BP to increase, which increased further with small Hct augmentations. Carrying out the same experiments in eNOS-knockout mice (B6.129P2-Nos3tm1Unc, Charles River Laboratories, Boston, MA) did not have an effect, which was otherwise evident in normal wild types.

Acute Hct augmentations within the physiological range lower BP, while maintaining or enhancing blood flow, suggesting the existence of a chronic

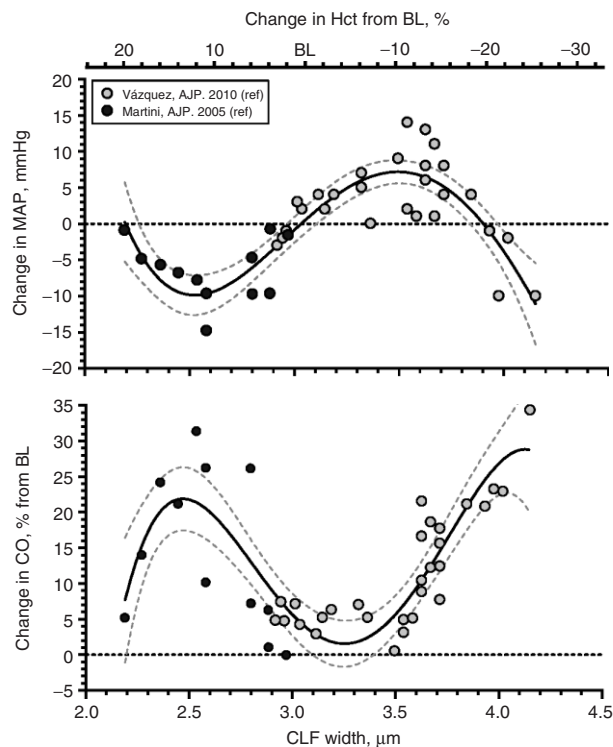


FIGURE 3 | Changes in blood pressure and cardiac output due to small changes in Hct according to the experimental results of Martini et al.^{78,80} and Salazar Vázquez et al.⁸¹ The data is presented as a function of the change in Hct and the corresponding changes in plasma layer width in arterioles according to the results of Yalcin et al.⁸²

optimal Hct, higher than normal, for conditions of healthy, normal endothelium. We hypothesize that there is an optimal Hct range and that maintenance of Hct at this level lowers cardiovascular risk. If cardiovascular protective effects of this optimal Hct range are substantiated, our work could provide a basis for prevention and treatment interventions for hypertension and other related vascular diseases. As a corollary, evidence for high Hct and BP would be indicative of endothelial dysfunction.

HEALTH RELATED SIGNIFICANCE OF CFL REGULATION

The Control of Blood Pressure

BP control is a significant determinant of longevity. This goal today is mostly achieved by pharmacological means where the first line treatment is by means of diuretics, followed by approaches that treat vasoconstriction by central mediation. However, treatment by diuretics, established before the discovery of the role of NO, is presumed to regulate BP by changing circulating blood volume. NO is a potent dilator of arterioles, the microvessels mostly responsible for setting PVR

and therefore BP. Vessel wall NO bioavailability is not centrally mediated, rather it is a local factor. We propose that this local regulation of BP is as important as central regulation, and may provide for an additional approach for treating hypertension that is less dependent on pharmacological interventions, and of potential long lasting health benefits. Fazio et al.⁸⁴ found that a significant decrease in BP (-6% , $P < 0.005$) corresponded to a significant increase in Hct in patients treated with the diuretic furosemide (6% , $P < 0.005$).

BP is determined by PVR where blood viscosity, a parameter strongly influenced by Hct is a factor. Although the association between blood viscosity, PVR, and BP was repeatedly investigated it has not led to conclusive results. It is now apparent that this is because the effect of increasing viscosity is negated by increased WSS, the mechano-transduction related increase of NO bioavailability and corresponding vasodilatation, and vice versa. Treatment of hypertension with diuretics and its related increase in Hct, blood viscosity, WSS and presumably increased NO bioavailability should contribute in lowering BP, and may be the major cause for their therapeutic efficacy. Furthermore, it should be of clinical interest to focus on patient's Hct levels given their importance in modulating the CFL width.

These considerations suggest that one of the initial triggers for hypertension could be the disruption of the balance of effects taking place in the plasma layer. This imbalance of NO bioavailability could be either positive or negative, and may not manifest initially as hypertension; however, it could be evident as a preclinical form of the disease in terms of paired small excursions from an optimal BP and Hct. Hypertension would then be in part due to the outcome of cumulative effects due to this initial imbalance, supported by the additional long range effects of associated with altered NO bioavailability promoting a lifelong pro-inflammatory condition. An intriguing aspect of this process is that the imbalance in the relationship BP and Hct could be initially corrected by simple interventions such as exercise and diet.

Plasma Expanders and Transfusion Medicine

Intravenous fluid administration is the initial treatment for blood losses, used up to the so called 'transfusion trigger' when a blood transfusion is necessary because fluid replacement via plasma expanders no longer provides sufficient oxygen delivery capacity to maintain tissue metabolism. Recent findings in the

field of shock resuscitation, related to the development of non-blood-based oxygen carriers show that restoration of microvascular function is as critical as maintenance of oxygen carrying capacity,⁸⁵ and that maintenance of microvascular function even in the presence of significant decrease in intrinsic oxygen carrying capacity can compensate for the decreased oxygen supply, as comparatively few RBCs are sufficient to oxygenate the tissue and maintain metabolism, if microvascular function is normal.

Using plasma expanders automatically lowers Hct, decreasing both NO scavenging and WSS and NO production by the endothelium. According to the model of Sriram et al. hemodilution favors the effect of NO scavenging over the production of NO by the shear stress stimulus thus lowering overall NO bioavailability and promoting vasoconstriction. The fact that plasma expanders are formulated with a viscosity similar to that of plasma further reduces WSS. Notably, the concept that lowering blood viscosity (a direct consequence of phlebotomy) is of therapeutic benefit is embedded in medical thinking since antiquity.⁸⁶ Its physiological rationale is based on the finding that the decrease in blood viscosity consequent to lowering Hct by moderate hemodilution maintains or improves tissue perfusion, without affecting oxygen carrying capacity according to earlier studies,^{76,77} showing how the circulation adjusts to maintain relatively constant the rate of delivery of RBCs to tissue during significant decreases of Hct, as a consequence to the significant lowering of blood viscosity.

Viewing hemodilution from the perspective of the effects that take place in the CFL it is apparent that the lowered blood viscosity does not produce sufficient WSS for producing NO, causing the circulation to constrict. This phenomenon is apparent in analyzing the relationship Hct/BP in the normal population when the effect of viscosity is factored out, showing that in general as Hct increases BP diminishes.⁸⁷

These considerations lead to the proposal of formulating plasma expanders with comparatively high viscosity first implemented by Tsai et al.⁸⁸ This study showed that extension of isovolemic iso-oncotic hemodilution beyond the transfusion trigger led to normal microcirculatory conditions if plasma viscosity was increased to 2.3 cp versus the normal value of about 1.1–1.2 cp, even if Hct was lowered to conditions of extreme hemodilution (Hct ~11%). Notably, high viscosity plasma also increased microvascular perfusion above baseline levels, and maintained acid base balance positive, which was the opposite finding in the same protocol when plasma viscosity was normal. These findings were corroborated using plasma expanders based on alginates

(Alginate, FMC BioPolymer, Brakrøya, Norway) the basic constituent of agar. This material in normal saline at a concentration of 0.7 g/dL has a viscosity of 7.6 cp and a colloidal osmotic pressure of 2.1 mmHg. Using a combination of alginate and dextran 70 kDa in extreme hemodilution (Hct 11%) and increasing plasma viscosity to 2.7 cp provided for improved microvascular conditions over those attained using a mixture of dextran 70 and 500 kDa with plasma viscosity of 2.2 cp. The same level of hemodilution attained with dextran 70 and plasma viscosity of 1.4 cp did not result in a level of functional capillary density (FCD) compatible with survival since base excess was negative.⁸⁹ Notably, in these experiments BP remained below normal.⁹⁰

In these experiments shear stress at the microvascular wall was higher than that found with low viscosity plasma in extreme hemodilution, leading to a significantly higher concentration of perivascular NO in the arterioles and venules.⁵⁹ Increasing plasma expander viscosity was also found beneficial in shock resuscitation, in comparison with using low viscosity plasma expanders.^{91–94}

CONCLUSIONS

Analyses and findings about the CFL lead to several related hypotheses. We know that BP increases with age and it is likely that endothelial function, defined as the ability to respond to shear stress, also declines with age. Since Hct decreases with age, there appears to be a natural tendency for decreasing the stimulus for vasodilatation promoting hypertension, which is in part associated with increased pro-inflammatory conditions.

Furthermore, one of the initial triggers for hypertension could be the disruption of the balance of effects taking place in the CFL. This imbalance of NO bioavailability could be either positive or negative, and may not manifest initially as hypertension; however, it could be evident as a preclinical form of the disease in terms of paired small excursions from an optimal BP and Hct. Hypertension would then be in part due to the outcome of cumulative effects due to this initial imbalance, supported by additional long range effects associated with altered NO bioavailability, promoting a lifelong pro-inflammatory condition. An intriguing aspect of this process is that the imbalance in the relationship BP and Hct could be initially corrected by simple interventions that influence Hct and NO bioavailability, including exercise and diet.

It is clear that a number of the critical phenomena that take place in the CFL must still be rigorously modeled and experimentally validated. Nonetheless,

the system of interactions presented appear to provide a plausible explanation for forms of cardiovascular regulation that are counterintuitive, like the high

sensitivity of BP to small Hct changes and the apparent beneficial effects of increased blood and plasma viscosity.

ACKNOWLEDGMENTS

This study was supported in part by the USPHS Bioengineering Research Partnership grant R24-HL 064395 (MI), R01-HL 062354 (MI) and R01-HL 076182 (PCJ).

REFERENCES

- Wang SK, Hwang NH. On transport of suspended particulates in tube flow. *Biorheology* 1992, 29:353–377.
- Segre G, Silberberg A. Behaviour of macroscopic rigid spheres in Poiseuille flow. *J Fluid Mech* 1962, 14:115–135.
- Secomb TW, Hsu R, Pries AR. A model for red blood cell motion in glycocalyx-lined capillaries. *Am J Physiol* 1998, 274:H1016–H1022.
- Munn LL, Dupin MM. Blood cell interactions and segregation in flow. *Ann Biomed Eng* 2008, 36:534–544.
- Frangos JA, Eskin SG, McIntire LV, Ives CL. Flow effects on prostacyclin production in cultured human endothelial cells. *Science* 1985, 227:1477–1479.
- Grabowski EF, Jaffe EA, Weksler BB. Prostacyclin production by cultured endothelial cell monolayers exposed to step increases in shear stress. *J Lab Clin Med* 1985, 105:36–43.
- McAllister TN, Frangos JA. Steady and transient fluid shear stress stimulate NO release in osteoblasts through distinct biochemical pathways. *J Bone Miner Res* 1999, 14:930–936.
- Lancaster JR. Simulation of the diffusion and reaction of endogenously produced nitric oxide. *Proc Natl Acad Sci USA* 1994, 91:8137–8141.
- Buerk DG. Can we model nitric oxide biotransport? A survey of mathematical models for a simple diatomic molecule with surprisingly complex biological activity. *Annu Rev Biomed Eng* 2001, 3:109–143.
- Butler AR, Megson IL, Wright PG. Diffusion of nitric oxide and scavenging by blood in the vasculature. *Biochem Biophys Acta* 1998, 1425:168–176.
- Wolin MS, Hintze TH, Shen W, Mohazzab-H KM, Xie YW. Involvement of reactive oxygen and nitrogen species in signalling mechanisms that control tissue respiration in muscle. *Biochem Soc Trans* 1997, 25:934–939.
- Xie YW, Shen W, Zhao G, Xu X, Wolin MS, Hintze TH. Role of endothelium-derived nitric oxide in the modulation of canine myocardial mitochondrial respiration in vitro. *Circ Res* 1996, 79:381–387.
- Förstermann U. Nitric oxide and oxidative stress in vascular disease. *Pflugers Arch* 2010, 459:923–939.
- Zhang J, Johnson PC, Popel AS. Effects of erythrocyte deformability and aggregation on the cell free layer and apparent viscosity of microscopic blood flows. *Microvasc Res* 2009, 77:265–272.
- Bagnold RA. Experiments on a gravity free dispersion of large solid spheres in a Newtonian fluid under shear. *Proc R Soc* 1954, 225:49–63.
- Vink H, Duling BR. Identification of distinct luminal domains for macromolecules, erythrocytes, and leukocytes within mammalian capillaries. *Circ Res* 1996, 79:581–589.
- Vink H, Constantinescu AA, Spaan JA. Oxidized lipoproteins degrade the endothelial surface layer: implications for platelet-endothelial cell adhesion. *Circulation* 2000, 101:1500–1502.
- van den Berg BM, Vink H, Spaan JA. The endothelial glycocalyx protects against myocardial edema. *Circ Res* 2003, 92:592–594.
- Rehm M, Zahler S, Lotsch M, Welsch U, Conzen P, Jacob M, Becker BF. Endothelial glycocalyx as an additional barrier determining extravasation of 6% hydroxyethyl starch or 5% albumin solutions in the coronary vascular bed. *Anesthesiology* 2004, 100:1211–1223.
- Pappenheimer JR, Renkin EM, Borrero LM. Filtration, diffusion and molecular sieving through peripheral capillary membranes; a contribution to the pore theory of capillary permeability. *Am J Physiol* 1951, 167:13–46.
- Weinbaum S. 1997 Whitaker distinguished lecture: models to solve mysteries in biomechanics at the cellular level; a new view of fiber matrix layers. *Ann Biomed Eng* 1998, 26:627–643.
- Florian JA, Kosky JR, Ainslie K, Pang Z, Dull RO, Tarbell JM. Heparan sulfate proteoglycan is a mechanosensor on endothelial cells. *Circ Res* 2003, 93:e136–e142.
- Zuurbier CJ, Demirci C, Koeman A, Vink H, Ince C. Short-term hyperglycemia increases endothelial glycocalyx permeability and acutely decreases lineal density of capillaries with flowing red blood cells. *J Appl Physiol* 2005, 99:1471–1476.
- Nieuwdorp M, van Haeften TW, Gouverneur MC, Mooij HL, van Lieshout MH, Levi M, Meijers JC, Holleman F, Hoekstra JB, Vink H, et al. Loss of

- endothelial glycocalyx during acute hyperglycemia coincides with endothelial dysfunction and coagulation activation in vivo. *Diabetes* 2006, 55:480–486.
25. Seal JB, Gewertz BL. Vascular dysfunction in ischemia-reperfusion injury. *Ann Vasc Surg* 2005, 19:572–584.
 26. Reitsma S, Slaaf DW, Vink H, van Zandvoort MA, oude Egbrink MG. The endothelial glycocalyx: composition, functions, and visualization. *Pflügers Archiv* 2007, 454:345–359.
 27. Mulivor AW, Lipowsky HH. Inflammation- and ischemia-induced shedding of venular glycocalyx. *Am J Physiol Heart Circ Physiol* 2004, 286:H1672–H1680.
 28. Barker AL, Konopatskaya O, Neal CR, Macpherson JV, Whatmore JL, Winlove CP, Unwin PR, Shore AC. Observation and characterisation of the glycocalyx of viable human endothelial cells using confocal laser scanning microscopy. *Phys Chem Chem Phys* 2004, 6:1006–1011.
 29. Potter DR, Jiang J, Damiano ER. The recovery time course of the endothelial cell glycocalyx in vivo and its implications in vitro. *Circ Res* 2009, 104:1318–1325.
 30. Long DS, Smith ML, Pries AR, Ley K, Damiano ER. Microviscometry reveals reduced blood viscosity and altered shear rate and shear stress profiles in microvessels after hemodilution. *Proc Natl Acad Sci U S A* 2004, 101:10060–10065.
 31. Henry CBS, Duling BR. Permeation of the luminal capillary glycocalyx is determined by Hyaluronan. *Am J Physiol* 1999, 277:H508–H518.
 32. Michel CC, Curry FR. Glycocalyx volume: a critical review of tracer dilution methods for its measurement. *Microcirculation* 2009, 16:213–219.
 33. Kim S, Kong RL, Popel AS, Intaglietta M, Johnson PC. A computer-based method for determination of the cell-free layer width in microcirculation. *Microcirculation* 2006, 13:199–207.
 34. Hightower CM, Hightower JD, Salazar Vázquez BY, Intaglietta M. Seasonal hematocrit variation and health risks in the adult population of Kinshasa, Democratic Republic of Congo. *Vasc Health Risk Manage* 2009, 5:1001–1005.
 35. Sriram K, Salazar Vázquez BY, Yalcin O, Johnson PC, Intaglietta M, Tartakovsky DM. The effect of small changes in hematocrit on nitric oxide transport in arterioles. *Antioxid Redox Signal* 2011, 14:175–185.
 36. Maeda N, Suzuki Y, Tanaka J, Tateishi N. Erythrocyte flow and elasticity of microvessels evaluated by marginal cell-free layer and flow resistance. *Am J Physiol* 1996, 271:H2454–H2461.
 37. Gretz JE, Duling BR. Measurement uncertainties associated with the use of bright-field and fluorescence microscopy in the microcirculation. *Microvasc Res* 1995, 49:134–140.
 38. Smith ML, Long DS, Damiano ER, Ley K. Near-wall micro-PIV reveals a hydrodynamically relevant endothelial surface layer in venules in vivo. *Biophys J* 2003, 85:637–645.
 39. Otsu N. A threshold selection method from grey level histograms. *IEEE Trans Syst Man Cybern* 1979, 9:62–66.
 40. Kim S, Kong RL, Popel AS, Intaglietta M, Johnson PC. Temporal and spatial variations of cell-free layer width in arterioles. *Am J Physiol Heart Circ Physiol* 2007, 293:H1526–H1535.
 41. Lazarev YN, Petrov PV, Tartakovsky DM. Interface dynamics in randomly heterogeneous porous media. *Adv Water Resour* 2005, 28:393–403.
 42. Xiu D, Tartakovsky DM. Numerical methods for differential equations in random domains. *SIAM J Sci Comput* 2006, 28:1167–1185.
 43. Tartakovsky DM, Xiu D. Stochastic analysis of transport in tubes with rough walls. *J Comput Phys* 2006, 217:248–259.
 44. Lin G, Tartakovsky AM, Tartakovsky DM. Uncertainty quantification via random domain decomposition and probabilistic collocation on sparse grids. *J Comput Phys* 2010, 229:6995–7012.
 45. Lomize M. *Filratsiya v Treshchinovatykh Porodakh (in Russian)*. Moscow: Gosenergoizdat; 1951.
 46. Scholander PF. Oxygen transport through hemoglobin solutions. *Science* 1960, 131:585–590.
 47. Hellums JD. The resistance to oxygen transport in the capillaries relative to that in the surrounding tissue. *Microvasc Res* 1977, 13:131–136.
 48. Hellums JD, Nair PK, Huang NS, Oshima N. Simulation of intraluminal gas transport processes in the microcirculation. *Ann Biomed Eng* 1996, 24:1–24.
 49. Tsai AG, Friesenecker B, Mazzoni MC, Kerger H, Buerk DG, Johnson PC, Intaglietta M. Microvascular and tissue oxygen gradients in the rat mesentery. *Proc Natl Acad Sci* 1998, 95:6590–6595.
 50. Briceno JC, Cabrales P, Tsai AG, Intaglietta M. Radial displacement of red blood cells during hemodilution and the effect on arteriolar oxygen profile. *Am J Physiol Heart Circ Physiol* 2004, 286:H1223–H1228.
 51. Tsai AG, Cabrales P, Intaglietta M. The physics of oxygen delivery: facts and controversies. *Antioxid Redox Signal* 2010, 12:683–691.
 52. Han YF, Weinbaum S, Spaan JAE, Vink H. Large-deformation analysis of the elastic recoil of fibre layers in a Brinkman medium with application to the endothelial glycocalyx. *J Fluid Mech* 2006, 554:217–235.
 53. Secomb TW, Pries AR. Mechanics of shear stress transmission to endothelial cells in blood vessels lined with an endothelial surface layer. *ASME Bioeng Conf* 2001, 50:389–390.
 54. Weinbaum S, Zhang X, Han Y, Vink H, Cowin SC. Mechanotransduction and flow across the endothelial glycocalyx. *Proc Natl Acad Sci U S A* 2003, 100:7988–7995.

55. Tarbell JM, Pahakis MY. Mechanotransduction and the glycocalyx. *J Intern Med* 2006, 259:339–350.
56. Tarbell JM, Weinbaum S, Kamm RD. Cellular fluid mechanics and mechanotransduction. *Ann Biomed Eng* 2005, 33:1719–1723.
57. Namgung B, Ong PK, Johnson PC, Kim S. Effect of cell-free layer variation on arteriolar wall shear stress. *Ann Biomed Eng* 2011, 399:359–368.
58. Liao JC, Hein TW, Vaughn MW, Huang KT, Kuo L. Intravascular flow decreases erythrocyte consumption of nitric oxide. *Proc Natl Acad Sci U S A* 1999, 96:8757–8761.
59. Tsai AG, Acero C, Nance PR, Cabrales P, Frangos JA, Buerk DG, Intaglietta M. Elevated plasma viscosity in extreme hemodilution increases perivascular nitric oxide concentration and microvascular perfusion. *Am J Physiol Heart Circ Physiol* 2005, 288:H1730–H1739.
60. Tsai AG, Cabrales P, Manjula BN, Acharya SA, Winslow RM, Intaglietta M. Dissociation of local nitric oxide concentration and vasoconstriction in the presence of cell-free hemoglobin oxygen carriers. *Blood* 2006, 108:3603–3610.
61. Kameneva MV, Watach MJ, Borovetz HS. Gender difference in rheologic properties of blood and risk of cardiovascular diseases. *Clin Hemorheol Microcirc* 1999, 21:357–363.
62. Salazar-Vázquez BY, Intaglietta M, Rodriguez-Morán M, Guerrero-Romero F. Blood pressure and hematocrit in diabetes and the role of endothelial responses in the variability of blood viscosity. *Diabetes Care* 2006, 29:1523–1528.
63. Pries AR, Neuhaus D, Gaehtgens P. Blood viscosity in tube flow: dependence on diameter and hematocrit. *Am J Physiol* 1992, 263:H1770–H1778.
64. Sharan M, Popel AS. A two-phase model for flow of blood in narrow tubes with increased effective viscosity near the wall. *Biorheology* 2001, 38:415–428.
65. Mashour GA, Boock RJ. Effects of shear stress on nitric oxide levels of human cerebral endothelial cells cultured in an artificial capillary system. *Brain Res* 1999, 842:233–238.
66. Roy B, Garthwaite J. Nitric oxide activation of guanylyl cyclase in cells revisited. *Proc Natl Acad Sci U S A* 2006, 103:12185–12190.
67. Arnold WP, Mittal CK, Katsuki S, Murad F. Nitric oxide activates guanylate cyclase and increases guanosine 3':5'-cyclic monophosphate levels in various tissue preparations. *Proc Natl Acad Sci U S A* 1977, 74:3203–3207.
68. Kavdia M, Popel AS. Wall shear stress differentially affects NO level in arterioles for volume expanders and Hb-based O₂ carriers. *Microvasc Res* 2003, 66:49–58.
69. Tsoukias NM, Popel AS. Erythrocyte consumption of nitric oxide in presence and absence of plasma-based hemoglobin. *Am J Physiol Heart Circ Physiol* 2002, 282:H2265–H2277.
70. Lamkin-Kennard KA, Buerk DG, Jaron D. Interactions between NO and O₂ in the microcirculation: a mathematical analysis. *Microvasc Res* 2004, 68:38–50.
71. Chen X, Jaron D, Barbee KA, Buerk DG. The influence of radial RBC distribution, blood velocity profiles, and glycocalyx on coupled NO/O₂ transport. *J Appl Physiol* 2006, 100:482–492.
72. Gundersen SI, Chen G, Palmer AF. Mathematical model of NO and O₂ transport in an arteriole facilitated by hemoglobin based O₂ carriers. *Biophys Chem* 2009, 143:1–17.
73. Yalcin O, Choi C, Chatpun S, Intaglietta M, Johnson PC. The dependence of cell-free layer thickness in arterioles on systemic hematocrit level. *FASEB J* 2009, 23:949.7.
74. Cheng C, Tempel D, Oostlander A, Helderman F, Gijzen F, Wentzel J, van Haperen R, Haitisma DB, Serruys PW, van der Steen AF, et al. Rapamycin modulates the eNOS vs. shear stress relationship. *Cardiovasc Res* 2008, 78:123–129.
75. Lindenfeld J, Weil JV, Travis VL, Horwitz LD. Regulation of oxygen delivery during induced polycythemia in exercising dogs. *Am J Physiol Heart Circ Physiol* 2005, 289:H1821–1825.
76. Messmer K, Sunder-Plassmann L, Klövekorn WP, Holper K. Circulatory significance of hemodilution: rheological changes and limitations. *Adv Microc* 1972, 4:1–77.
77. Richardson TQ, Guyton AC. Effects of polycythemia and anemia on cardiac output and other circulatory factors. *Am J Physiol* 1959, 197:1167–1170.
78. Martini J, Tsai AG, Cabrales P, Johnson PC, Intaglietta M. Increased cardiac output and microvascular blood flow during mild hemoconcentration in hamster window model. *Am J Physiol Heart Circ Physiol* 2006, 291:H310–317.
79. Kuo L, Pittman RN. Influence of hemoconcentration on arteriolar oxygen transport in hamster striated muscle. *Am J Physiol* 1990, 259:H1694–H1702.
80. Martini J, Carpentier B, Chavez Negrete A, Frangos JA, Intaglietta M. Paradoxical hypotension following increased hematocrit and blood viscosity. *Am J Physiol Heart Circ Physiol* 2005, 289:H2136–H2143.
81. Salazar Vázquez BY, Martini J, Tsai AG, Johnson PC, Cabrales P, Intaglietta M. The variability of blood pressure due to small changes of hematocrit. *Am J Physiol Heart Circ Physiol* 2010, 299:H863–H867.
82. Yalcin O, Choi C, Chatpun S, Intaglietta M, Johnson PC. The dependence of cell-free layer thickness in arterioles on systemic hematocrit level. *FASEB J* 2009, 23:949.7 (Abstract).
83. Salazar Vázquez BY, Cabrales P, Tsai AG, Johnson PC, Intaglietta M. Lowering of blood pressure by increasing hematocrit with non nitric oxide scavenging red blood cells. *Am J Respir Cell Mol Biol* 2008, 38:135–142.

84. Fazio M, Bardelli M, Cominotto F, Fiammengo F, Fabris B, Fischetti F, Candido R, Pascazio L, Lapasin R, Carretta R. Haemoconcentration, shear-stress increase and carotid artery diameter regulation after furosemid administration in older hypertensives. *Exp Gerontol* 2001, 36:571–581.
85. Salazar Vázquez BY, Wettstein R, Cabrales P, Tsai AG, Intaglietta M. Microvascular experimental evidence on the relative significance of restoring oxygen carrying capacity vs. blood viscosity in shock resuscitation. *Biochim Biophys Acta* 2008, 1784:1421–1427.
86. Kuriyama S. Interpreting the history of bloodletting. *J Hist Med Allied Sci* 1995, 50:11–46.
87. Salazar Vázquez BY, Martini J, Chávez Negrete A, Tsai AG, Forconi S, Cabrales P, Johnson PC, Intaglietta M. Cardiovascular benefits in moderate increases of blood and plasma viscosity surpass those associated with lowering viscosity: experimental and clinical evidence. *Clin Hemorheol Microcirc* 2010, 44:75–85.
88. Tsai AG, Friesenecker B, McCarthy M, Sakai H, Intaglietta M. Plasma viscosity regulates capillary perfusion during extreme hemodilution in hamster skin fold model. *Am J Physiol* 1998, 275:H2170–H2180.
89. Cabrales P, Tsai AG, Intaglietta M. Alginate plasma expander maintains perfusion and plasma viscosity during extreme hemodilution. *Am J Physiol* 2005, 288:H1708–H1716.
90. Cabrales P, Tsai AG. Plasma viscosity regulates systemic and microvascular perfusion during acute extreme anemic conditions. *Am J Physiol* 2006, 291:H2445–H2452.
91. Cabrales P, Intaglietta M, Tsai AG. Increase plasma viscosity sustains microcirculation after resuscitation from hemorrhagic shock and continuous bleeding. *Shock* 2005, 23:549–555.
92. Cabrales P, Intaglietta M, Tsai AG. Transfusion restores blood viscosity and reinstates microvascular conditions from hemorrhagic shock independent of oxygen carrying capacity. *Resuscitation* 2007, 75:124–134.
93. Cabrales P, Tsai AG, Intaglietta M. Hyperosmotic-hyperoncotic vs. hyperosmotic-hyperviscous small volume resuscitation in hemorrhagic shock. *Shock* 2004, 22:431–437.
94. Cabrales P, Tsai AG, Intaglietta M. Is resuscitation from hemorrhagic shock limited by blood oxygen-carrying capacity or blood viscosity? *Shock* 2007, 27:380–389.