Supporting Text

Summary of Analysis for the Microviscometric Method in Glass Tubes. Here, we summarize the essential analytical elements of the microviscometric method in glass tubes. Full details are given in ref. 1.

In all of our glass tube studies, the microparticle image velocimetry (μ-PIV) data were obtained $\approx$1.5 cm from the inlet of the glass tube. With a typical Reynolds number on the order of 10^{-2} for these experiments, it is reasonable to assume that entrance effects are negligible. We, therefore, assume the flow to be fully developed and axisymmetric. In terms of the axial velocity component, $v_{\rm z}$, and pressure, p, the momentum equation for an axisymmetric, fully developed, steady incompressible flow of a spatially varying, linearly viscous fluid in a cylindrical tube of radius R is given in cylindrical coordinates (r, θ, z) by

$$\frac{1}{r}\frac{d}{dr}\left(\mu(r)\, r\frac{dv_{\rm z}}{dr}\right) = \frac{dp}{dz}, \quad 0 < r < R, \tag{1}$$

where it can be shown (1) that the axial pressure gradient,

$$\frac{dp}{dz} = \frac{2\mu_{\rm R}}{R}\frac{dv_{\rm z}}{dr}\bigg|_{\rm r=R}, \tag{2}$$

and the local normalized dynamic viscosity of blood is given by

$$\frac{\mu(r)}{\mu_{\rm R}} = \frac{r}{R}\frac{(dv_{\rm z}/dr)|_{\rm r=R}}{dv_{\rm z}/dr} = \frac{r}{R}\frac{\dot\gamma(R)}{\dot\gamma(r)}, \quad 0 \le r \le R. \tag{3}$$

Here, the viscosity evaluated at the tube wall, $\mu_{\rm R} = \mu(R)$, is determined by minimizing the least-squares error in the difference from unity of the ratio of the measured to predicted values of dp/dz for all of the *in vitro* data sets analyzed, excluding the saline-perfused tubes. From this calibration we estimate $\mu_{\rm R}$ as being very nearly equal to that of plasma viscosity, $\mu_{\rm p}$, obtained from a capillary viscometer ($\mu_{\rm R} \simeq 0.95\,\mu_{\rm p}$, where $\mu_{\rm p}$ was found to have an average value of 1.20 $\pm$ 0.07 cP at 23°C).

In terms of the viscosity distribution, the relative apparent viscosity, $\eta_{\rm rel}$, defined as the ratio of steady volume-flow rates per unit pressure drop of blood plasma relative to whole blood, is given by

$$\eta_{\rm rel} = \left(8\int_0^1\int_{\rm r/R}^1 \frac{\sigma d\sigma}{\mu(\sigma)/\mu_{\rm R}}\tilde r d\tilde r\right)^{-1}. \tag{4}$$

The tube hematocrit, defined as the mean instantaneous red-cell concentration in the vessel, and the discharge hematocrit, defined as the mean red-cell flux fraction through the vessel, are given, respectively, by

$$H_{\mathrm{T}} := \frac{1}{A}\iint_{\mathrm{A}} H(r)\,dA \quad \text{and} \quad H_{\mathrm{D}} := \frac{1}{Q}\iint_{\mathrm{A}} H(r)\,v_{\mathrm{z}}(r)\,dA, \tag{5}$$

where A is the cross-sectional area of the tube lumen, Q is the volume flow rate in the tube, and $H(r)$ is the local hematocrit derived from Eq. **3** and a species-specific transport relationship, $H(\mu)$, obtained from rotational viscometric data (2). The hematocrit distribution, $H(r)$, reflects the time-averaged hematocrit at any point in the tube cross section, and as such, it is predicted to vanish only at the tube wall. Even in an approximately steady flow, experimental observations have shown (3–6) that red cells transiently invade the plasma-rich region of microvessels, and contribute to the local hematocrit and viscosity there. Thus, in a time-averaged sense, the local hematocrit is considered to be nonvanishing throughout the vessel lumen.

In principle, the tube and discharge hematocrits are derivable from the viscosity distribution given by Eq. **3** if $H(\mu)$ were known for a particular species. However, even without $H(\mu)$, one can nevertheless estimate the fractional change in H_{T} and H_{D} by considering the fractional change in μ^*_{T} and μ^*_{D}, which we define in glass tubes as

$$\mu^*_{\mathrm{T}} := \frac{1}{A}\iint_{\mathrm{A}} \frac{\mu(r)}{\mu_{\mathrm{R}}}\,dA \quad \text{and} \quad \mu^*_{\mathrm{D}} := \frac{1}{Q}\iint_{\mathrm{A}} \frac{\mu(r)}{\mu_{\mathrm{R}}}\,v_{\mathrm{z}}(r)\,dA. \tag{6}$$

It is evident from Eq. **6** that μ^*_{T} corresponds to the mean instantaneous normalized viscosity over the vessel cross section, and is therefore analogous to H_{T}. Likewise, μ^*_{D} is analogous to H_{D}. Just as the local temperature is determined by the energy content in a volume of fluid (and can be used to predict energy flux if the material-specific equation of state for the heat capacity is known), so too is the local value of $\mu(r)$ determined by the number of red cells in a volume of blood [and can be used to predict red-cell flux if the species-specific transport relationship, $H(\mu)$, is known]. As such, μ^*_{D} is an indicator of the red-cell flux fraction through the vessel.

Summary of Analysis for the Microviscometric Method in Microvessels. In microvessels, we again assume the flow to be axisymmetric and fully developed, the latter being reasonable *in vivo* for profiles

measured more than one vessel diameter away from bifurcations (7, 8). In independent studies using endogenous particle tracers (5, 8), a parameter used to quantitatively determine asymmetry in the velocity profile was not significantly different from zero, suggesting that velocity profiles were nearly axisymmetric in most microvessels. Other groups have reported significantly asymmetric red-cell profiles in venules of the rabbit omentum (9) and hamster retractor muscle (10). However, in both of these studies, velocity distributions were determined from a small number of endogenous red-cell tracers that are likely to be too large to provide the necessary spatial resolution required to draw conclusions about symmetry. It has also been reported that red blood cells exhibit erratic deviations in radial position and velocity during transit through 400-μm-long sections of venules in rat spinotrapezius muscle (6). Despite these deviations, erythrocytes from daughter branches of a confluent venular bifurcation remained separated for up to 250 μm downstream of the bifurcation (11). However, redistribution of plasma over the vessel cross-section would likely lead to axisymmetric velocity profiles even in these cases. The fluorescent microspheres used in the present study, being much smaller and more regularly shaped than endogenous particle tracers, provided very high spatial resolution for the velocity profiles we were able to extract. In most of the vessels that we examined, distributions appeared nearly axisymmetric.

In order to extend the microviscometric method to blood flow in microvessels *in vivo*, a generalization is introduced (1) to account for the hemodynamic influence of the endothelial surface layer (ESL) on the luminal vessel wall (12–14). In particular, we identify two distinct regions, which we refer to as the free lumen, where $0 \leq r \leq a$, and the annular porous layer (corresponding to the ESL), where $a \leq r \leq R$. Here, a is the radial location of the effective hydrodynamic interface between blood in the free lumen and the ESL (14). In terms of the axial velocity component, v_z, in the free lumen, and pressure, p, the momentum equation in the free lumen for axisymmetric, fully developed, steady incompressible flow of a linearly viscous fluid having radially varying viscosity, $\mu(r)$, is given by Eq. **1** if the tube radius, R, is replaced by a. Furthermore, expressions for dp/dz, $\mu(r)$, H_T, and H_D, given by Eqs. **2**, **3**, and **5**, also apply in microvessels if R is replaced by a in Eqs. **2** and **3**.

Assuming that red cells cannot penetrate the ESL (12), we model flow in the porous layer with the Brinkman equation (15). Under these flow conditions, the axial fluid velocity component, v_z^f, in the porous layer is governed by

$$\frac{\mu_a}{r}\frac{d}{dr}\left(r\frac{dv_z^f}{dr}\right) = \frac{dp}{dz} + K\, v_z^f, \quad a < r < R, \tag{7}$$

where $\mu_a = \mu(a)$ is the viscosity at the interface between the ESL and blood in the free lumen, and K is the spatially averaged effective hydraulic resistivity of the layer, which is inversely proportional to its permeability.

A generalization of Eq. **4** for the relative apparent viscosity, η_{rel}, in microvessels is found to be

$$\eta_{rel} = -\frac{1}{8\,G(\alpha)}\left(\int_0^\alpha \left(2 - \frac{1}{G(\alpha)}\int_{r/R}^\alpha \frac{\sigma d\sigma}{\mu(\sigma)/\mu_a}\right)\tilde{r}d\tilde{r} + (1-\alpha^2)\bar{v}^f\right)^{-1} \tag{8}$$

where $\alpha = a/R$ and $\bar{v}^f$ is related to the dimensionless mean velocity of plasma flow through the porous layer (1). The function $G(r)$ and the quantity $\bar{v}^f$ are given in ref. 1 and depend parametrically only upon the dimensionless ESL thickness, $1-\alpha$, and the dimensionless hydraulic resistivity of the ESL, KR^2/μ_a.

Procedure for Obtaining the Velocity Distribution from the μ-PIV Data. We accounted for the hydrodynamic interaction, arising between the microsphere and the vessel or tube wall, that causes the translational speed of the center of a microsphere very near the wall to lag the velocity a fluid particle would have at the same radial distance from the wall if the microsphere were not present in the flow. Following a previously described method (14), the three-dimensional analyses of the free motion of a neutrally buoyant sphere in a uniform shear field adjacent to a planar confining boundary (16) or a Brinkman half space (17) were used to infer, from the measured translational speed of each microsphere, the true fluid particle velocity that would arise in the absence of the particle tracer. The velocity profile over the vessel cross-section was extracted from the estimated fluid particle velocities rather than directly from the measured microsphere velocities.

Because not all of the recorded beads travel in the midsagittal plane, we used a previously described monotonic filter (1, 14) and considered only the fastest beads at a given measured radial

location, where it is understood that a bead in the midsagittal plane travels faster than any other bead at that measured radial location. Only monotonically filtered data were included in the analysis.

Following methods described in ref. 1, an axisymmetric velocity profile, $v_z(r)$, was extracted from each μ-PIV data set. All velocity profiles *in vitro* and *in vivo* were obtained using the function defined in ref. 1, which identically satisfies the momentum equation and boundary conditions, and is given by

$$v_{\mathrm{fit}}(r) = v_{\max}\left(2G(\alpha) - \int_{r/R}^{\alpha} f(\sigma)\,d\sigma\right)\left(2G(\alpha) - \int_{0}^{\alpha} f(\sigma)\,d\sigma\right)^{-1}, \quad 0 \le r/R \le \alpha. \tag{9}$$

Here, $f(\sigma) = \sigma(1 - c_1 \sinh c_2\,\alpha) + \alpha\, c_1 \sinh c_2\,\sigma$ is the fitting function and c_1 and c_2 are found by means of nonlinear-regression analysis that uses Eq. **9** to minimize the least-squares error in the fit to the monotonically filtered data points identified above. The parameter $v_{\max}$, corresponding to the axial centerline velocity, is found by solving Eq. **9** for $v_{\max}$, substituting for r and v_{fit}, the measured radial position and velocity of each microsphere (from the monotonically filtered data set after correcting for fluid drag), and then taking the average of those values. It should be noted that the first term on the right side of Eq. **9** represents the slip velocity at $r = \alpha$. For flow in a glass tube, $\alpha = 1$, and because $G(1) = 0$ (1), the slip velocity in Eq. **9** vanishes and Eq. **9** satisfies the no-slip condition at the tube wall. The function v_{fit} and its derivatives are continuous on $0 \le r^* \le \alpha$.

The primary error source in our prediction of dp/dz is attributable to the uncertainty in our estimate of wall shear rate *in vitro*, because this quantity depends on the first derivative of our fit to the data. We have found that this quantity can be accurately estimated from the data used to extract the velocity distribution as long as (*i*) the true radial position of at least one particle tracer is within a distance of $\approx 0.1R$ from the tube wall, (*ii*) at least one particle tracer is within a distance of $\approx 0.2R$ from the tube centerline, and (*iii*) no two adjacent particle tracers are more than a distance of $\approx 0.4R$ apart if either one of the two tracers has a radial position, r, of more than $\approx 0.5R$. Any data set that did not meet all three of these criteria (after the optical correction procedure) was excluded from our analysis. Furthermore, in order to achieve sufficient spatial resolution to accurately predict the velocity distribution near the tube wall, we have found that the microsphere-to-vessel diameter

ratio should be less than ≈2%.

Materials and Methods for Glass-Tube Experiments. Perfusion experiments were performed at room temperature (23–24°C), which was continuously monitored at the microscope stage. Capillary tubes were pretreated with 1% Tween 20 in PBS for 2 hours to prevent cell adhesion to the tube wall. The downstream reservoir and silastic tubing were prefilled with degassed saline, and all air bubbles were removed. The fluids were perfused through each glass tube in the following order: saline, plasma, and red-cell suspensions. A high perfusion pressure (≈100 cm H_20) was applied for 1 minute at the start of each new perfusate. The perfusion pressure was quickly reduced to establish the target perfusion pressure. The velocity was measured ≈1.5 cm from the inlet of the glass tube, and the flow was measured for 1–2 minutes at each perfusion pressure.

Transillumination was maintained to keep the luminal glass capillary wall clearly visible. The midsagittal plane was defined as corresponding to the focal plane at which the contrast of the edge of the intraluminal wall reversed (14). The diameters of the upstream and downstream ends of the glass tube were determined by end-on microscopy. Capillary tubes were immersed in phthalic acid dibutyl ester (ND = 1.48, Sigma), which has an index of refraction that effectively eliminates optical refraction at the outer tube wall. Optical refraction at the inner tube wall, which arises from the mismatch in the refractive indices of the perfusate and the glass, introduces an optical artifact, as manifested by the disparity between the measured and actual radial positions of the microsphere. This disparity increases with increasing radial position. Applying Snell's law, the disparity, δx, between the measured radial position, d_m (open symbols in Figs. 1 and 5–14), and actual radial position, d_a (closed symbols in Figs. 1 and 5–14), of a microsphere in the midsagittal plane of the glass capillary tube is given by (14)

$$\delta x = d_\mathrm{m} - d_\mathrm{a} = \sqrt{d_\mathrm{m}(2R - d_\mathrm{m})}\,\tan\left(\sin^{-1}\left(\frac{N_\mathrm{g}}{N_\mathrm{p}}\sin\theta_1\right) - \theta_1\right) \tag{10}$$

where N_g and N_p are the indices of refraction of the glass tube wall and perfusate, respectively, and

$$\theta_1 = \frac{\pi}{2} - \tan^{-1}\left(\frac{\sqrt{d_\mathrm{m}(2R - d_\mathrm{m})}}{R - d_\mathrm{m}}\right). \tag{11}$$

The refractive indices of plasma and saline were determined by using a hand-held refractometer (Atago, Kirkland, WA), and the viscosity of plasma was determined by using a capillary viscometer (Cannon-Manning Semimicro Viscometer, Cannon Instrument, State College, PA).

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