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## ALTERNATIVE VIEW ON BLOOD AND BLOOD PLASMA IN THE VASCULATURE

U. Windberger<sup>a,\*</sup> and L. Noirez<sup>b</sup>

UDC 611.1; 532.5

**Abstract:** Results of an experimental study of blood properties are reported. To approach the boundary conditions of blood biophysics in a real flow, the viscoelastic measurements are carried out on surfaces coated with a 10-nm thick fibrous layer composed of extracellular matrix protein. For native whole blood, a plateau of the shear elastic modulus as a function of frequency is observed. The immediate consequence of the shear elasticity is that it is necessary to exceed the stress threshold so that blood can flow. This elastic threshold depends on the boundary conditions.

*Keywords:* blood plasma, rheology, interfacial forces, boundary conditions, shear elasticity, endothelial surface layer.

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### INTRODUCTION

The liquid state is the most widely spread state on the Earth's surface; it plays the most crucial role up to the ultimate scales of life. It is also the least understood state. The microfluidic scale is certainly a scale at which these puzzling effects are more visible. It is a scale at which the mass flux occurs in the vasculature, and it is, therefore, crucial for living bodies. The role of blood plasma in this regard appears to be still poorly explored in relation to studies on red blood cells (RBCs), although the plasma transmits the shear stress of the flow to RBC membranes forcing their mobility and deformation and, thus, couples the RBC flow with its own flow [1]. The plasma also interacts with the vessel wall and forms a marginal cell-free layer that lubricates the RBC flow [2, 3]. In general, flowing red blood cells do not touch the wall, whether in vitro or in vivo. RBCs are always located at a certain distance from the wall [4]. The biophysics of this cell-free layer justifies investigations because of its impact on the flow resistance. This was documented in a meta-analysis of patient data [5] and also experimentally [6] by diluting blood or by changing its composition. The marginal plasma layer cannot be purely fluidic over its entire width. It contains a luminal part in which the plasma flows with the RBCs and a marginal part where plasma flow is retarded until a situation is reached where the plasma becomes stationary. The presence of an immobile layer close to the endothelial cells was evidenced in early studies by the differences between the inflow (tube) hematocrit and outflow (discharge) hematocrit [7]. This layer was later visualized in hamster cremaster muscle capillaries [8]. Typical properties of this layer [9–11] include sieving of molecules [12] and prevention of excessive fluid filtration into the interstitium [13, 14]. The closest layer on endothelial cell surfaces (endothelial glycocalyx layer; EGL) confines the plasma within its fibrous network and generates a matrix. It protects the endothelium against the adherence of prothrombotic and inflammatory agents [15, 16] and can diminish in diseases [17]. Its stiffness is slightly more than 0.3 pN/nm [18], and its apparent Young's modulus is around 40 Pa [19]. On the basis of the flow resistance, the calculated hydraulic resistivity identifies it as a very dilute matrix of macromolecules [20]. To date it is not known

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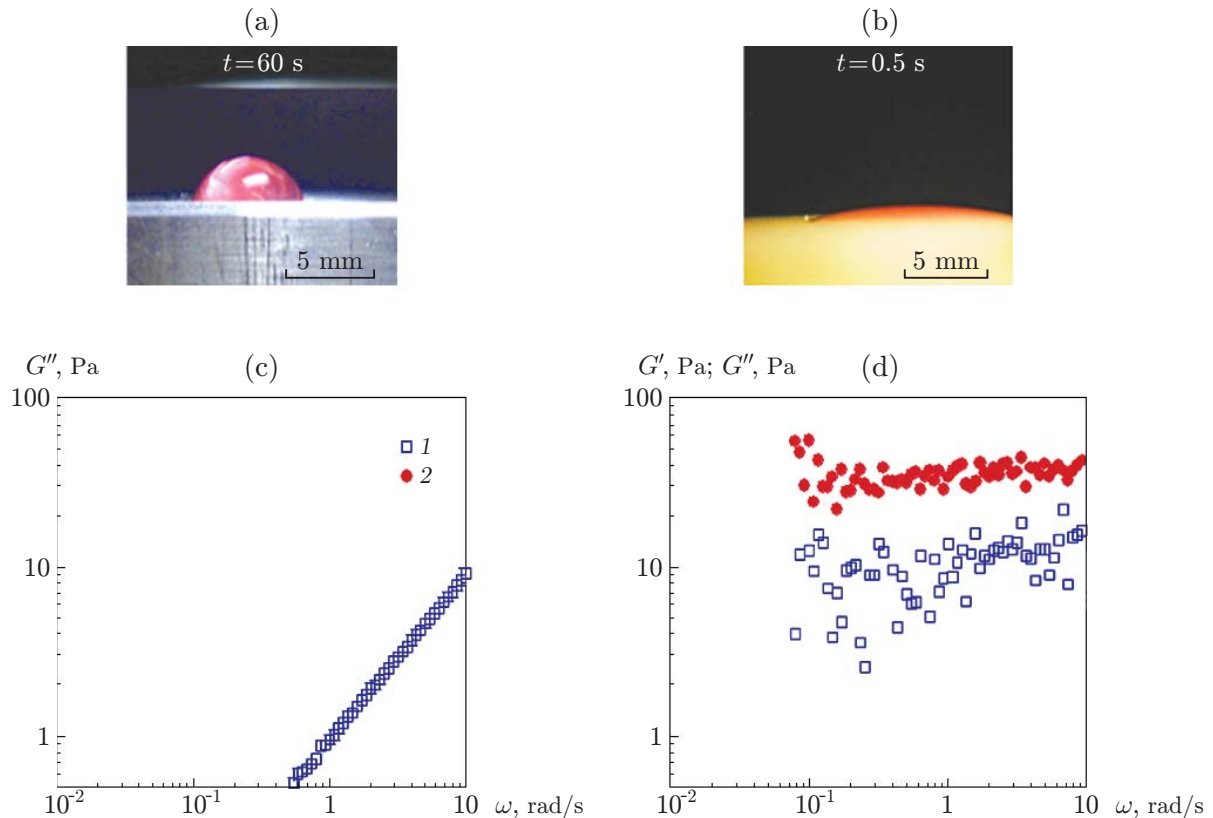
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whether the matrix layer exceeds luminally through adsorption of macromolecules to generate the total width of the endothelial surface layer (ESL) [21]. The difficulty in deciding the composition of the immobile layer on vessel walls arises from the experimental difficulty in determining the length of the fragile glycocalix. Reported data range from several tens to several hundreds of nanometers wide [22]. The flow resistance, however, indicates an ESL width to be in the range of several micrometers [4], which strongly suggests that the plasma layer is so much retarded that it no longer flows.

Our motivation was to find gel-like properties in blood and blood plasma. With plasma, we carried out dynamic mechanical tests in which the conventional steel surfaces [23] were replaced by patented ceramic fixtures [24] to improve the interactions between the sample and the substrate and, thus, optimize the shear stress transmission across the gap. With blood, we chose a coating that simulates the boundary of a dissected arterial wall. The principle of rheometry consists in measuring the time-dependent response transmitted by the fluid subjected to a dynamic mechanical shear stress versus the frequency  $\omega$ . The fluid is confined between two surfaces; the mechanical stress is transmitted via the interfacial interactions between the fluid and substrate; therefore, it depends on the energy of interaction between the sample and the test surface in contact with the fluid.

Although the principle of rheometry is well known, the boundary conditions between the substrate and fluids are often ignored [25]. By enhancing the force of interaction of the fluid with the substrate by using the excellent wetting properties of a high-energy substrate (ceramic), we accessed the shear elasticity of blood plasma and showed that the elastic property was reinforced at small scales (few hundreds of microns) [26]. The consideration of interfacial forces [27, 28] is recent and has revealed that the boundary conditions play a major role in the quality of rheological measurements. The dynamic response of fluids (including viscous liquids) under total wetting conditions can be much stronger and can make a viscoelastic behaviour of a solid-like type appear (Fig. 1).



**Fig. 1.** Wetting and liquid elasticity: (a and b) formation of a glycerol droplet ((a) nearly complete wetting after 60 s (deposition of the liquid onto the aluminum surface) and (b) complete wetting (deposition of the liquid onto the non-porous ceramic surface)); (c and d) viscous modulus  $G''$  (1) and shear elastic modulus  $G'$  (2) versus frequency in the case of glycerol deposition onto the aluminum (c) and ceramic (d) surfaces.

This identification of “static” shear elasticity in confined geometry challenges the classical viscoelasticity model (Maxwell’s model) according to which the liquid differs from the solid by the absence of resistance to the shear stress:  $G'_{|\omega \rightarrow 0} = 0$ .

The impact of boundary conditions in the viscoelastic response demonstrates that fluids cannot be characterized by a universal viscoelastic signature [25]. The identification of shear elastic properties of blood plasma is in agreement with its role in forming an elastic layer on vertebrate RBCs. The halo that was detected in atomic force microscopy/single cell force spectroscopy (AFM-SCFS) tests not only enlarged the functional cell size, but also blunted the thermal input into the RBC membrane [29]. The plasma components can, thus, accumulate on several cell surfaces to create an elastic envelope.

## MATERIALS AND METHODS

A typical viscoelastic measurement was carried out using a stress-controlled Physica MCR301 rheometer (Anton Paar, Austria) to impose the frequency, the shear stress, and the gap thickness. The blood confined between two parallel plates 50 mm in diameter was submitted to a dynamic shear stress generated from the sinusoidal movement of the plate

$$\sigma = \sigma_0 \sin(\omega t),$$

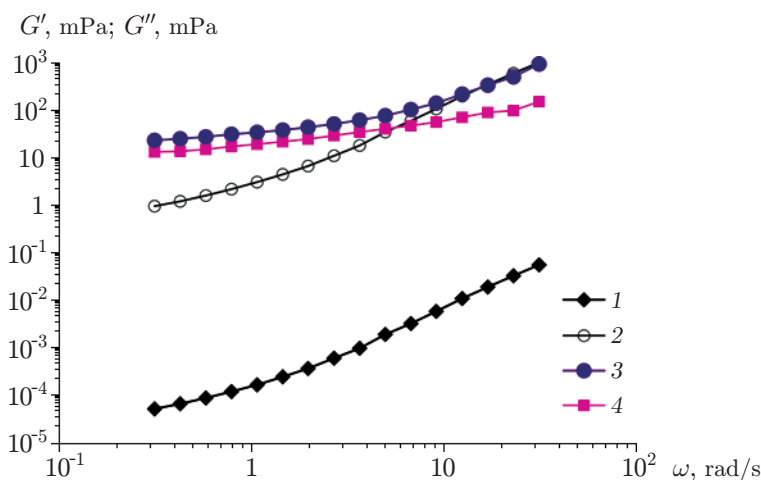
where  $\sigma_0$  is the imposed shear stress, and  $\omega$  is the frequency. By varying the frequency of the imposed sinusoidal wave stress

$$\sigma(\omega) = \sigma_0 \sin(\omega t + \Delta\varphi),$$

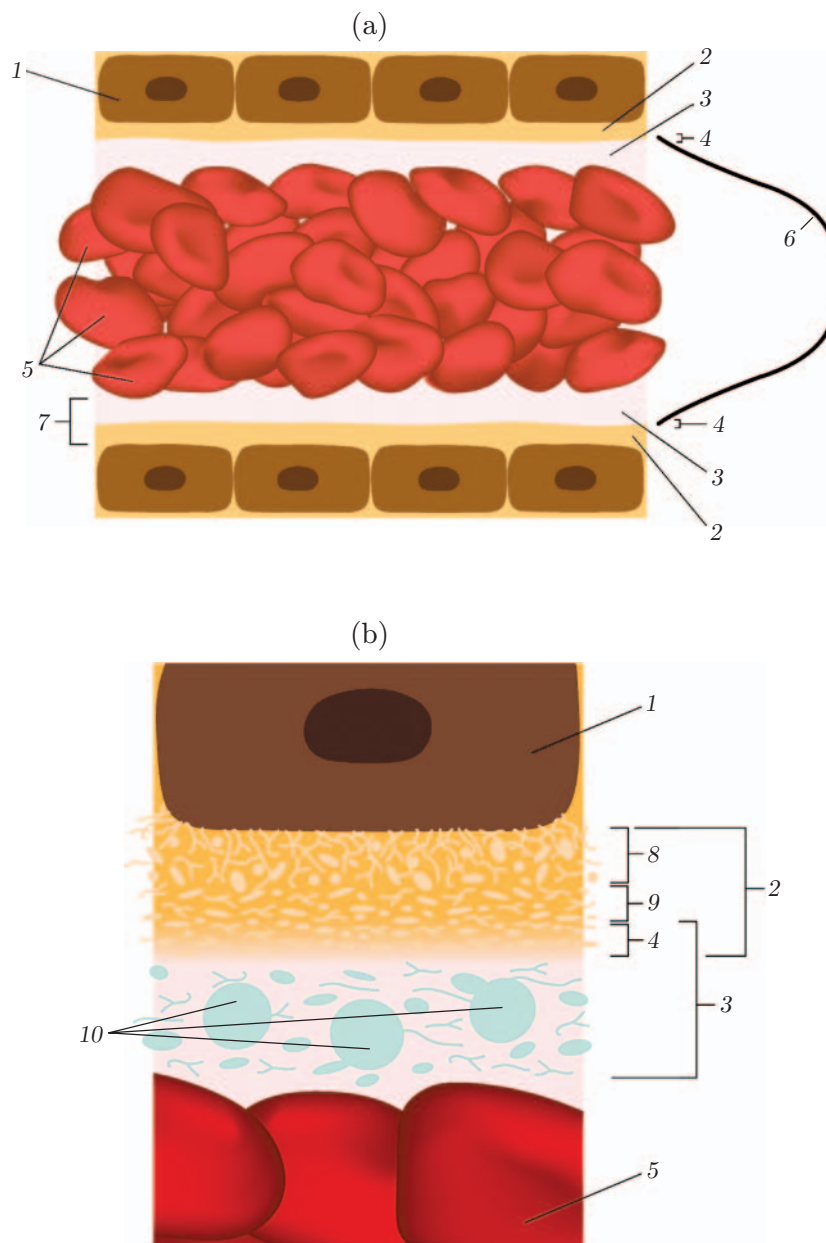
( $\sigma(\omega)$  is the imposed shear stress, and  $\Delta\varphi$  is the phase shift between the input and output waves), the impact of the incoming shear stress on the fluid dynamics can be analyzed in terms of the shear elastic  $G'(\omega)$  and viscous  $G''(\omega)$  moduli as

$$\sigma(\omega) = \gamma_0(G'(\omega) \sin(\omega t) + G''(\omega) \cos(\omega t)).$$

Here  $\gamma_0$  is the strain amplitude defined as the ratio of the displacement to the sample gap thickness. It should be emphasized that the formalism in terms of  $G'$  and  $G''$  implies that the resulting stress wave keeps the shape of the imposed sinusoidal stress wave. A constant shear stress amplitude of 10 mPa was applied, the frequency was varied in the interval 0.3–30.0 rad/s, and the chosen gap thickness was 0.7 mm.



**Fig. 2.** Experimental curves of the shear elastic (1, 3) and viscous (2, 4) moduli versus frequency obtained for a native human blood sample by using conventional stainless steel plates (1, 2) and coated plates (3, 4).



**Fig. 3.** Stationary and mobile layers adjacent to the endothelial cell lining in blood vessels: (a) general view; (b) detailed view; (1) endothelial lining; (2) static cell-free layer; (3) fluidic cell-free layer; (4) dynamic interface; (5) red blood cells; (6) flow profile; (7) cell-free layer width; (8) glycocalyx; and (9) adsorbed plasma molecules; (10) platelets.

Due to the importance of the flow inside the false lumen of a dissected aortic wall [30], we coated our plates with a layer that mimics the connective tissue. We used a purified and decellularized chorion-derived extracellular matrix hydrogel powder [31] and diluted it in a phosphate buffer (0.2% powder concentration). We placed a portion of it on top of our rheometer plates (bottom fixture: borate glass; upper fixture: disposable plates fabricated of aluminium-coated tinplate) and submerged the surfaces afterwards slowly into the phosphate buffer at 37°C in a Petri dish. After two hours of incubation, the buffer was removed by placing the plates upright and letting the buffer drain into the absorbent material, which was visible by a slightly opaque film on the glass surface. We mounted the plates, adjusted the motor and inertia of the upper plate due to the additional weight, and performed frequency sweep tests of a native human blood sample (hematocrit: 42%). Conventional viscoelastic measurements with stainless steel substrates were carried out under the same conditions for reference and comparison. Both series of measurements were performed at 37°C.

## RESULTS AND CONCLUSIONS

Figure 2 displays the shear elastic and viscous moduli of a 0.7 mm human blood layer as functions of frequency.

The viscoelastic response obtained on the coating is compared to the conventional rheometry protocol using stainless steel surfaces under the same dynamic conditions. The results show that blood exhibits a shear elastic plateau around 30 mPa, and oscillates as a whole almost in phase in the gap when it is in contact with extracellular matrix proteins. The absence of shear elasticity in the control experiment with steel plates exhibiting a viscous-like behaviour demonstrates the influence of the coating. Defining the intimate contact area of the flowing blood suspension with the endothelium is extremely challenging. Blood always begins to flow at a certain distance away from the endothelial cell membrane, because there is a solid-like layer in between (Fig. 3). This layer reduces the available space for the flow [3]. What first comes into mind is an albumin coating due to its strong tendency to bind to cell surfaces [32, 33]. The albumin coating is, on the one hand, effective in protecting RBCs from lysis due to hydrodynamic shear forces [34]. On the other hand, albumin alone cannot generate a full-length surface layer [35]. We showed recently that blood plasma (and not the concentration of the structures that it contains) can form a stationary layer when the blood flow is retarded and when there is sufficient adhesion to the substrate below [26]. Thus, we support the concept that the EGL can be extended lumenally through the interaction with plasma components [21]. This finding is in agreement with new theoretical models integrating the effects of confinement [25, 36].

To conclude, we have evidenced that submillimeter thickness blood layers confined between two thin sheaths of extracellular matrix proteins exhibit a finite elastic threshold. The identification of shear elasticity in blood has the immediate consequence that it is necessary to exceed the stress threshold so that blood can flow. This elastic threshold depends on the boundary conditions.

From a fundamental point of view, this dynamic study points out the importance of interfacial conditions, which certainly modify the flow in microchannels and open the question of personalization of hemodynamic models for practical applications.

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