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Developing Rheological Characterization Methods for Bovine Blood and Hydrogel-Based Artificial Blood

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ABSTRACT

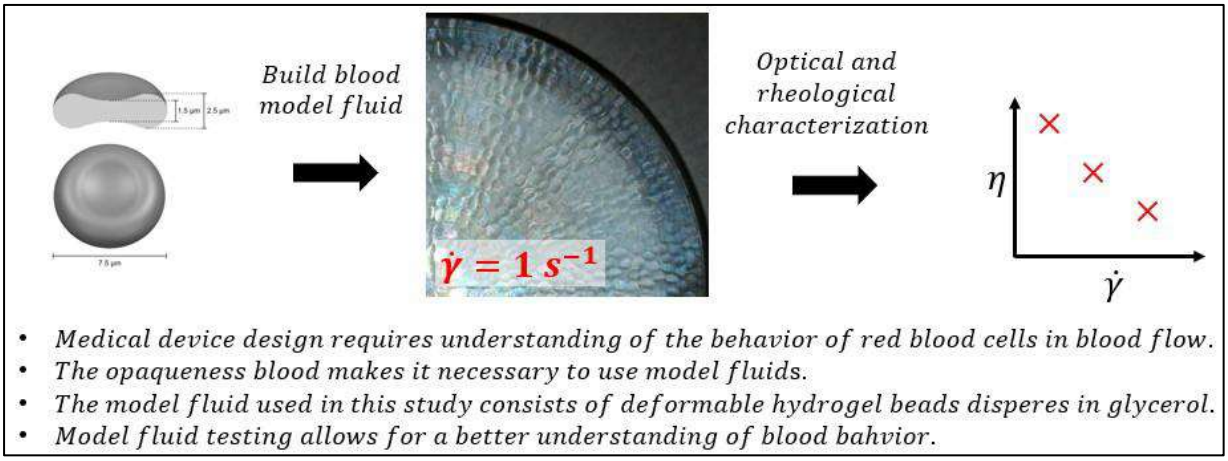

FIGURE 1: Graphical abstract: Rheological understanding of blood is relevant for the design of cardiovascular medical devices and implants. Erythrocytes are represented by hydrogel beads dispersed in a continuous glycerol phase. The model system is characterized by aims of optical and rheological characterization. The erythrocyte schematic has been taken from [1]. The deformable nature of the hydrogel particles can be seen from the pictures taken during the squeezing experiment (see Fig. 4).

INTRODUCTION TO BLOOD RHEOLOGY AND ARTIFICIAL BLOOD

Understanding the rheological behavior of blood is relevant for the design of medical devices or implants such as blood pumps, grafts, and stents[2]. Blood is a complex multiphasic fluid consisting of blood plasma and blood cells (see also Fig. 1 depicting red blood cell measuring 7.5 µm in diameter, 1.5 µm in inner and 2.5 µm in outer cross section[1]). Its structure yields a complex flow behavior. Due to similar cell sizes and volume fraction (hematocrit), bovine blood is often used as substitute for human blood, thus, it is an interesting material for bio-rheological characterization[3]. Due to its limited stability and its opaqueness, the flow-induced behavior of

blood cells is difficult to study[4]. This is our motivation for the development of a blood substitute fluid with similar rheological behavior as bovine blood with a transparent continuous phase.
In this study, we investigate several approaches for the shear rheological and optical characterization of artificial blood with hydrogel-based particles as model erythrocytes, dispersed in a glycerol-water phase. The results are compared with those gained with bovine blood.

MATERIAL AND METHODS

Artificial blood

Erythrocytes were represented by hydrogel beads made from poly-sodiumacrylate-co-acrylamide obtained with a microfluidic setup. The beads were dispersed in a 36 % v/v glycerol solution at a concentration of 40 vol.%. A fraction of the hydrogel beads was functionalized with hemoglobin and dispersed in a phosphate-buffered saline solution (PBS) at a concentration of 20 vol.%.

Bovine blood samples

Bovine whole blood (hematocrit of 45 vol.%), bovine erythrocyte concentrate (100%v/v), and bovine platelet rich plasma (PRP) samples were used as references in this study.

Rheological characterization of the artificial blood

A Kinexus Prime ultra+ rotational rheometer (NETZSCH-Gerätebau GmbH, Selb, Germany) equipped with a cylinder Peltier-cartridge and a quartz-glass plate was used for the rheological measurements. A USB camera with up to 200 x magnification allowing for optical observation of the sample was present inside the cylinder cartridge. An upper plate made out of stainless steel with a polished surface with 40mm in diameter was used. Shear viscosity measurements at constant shear rates (referred to as “Viscometry Single Value” in Fig. 2) of 1 s^{-1}, 10 s^{-1}, and 100 s^{-1} were performed.
Functionalized artificial blood was squeezed between the upper plate and the lower plate by lowering the upper plate at a speed of 0.1 $mm \cdot s^{-1}$.
Bovine blood measurements were carried out with the same instrument, using an active hood Peltier-cartridge and stainless steel plate-plate geometries.
All measurements were carried out at 25°C to minimize drying of the samples. The instrument was set to this temperature for thermal equilibrium of the system prior to determining the zero gap. Complementary measurements with a concentric cylinder geometry (C14) were also carried out.

RESULTS AND DISCUSSION

The shear rheological measurements were carried out at different shear rates to study the transient behavior of the samples. Fig. 2 depicts the results of the shear viscosity measurements. The change in the measured shear viscosity over time for the investigated shear rates could be explained by sedimentation of relatively big particles or by time-dependent structural changes in the sample.

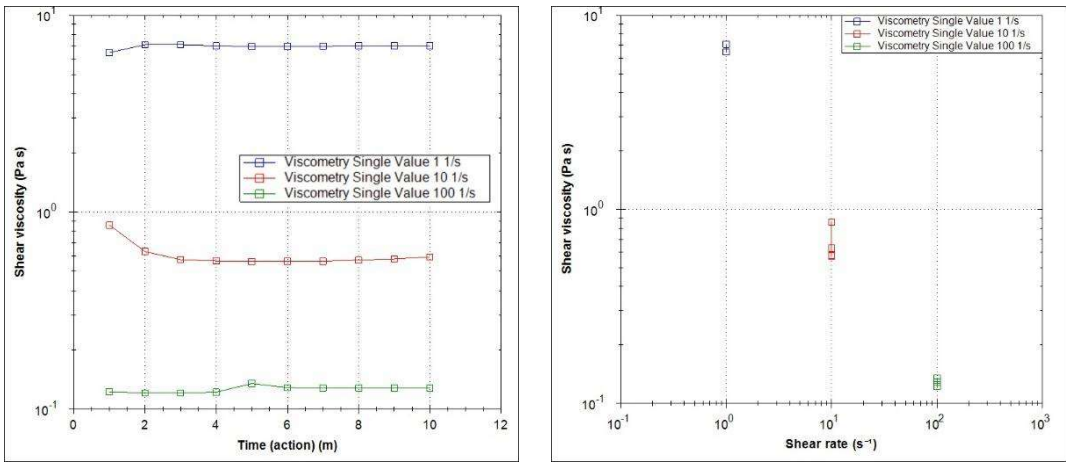

FIGURE 2: Shear viscosity of glycerol-based artificial blood at different shear rates (right). Time dependent shear viscosity (left) vs. time as the respective shear rate. Right: Shear viscosity vs. shear rate.

Pictures obtained with the USB camera video recording at different shear rates are depicted in Fig. 3. Each picture was taken after 20 s of constant shear. The images show the artificial blood sample at different shear rates. For all shear rates, the same image section (upper right) was chosen and consequently, the images of all shear rates except for shear rate 1 s^{-1} are displayed in a rotated way. The particularly bright spots at lowest plate diameters are reflections of the camera illumination on the upper plate. At a shear rate of 1 s^{-1}, the visible non-spherical hydrogel particles tend to be oriented from the inside to the outside of the plate-plate geometry. This orientation can be attributed to the squeeze-flow-like flow when the upper plate is lowered before starting the measurement. There was only a slight movement visible of the particles close to the lower quartz-glass plate which can be seen as an indication of wall adhesion. At higher shear rates (10 s^{-1} and 50 s^{-1}), the particles close to the lower quartz-glass plate tend to move. This is more pronounced with bigger plate diameters which can be explained by the shear rate distribution within the shear gap of a plate-plate geometry which has higher shear rates at bigger plate diameters or possible edge effect. At highest shear rates (100 s^{-1}), particles stick to the lower quartz-glass surface only occasionally. This behavior can be explained by hydrodynamic forces induced by the shear velocity gradient over the particle. From the video recordings, a direct particle-particle contact between the dispersed particles can be seen, which can be expected at the relatively high solid volume fraction of the artificial blood. The deformable nature of the hydrogel particles can be seen from the pictures taken during the squeezing experiment (cf. Fig. 4).

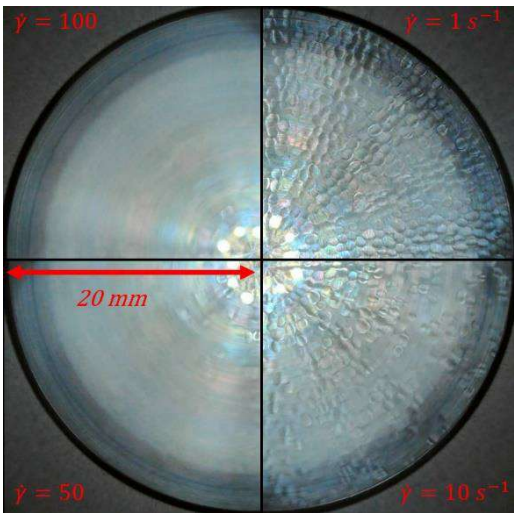

FIGURE 3: Artificial blood at different shear rates. The pictures were recorded from underneath the lower plate.

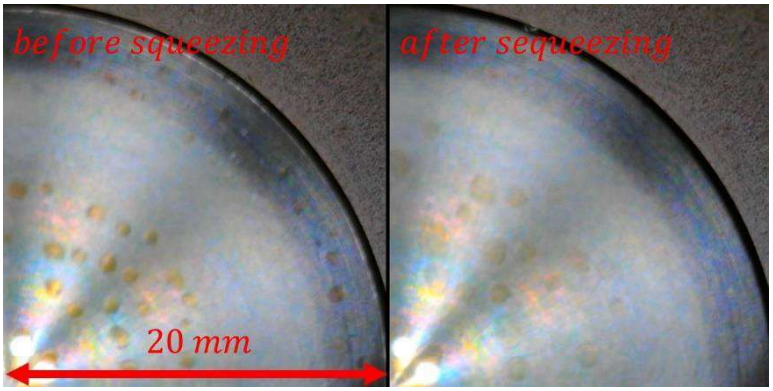

FIGURE 4: Artificial blood functionalized with hemoglobin before and after squeezing.

The results of the shear viscosity measurements at shear rates of 10 s^{-1} and 100 s^{-1} are shown in Fig. 5. Over the measurement time of 10 min for both shear rates, no pronounced changes in shear viscosity were observed. For both the bovine erythrocyte concentrate and the bovine whole blood sample, there was a clear decrease in shear viscosity at 100 s^{-1} compared to 10 s^{-1}. This shear-thinning behavior was not observed for the plasma sample as well as for the bovine platelet rich plasma sample. The latter sample showed similar shear viscosity values.

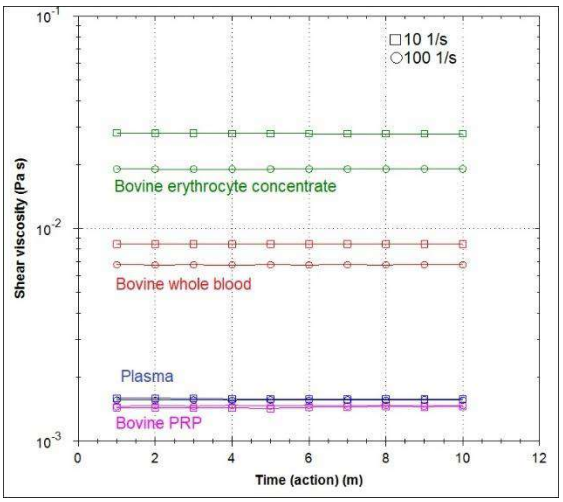

FIGURE 5 Results from transient shear viscosity measurements of bovine blood samples.

CONCLUSION AND OUTLOOK

In this study, shear thinning behavior of the model blood fluid, bovine whole blood and bovine erythrocyte concentrate could been well-observed by rotational rheology. The shear viscosity of the model fluid was higher as compared to those of the bovine blood samples. Additional measurements are required to investigate shear viscosity values comparing bovine plasma and platelet-rich plasma. Lower absolute shear viscosity values of the model blood fluid would be desirable to be closer to the real blood sample. However, a major challenge when working with artificial blood is the sedimentation of the hydrogel beads. The beads should be further reduced in size to get closer to the size of erythrocytes and to reduce sedimentation during rheological measurements. To further improve the introduced measurement methods, the authors will implement optical methods with higher time and spatial resolution.

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