MICROHEMOFLUIDICS IN POST-CAPILLARY VENULES DETERMINED FROM FLUORESCENT INTRAVITAL MICRO-PARTICLE IMAGE VELOCIMETRY

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INTRODUCTION

It is well known that blood, consisting primarily of red cells and plasma, undergoes a phase separation in these two constituents under steady flow in microvessels and narrow glass capillary tubes. This non-uniform distribution of red cells over the vessel cross section leads to a cell-rich core flow and a plasma-rich region near the vessel wall. Fluid drag in the plasma-rich region gives rise to the so-called Fahraeus effect in which the tube hematocrit, H_T, in the vessel is decreased relative to the discharge hematocrit, H_D. The ratio H_T/H_D is seen to decrease with decreasing vessel diameter in glass tubes ranging between 20 and 1000 μm in diameter[1]. Furthermore, since a disproportionate amount of the integrated shear stress over the vessel cross section is borne by the flow in the less viscous plasma-rich region, the relative apparent viscosity, η_{rel}, defined as the ratio of steady volume-flow rates per unit pressure drop of blood plasma relative to whole blood, is also seen to decrease with decreasing vessel diameter in glass tubes ranging between 10 and 1000 μm in diameter. This phenomenon, known as the Fahraeus-Lindqvist effect, has been fairly well established *in vitro* for blood flow under high shear rates through smooth-walled glass capillary tubes[2].

For steady flow in microvessels and glass capillary tubes, vessel diameter and discharge hematocrit are the most important determinants of a variety of flow parameters, including H_T, η_{rel}, volume-flow rate, Q, pressure-gradient amplitude, dp/dz, and local shear stress, τ_z. Owing to the significant heterogeneity in red-cell distributions that arise within microvascular networks *in vivo*, there typically exists a broad range of values H_D can assume for the many vessels in a given network, and even a significant variability in its value for a particular vessel in the network over time. This heterogeneity, coupled with the inability to accurately measure pressure gradients *in vivo*[3], has confounded attempts at quantitatively characterizing blood flow in the microcirculation.

We present a method for easily and accurately estimating all of the aforementioned rheological parameters, in both glass capillary tubes and individual post-capillary venules within a microvascular network, which is based on analytically determined expressions for the viscosity and hematocrit profiles that are derived from experimental velocity-distribution data obtained over the vessel cross section *in vitro* and *in vivo* using fluorescent micro-particle image velocimetry (μ-PIV).

ANALYSIS AND METHODS

Model Formulation. The model that forms the basis of our viscosity- and hematocrit-profile estimates depends upon the assumption that in tubes and vessels larger than 30 μm in diameter, the rheological properties associated with a heterogeneous red blood cell suspension can well be approximated by a continuous, linearly viscous fluid having a spatially non-uniform viscosity. Whereas most two-phase and variable-viscosity models of blood flow in the microcirculation that invoke the continuum approximation have started with assumptions about the viscosity and/or hematocrit distributions that led to predictions of the velocity profile, we have proceeded by solving the inverse problem. Assuming the velocity distribution to be known, as that can be directly measured using fluorescent μ-PIV *in vitro* and *in vivo*, we have derived simple analytical expressions for the viscosity and hematocrit profiles over the vessel cross section that satisfy the conservation principles of mass and momentum and depend only upon the experimentally obtained velocity distribution. Once obtained, these profiles provide a wealth of information about dynamic, kinematic, and rheological properties of the flow.

For a fully developed, axisymmetric, steady flow in a cylindrical tube of a linearly viscous, incompressible, isotropic fluid with radially varying viscosity, $\eta(r)$, the momentum equation for the axial velocity component, $v_z(r)$, is second order in $v_z(r)$, first order in $\eta(r)$, and integrable in cylindrical coordinates (r, θ, z). An expression for the viscosity distribution can be found in terms of $v_z(r)$ and dp/dz by integrating the momentum equation with respect to r, imposing symmetry about the tube axis, and noting that the viscosity is bounded and nonzero everywhere. If we assert that, at the tube wall, the local viscosity is equal to plasma viscosity, then we can obtain an

expression for $\mu(r)$ that depends only on $v_z(r)$. In microvessels, the effect of the glycocalyx surface layer on the vessel wall is accounted for in the analysis by modeling the layer as a uniformly thick Brinkman medium. Velocity distributions in glass tubes and post-capillary venules were obtained using μ-PIV as described below.

Glass Tube Experiments. Glass capillary tubes (2–4 cm in length with inside diameters varying between 30 and 100 μm) were mounted horizontally on a horizontal microscope stage. A modular flow chamber assembly, which incorporates a removable glass tube, was immersed in phthalic acid. The indices of refraction of the glass tube and phtalic acid were sufficiently close so as to effectively eliminate optical refraction at the outer tube wall. Blood samples were centrifuged and the buffy coat was aspirated and discarded. After fluorescently labeling a small fraction of the red blood cells, both labeled and unlabeled red cells were resuspended in the plasma at several different concentrations (hematocrits being approximately 20, 30, 40, 50, and 60%). Following each experiment, samples were analyzed using a Hemavet 850 to precisely determine hematocrit and mean corpuscular volume. The glass tubes were perfused from a feed reservoir containing the perfusion solution, which was stirred to prevent red-cell sedimentation. The pressure gradient was controlled by varying the hydrostatic pressure head between the feed reservoir and a collection reservoir, the height of which was adjustable from a micrometer scale.

Intravital Experiments. The cremaster muscle of WT C57Bl6 mice was prepared for intravital microscopy and visualized on a Zeiss intravital microscope with a X100 saline immersion objective. Venules 24 to 41 μm diameter with clear focus were selected. A small volume of 470 nm fluorescent microspheres was slowly injected through the carotid cannula until 10 to 20 beads per second passed through the vessel. In some mice, FITC-dx 70 in saline was slowly infused through the carotid cannula until the venular lumen was sufficiently bright without obscuring visualization of fluorescent microspheres. After 10 minutes, the cremaster muscle was continuously epi-illuminated for 5 minutes at 450 to 490 nm with a mercury vapor short-arc lamp and a X20 objective to partially degrade the glycocalyx surface layer[4]. Microsphere velocity recordings were then made as described below using the X100 objective.

μ-PIV and Data Analysis. The microspheres were visualized using stroboscopic double-flash epi-illumination, and recordings were made through a CCD camera. This technique yields, in one picture, two images of the same microsphere displaced a measurable distance over a known time interval. Transillumination was maintained to keep the vessel wall (or inner tube wall in the case of glass tube experiments) clearly visible.

For each vessel/tube, μ-PIV data were collected and axisymmetric curve fits were performed on a subset of the data using a standard nonlinear regression analysis. The fits themselves made use of the closed-form expression for the axial velocity component, $v_z(r)$, which can be determined to within an arbitrary function, $f(r)$. In glass tubes, $v_z(r)$ satisfies the no-slip condition at the tube wall, whereas in microvessels, $v_z(r)$ satisfies the continuity conditions of velocity and stress at the interface between the blood and the glycocalyx surface layer. Thus by determining the function $f(r)$ that results in the best fit of $v_z(r)$ to the reduced data set, the resulting fit not only minimizes the chi-squared error, but also identically satisfies the axial momentum equation and boundary conditions.

In order to account for the effect of the glycocalyx surface layer, its mean thickness and mean permeability to plasma were estimated from the μ-PIV data. Initially, the most conservative estimate of its thickness was made by assuming no flow through the layer. This thickness estimate was obtained iteratively by finding the best fit to the reduced data set for a given vessel. The distance from the vessel wall where this best fit to the data set corresponded to zero velocity was taken as our initial guess of the glycocalyx thickness. Progressively thicker estimates of the layer were made for increasing values of hydraulic permeability following a similar procedure. These estimates were made using μ-PIV data taken in vessels before and after light-dye treatment to degrade the glycocalyx.

RESULTS AND DISCUSSION

Parameters predicted by the analysis are in very good agreement with directly measured values in glass tubes. In most cases, measured values of H_D, μ_{rel}, and dp/dz were within 10% of predicted vaules. An interesting observation we have made is that predicted centerline hematocrit very slightly exceeds the discharge hematocrit, H_D, of the vessel. This finding is particularly useful since it offers the potential for direct and accurate estimation of an important, and up until now, elusive rheological parameter *in vivo*. With knowledge only of the velocity distribution, other important properties including the viscosity distribution, relative apparent viscosity, and ratio of tube to discharge hematocrit can be obtained. The relationships we obtained for these quantities satisfy the conservation principles of mass and momentum and have no free parameters. Furthermore, they do not depend on the magnitude of the axial pressure gradient or on the absolute value of the local hematocrit on any interior point within the vessel. If one further makes use of viscometric data of whole blood at high shear rates[5], our model offers a way of accurately estimating not only H_T/H_D, but H_T and H_D separately as well.

Since dp/dz, H_D, and μ_{rel} are, in general, extremely difficult, if not impossible, to directly measure *in vivo*, this approach provides a means of accurately estimating these quantities from empirical data of microvascular velocity distributions *in vivo*. However, while direct measurement of these quantities has remained elusive *in vivo*, they can all be directly measured *in vitro*. Thus, another great virtue of this approach is the opportunity it offers to interrogate the validity of the model through comparison of theoretical predictions with a variety of quantities that are measurable *in vitro*. We have therefore established a model that can be thoroughly tested *in vitro* on the one hand and has tremendous predictive potential *in vivo* on the other.

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