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Influence of red blood cell aggregation on perfusion of an artificial microvascular network

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Abstract

Red blood cells (RBCs) suspended in plasma form multicellular aggregates under low flow conditions, increasing apparent blood viscosity at low shear rates. It has previously been unclear, however, if RBC aggregation affects microvascular perfusion. Here we analyzed the impact of RBC aggregation on perfusion and 'capillary' hematocrit in an artificial microvascular network (AMVN) at driving pressures ranging from 5 to 60 cmH_2O to determine if aggregation could improve tissue oxygenation. RBCs were suspended at 30% hematocrit in either 46.5 g/L dextran 40 (D40, non-aggregating medium) or 35 g/L dextran 70 (D70, aggregating medium) solutions with equal viscosity. Aggregation was readily observed in the AMVN for RBCs suspended in D70 at driving pressures ≤ 40 cmH_2O. The AMVN perfusion rate was the same for RBCs suspended in aggregating and non-aggregating medium, at both 'venular' and 'capillary' level. Estimated 'capillary' hematocrit was higher for D70 suspensions than for D40 suspensions at intermediate driving pressures (5 – 40 cm H_2O). We conclude that although RBC aggregation did not affect the AMVN perfusion rate independently of the driving pressure, a higher hematocrit in the 'capillaries' of the network for D70 suspensions suggested a better oxygen transport capacity in the presence of RBC aggregation.

Keywords

Artificial microvascular network; Dextran; Hematocrit; Microvascular perfusion; Red blood cell aggregation

Introduction

Red blood cells (RBCs) suspended in plasma tend to aggregate among each other, forming multi-cellular branched or linear aggregates called rouleaux [5]. RBC aggregation occurs

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Disclosures

All authors declare no potential conflicts of interest.

Data and materials availability

All data and described custom-written scripts are available from the authors upon reasonable request.

under conditions with low shear rates during stagnant or low flow. It is a reversible process, i.e., aggregates are dispersed by higher shear forces and reform within seconds when those forces have ceased. RBC aggregation depends on the concentration of high molecular weight proteins such as fibrinogen in plasma [5].

Increased RBC aggregation has been reported in many different diseases such as bacterial infections [2,26] and sepsis [1], myocardial ischemia and infarction [35,73], cerebral infarction [60], peripheral vascular disease [33], hypertension [19], diabetes mellitus [72], metabolic syndrome [61], hypergammaglobulinemias [34], and inflammatory rheumatological diseases [57,65].

Inflammatory disease is accompanied by a systemic acute phase reaction characterized by an increased production of C-reactive protein, fibrinogen, alpha-2-macroglobulin, and immunoglobulins [23,25,32]. Increased concentration of fibrinogen and other high molecular weight proteins in plasma leads to increased RBC aggregation [9] which becomes apparent through elevated RBC sedimentation rate, a very old and time-honored laboratory test in clinical medicine [21]. It is still a matter of controversy whether increased RBC aggregation is beneficial and contributes to recovery from a disease [30,70], whether it is a harmful process and thus perhaps a mediator of disease [3,62,67], or an irrelevant parameter and only a marker of disease [15,28].

RBC aggregation affects hemorheology by increasing blood viscosity at low shear rates [16]. In tube flow, RBC aggregation occurs in the center axis of the tube, which leads to an increased cell-free layer near the wall [42,59]. Whether RBC aggregation also affects the flow of blood in the microvasculature is still a matter of debate. Current experimental data are confounded by the fact that a change in RBC aggregation is accompanied by a concomitant change in plasma viscosity, which itself is a major determinant of capillary blood flow [4,28]. In the present study we suspended normal human RBCs in two solutions of dextran with equivalent viscosities, but different capacity to cause RBC aggregation, namely dextran 70 as aggregating medium and dextran 40 as non-aggregating medium. We measured the ability of RBCs suspended in these to different media to perfuse an artificial microvascular network (AMVN). We also estimated the hematocrit in the “capillary” and “venular” portions of the AMVN for both suspensions, which in combination with the perfusion rate data, allowed us to test whether RBC aggregation increases or decreases tissue oxygenation capacity.

Material and Methods

Dextran solutions

Dextrans with molecular weights of 70,000 Daltons (dextran 70) and 40,000 Daltons (dextran 40) were used (Tokyo Chemical Industry Co., Tokyo, Japan). Both dextran 70 and dextran 40 were dissolved in saline for measuring RBC aggregation and viscosity, or in GASP buffer (1.3 mM NaH_2PO_4, 9 mM Na_2HPO_4, 140 mM NaCl, 5.5 mM glucose, 1% bovine serum albumin; osmolality 290 mOsm/kg; pH 7.4) for measuring AMVN perfusion rates. Dextran 70 was dissolved at a concentration of 35 g/L based on our previous experience studying RBC aggregation [41]. A series of dextran 40 concentrations was

prepared and their viscosities were measured (see below). From these viscosity measurements it was calculated that 46.5 g/L dextran 40 solution matched the viscosity of the 35 g/L dextran 70 solution, and therefore these concentrations were used in all experiments.

Blood samples

Samples of whole blood were obtained from healthy consenting volunteers via venipuncture using EDTA as an anticoagulant (4 mL, 7.2 mg K_2EDTA, Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA). The collection of blood samples was approved by the institutional review board (IRB) at the University of Houston (Houston, TX). For measurements of RBC aggregation (n = 5) and suspension viscosity (n = 10), whole blood was centrifuged at 2500 × g for 5 min (Allegra X-15R, Beckman Coulter, Schaumburg IL, USA), and plasma, buffy coat and the uppermost RBC layer were discarded. The pelleted RBCs were washed twice in saline and then resuspended in either dextran 40 or dextran 70 solutions described above with final hematocrit adjusted to 30%.

For measuring AMVN perfusion (n = 7), whole blood was centrifuged at 1300 × g for 5 min, plasma was discarded, and pelleted RBCs were diluted with GASP buffer down to ~5% hematocrit. The diluted RBC suspension was passed through a high-efficiency pediatric leukocyte reduction filter (Purecell NEO, Pall Corp., Port Washington, USA). The filtrate was centrifuged at 3080 × g for 5 min, the supernatant was discarded, and packed RBCs were re-suspended in either dextran 40 or dextran 70 solution described above at 30% hematocrit. All measurements of hematocrit were performed in duplicate on a hematology analyzer (Medonic M-series, Boule Medical AB, Stockholm, Sweden). Samples were kept on a mixer (Labquake, Barnstead Thermolyne, Dubuque, Iowa, USA) until AMVN experiments were performed. Each RBC suspension was tested once then discarded, and all experiments were completed within 4 hours of drawing samples.

Viscosity measurements

A coaxial Couette-type viscometer (Contraves LS 30, ProRheo, Althengstett, Germany) was used to measure solution viscosities. Measurements of viscosity of dextran 40 and dextran 70 solutions were done at a shear rate of 11 s^{-1}. Viscosities of RBC suspensions were measured over a wide range of shear rates in the following order: 69.5, 27.7, 11.0, 3.23, 0.95, 0.28 and 0.11 s^{-1}. Viscosity values were calculated from the plateau value of the stress-strain curve generated by the viscometer. All measurements were performed at room temperature within 4 hours.

Measurements of RBC aggregation

An aggregometer with a cone-plate shearing system and integrated infrared light transmission measurement (Myrenne MA-1, Roetgen, Germany) was used to quantify RBC aggregation. RBC aggregates were dispersed at a shear rate of 600 s^{-1} in an initial phase, which was followed by an integration of light transmission over 10 s either at stasis (M mode) or at a low shear rate of 3 s^{-1} (M1 mode). Measurements were performed at room temperature in triplicate.

Measurements of the AMVN perfusion rate

The scientific background, design and fabrication of the artificial microvascular network (AMVN) devices, and the methods used for measuring the AMVN perfusion rate have been described previously [13,24,50,51,54,55]. Each AMVN device contained three identical networks of “capillary” microchannels (widths 5-70 μm) arranged in a pattern inspired by rat mesentery microvasculature, and each having an independent inlet port connected to the “capillary” network via a 70 μm wide channel (“arteriole”) and converging to a common outlet port by a 70 μm wide channel (“venule”) [13]. All microchannels comprising the AMVN were 5 μm deep. AMVN devices were manufactured using polydimethylsiloxane (PDMS; Sylgard 184, Dow Corning Corp., Midland, MI) replicas of a patterned silicon wafer fabricated using soft lithography as described previously [13]. Patterned casts were then sealed to PDMS coated glass slides after 100 second exposure to air plasma. The assembled AMVN devices were treated for 30 min with a 1% solution of mPEG-silane (Laysan Bio, Inc., Arab, AL) in GASP, and then flushed with GASP buffer prior to use.

GASP buffer was removed from the AMVN inlets by drying (Kimwipes, Kimberly-Clark Professional, Roswell, USA) and 25 μL of either dextran 40 or dextran 70 samples were deposited in each inlet. A 2 mm micro-stirring bar was inserted into each inlet, and a magnetic stirrer (Model 1060, Instech Laboratories, Inc., Plymouth Meeting, USA) placed above the inlets was used to actuate micro-stirring bars in the inlets to prevent aggregation and sedimentation of RBCs within the inlets. The driving pressure applied to the system was controlled by a water column connected to the AMVN device outlet. RBCs were allowed to fully perfuse the AMVN device, then the driving pressure was set to 0 cmH_2O by adjusting the height of the water column until the RBCs came to a complete stop. Thereafter the system driving pressure was set to 60, 40, 20, 10 and 5 cmH_2O for 3 min at each pressure (Note: a driving pressure of 2.5 cmH_2O was attempted, however obstruction of microchannels, due to RBC adherence to channel walls – especially with dextran 70, resulted in inconsistent RBC velocities; therefore these data were not included in the final analysis).

Images were captured with a high-speed camera (100 fps, Flea3, Point Grey Research, Inc., Richmond, Canada) and analyzed offline with a custom image analysis algorithm implemented in MATLAB 2014b (The Math Works Inc., Natick, MA, USA) [13,51]. The image analysis algorithm compared subsequent images to determine the change in position of RBCs between images, thereby enabling determination of RBC solution velocity. The AMVN perfusion rate was then calculated by multiplying the solution velocity by the cross-sectional area of the microchannel (5 μm × 70 μm = 350 $μm^2$ for “venules”).

Estimation of hematocrit in microchannels

Microscopic observations of the flow through the AMVN suggested that the hematocrit was higher with dextran 70 suspensions than with dextran 40. This prompted a *post-hoc* analysis as follows. The same images used to measure AMVN perfusion rate were also used to estimate hematocrit in individual microchannels of the AMVN. To improve contrast and simplify the measurements of RBC velocity, we imaged the flow of blood through the AMVN in blue light (RBCs appear dark in blue light) using a band-pass blue-violet filter

(394 ± 50 nm, B-390, Hoya Corp. USA, Fremont, CA). We reasoned that the hematocrit within each microchannel, therefore, was proportional to the intensity of light transmitted through the channel, based on the principles of spectrophotometry. The average grayscale color intensity (0 to 255 au) of the interior area within each microchannel and of the device background area around the microchannels was measured using a custom image analysis algorithm implemented in MATLAB (The Math Works Inc.). The average grayscale color intensity of each microchannel was subtracted from the average grayscale color intensity of the device background in order to correct for lighting variations resulting from the thickness of the PDMS device and the microscope settings. The corrected average grayscale color intensities for individual microchannels were then compared to determine the differences in mean estimated hematocrit between different microchannels relative to one another. Importantly, while we were able to determine whether hematocrit in one channel was higher or lower than in another channel using this approach, we could not measure the absolute values of hematocrit in the channels. Therefore all hematocrit data in **Figure 6** and **Figure 7** is presented as the difference between the two estimated hematocrits (as a percent) and no absolute hematocrit values are given.

Statistical analysis

Statistical analysis was performed using built-in functions of MATLAB 2014b statistics toolbox. Paired Student's t-tests were used for comparisons of dextran 40 and dextran 70 data. The results are given as mean values ± standard deviations (SD). A p-value of < 0.01 was considered significant.

Results

The viscosities of 46.5 g/L solution of dextran 40 and 35 g/L solution of dextran 70 in saline were very similar (**Table 1**). Aggregometry revealed RBC aggregation in dextran 70 suspensions, but not in dextran 40 suspensions, both at stasis (M mode) and under low shear flow (M1 mode), which is also shown in **Table 1**. **Figure 1** shows viscosities of RBC suspensions in either dextran 40 or dextran 70 with a hematocrit of 30% for a wide range of shear rates. Whereas the viscosity of RBC suspensions in dextran 40 was almost independent of the applied shear rate (so called Newtonian behavior), the viscosity of RBC suspensions in dextran 70 increased exponentially with decreasing shear rates, indicating RBC aggregation.

Figure 2 illustrates the microscopic appearance of RBC aggregates in an AMVN at various driving pressures. In contrast to dextran 40, which did not exhibit RBC aggregation even at the lowest driving pressure (far left side **Fig. 2**), stacks of RBCs without apparent gaps between adjacent cells were seen for dextran 70 suspensions in "arterioles", "venules" and in "capillaries", best visible by single files of aggregated RBCs moving like trains into a first order "venule" (e.g., see right "capillary" at driving pressures of 10 – 40 cmH_2O in **Fig. 2**). A driving pressure of 60 cmH_2O resulted in such high velocities that we cannot make statements about the occurrence of RBC aggregation. Adhesion of RBCs to the mPEG-silane treated walls of the microchannel was very rarely observed, and was always transient, across all driving pressures evaluated.

Figure 3 shows characteristic traces of the AMVN perfusion rate, calculated from the mean RBC velocity in “venular” microchannels. The perfusion rate traces for RBCs suspended in dextran 40 (without aggregation) were relatively constant for any given driving pressure, while those for RBCs suspended in dextran 70 (with aggregation) showed a considerable undulating variation over time, which most likely reflected the intermittent passage through the network of large aggregates with aberrant, rotational movements.

Figure 4 shows the AMVN perfusion rates (calculated using the measured mean RBC velocities in “venular” microchannels) for RBC suspensions in dextran 40 and dextran 70, for a range of driving pressures. There was a linear relationship between the driving pressure applied to the AMVN and the AMVN perfusion rate. The AMVN perfusion rates were similar for RBC suspensions in dextran 70 (with aggregation) and in dextran 40 suspensions (without aggregation) for all driving pressures.

We also measured RBC velocities in two different 5 μm “capillaries”. **Figure 5** shows the results of these measurements. The two “capillaries” (**Fig. 5 inset**) had very different RBC velocities despite having the same dimensions, because the right “capillary” branched from a larger feeding vessel than the left “capillary” (see schematic drawing of an AMVN on the left side of **Fig. 2**). A linear relationship between driving pressure and RBC velocity was also seen in these two different “capillaries”, and no difference in RBC velocity was observed between RBC suspensions in dextran 70 with aggregation and in dextran 40 suspensions without aggregation, even at low pressures, when linear RBC aggregates were clearly visible within the “capillaries” (see **Fig. 2**).

Figure 6 shows the estimated hematocrits in “capillaries” and “venules” for RBCs suspended in dextran 70 with respect to RBCs suspended in dextran 40, across the same range of driving pressures at which AMVN perfusion rates were evaluated. There was no difference in hematocrit at high driving pressure (60 cmH_2O). With decreasing driving pressures down to 10 cmH_2O, estimated hematocrits in aggregating suspension medium dextran 70 became gradually higher with respect to non-aggregating dextran 40. This difference in estimated hematocrit was more pronounced in “capillaries” (up to 30 – 40% at driving pressures of 5 – 10 cmH_2O) than in “venules” (up to 10% at driving pressures of 10 – 20 cmH_2O).

Compared with feeding arterioles or draining venules, capillaries have a lower hematocrit, which is called the Fahraeus effect [19]. We have analyzed the difference in estimated hematocrit between “capillaries” and draining “venules” of the AMVN (**Figure 7**). For RBCs in dextran 40, i.e. without RBC aggregation, “capillary” hematocrit was 30 – 40% below “venular” hematocrit and showed a tendency to become more pronounced with decreasing driving pressure. In contrast, aggregation-inducing dextran 70 reduced the “capillary” hematocrit to only 20 – 25% of the “venular” hematocrit at driving pressures of 5 – 40 cmH_2O, where RBC aggregation was visually observed (**Fig. 2**).

Discussion

The key finding of this study is that RBC aggregation did not affect the RBC flow velocity through microchannels of the AMVN. Blood flow is determined by plasma viscosity, hematocrit, RBC deformability and RBC aggregation [58]. In our *in vitro* experiments, we kept every parameter except RBC aggregation constant, which allowed an appraisal of the influence of RBC aggregation by itself without confounding factors: 1) the viscosities of 46.5 g/L dextran 40 and 35 g/L dextran 70 solutions were equivalent, 2) the hematocrit was 30% for both suspensions, and 3) RBC deformability is not known to be affected by either version of dextran. The only difference between the two types of suspensions was presence of absence of RBC aggregation (**Table 1**), as documented by light transmission aggregometry either at stasis or at low flow (at a shear rate of 3 s^{-1}) as well as by the increased viscosity at low shear rates (**Fig. 1**). In contrast to RBC suspensions in dextran 40, strong RBC aggregation was found in dextran 70, which confirms the results of the many previous studies done with dextran 70 [5,18,41,47,48,56,68].

As expected, RBC aggregation affected suspension viscosities at shear rates <10 s^{-1}; in contrast to RBC suspensions in dextran 40, RBC suspensions in dextran 70 behaved as non-Newtonian fluids characterized by a progressive increase of viscosity with decreasing shear rate. At shear rates ≥10 s^{-1}, suspension viscosities were similar for RBC suspensions in dextran 70 and dextran 40, indicating that RBC aggregates were disrupted at these higher shear rates. The shear rate threshold around 10 s^{-1} for RBC aggregation in a rotational viscometer is in agreement with earlier observations [12,16,58].

So far it has not been clear whether RBC aggregation occurs at all in the microcirculation *in vivo*, which is known to be a high shear environment. We have calculated pseudo-shear rates at capillary walls for our AMVN as the quotient of mean RBC velocity in the channel and channel diameter [31]. For RBC suspensions flowing through the faster capillary (shown on the right in **Fig. 5 inset**), pseudo-shear rates of 22, 48, 104, 220, and 350 s^{-1} correspond to driving pressures of 5, 10, 20, 40 and 60 cmH_2O respectively. We observed RBC aggregates even at these high wall shear rates (**Fig. 2**). This observation could be explained by the fact that shear forces act differently on RBC aggregates in the relatively large gaps of rotational viscometers (500 μm) than in 5 μm microchannels. Shear forces disrupt aggregates only when they produce velocity gradients between neighboring cells, e.g., when one plane of flow is moved relative to the adjacent plane, as is the case in rotational viscometers. In the case of single-file flow through 5 μm microchannels, the velocity gradient between adjacent RBCs is virtually zero and hence shear forces are negligible. This could potentially explain how RBC aggregates may form during flow through the microcirculation [31]. The occurrence of RBC aggregation in microcirculatory flow under physiologically relevant flow rates agrees with observations *in vitro* in microfluidic channels with a diameter of 4.5 μm and in capillaries *in vivo* [12,38].

RBC aggregation induced by dextran 70 did not affect RBC flow velocity in “capillaries” and “venules” of the AMVN, and thus had no influence on AMVN perfusion rates at any driving pressure. This observation is in agreement with the work of others [6,11,15,17,30,66,69,70], but contradicts many studies, which suggested a negative influence

of RBC aggregation on microvascular perfusion [3,7,10,63,67]. The difference could be due to the difference in experimental approaches. In the discordant studies, RBC aggregation was changed by the addition of an aggregating substance, which concomitantly also increased the viscosity of plasma or other suspending medium. Since the viscosity of the suspending medium is a major determinant of microvascular perfusion, these studies could not separate between the influence of RBC aggregation alone and viscosity of the suspending medium [4,28,49]. Carefully matching viscosity of both aggregating and non-suspending media in our experiments allowed us to draw the conclusion that RBC aggregation induced by dextran 70 *per se* did not affect AMVN perfusion.

The microscopic impression of a lower hematocrit in AMVN microchannels for dextran 40 suspensions compared with dextran 70 prompted further investigations. To this end we estimated changes in hematocrit based on differences in color density in “capillaries” and “venules” of the AMVN. It was confirmed that the hematocrit was lower, for RBCs suspended in dextran 40 (i.e., without aggregation) as compared to aggregating dextran 70 suspensions, in “capillaries”, and to a lesser degree also in “venules”. RBC aggregation increases sedimentation rate and could theoretically explain higher hematocrit values with dextran 70 in AMVNs. RBC sedimentation is, however, characterized by a lag phase of several minutes (usually 10 – 20 min) before it becomes visible [21]. In addition, we prevented RBC sedimentation in the inlets of the AMVN devices with magnetically actuated micro-stirring bars. Furthermore, the fact that the changes in hematocrit were dissimilar in “capillaries” and “venules” (up to a 4-fold difference) make an artefact rather unlikely. These observations and data presented in **Fig. 7** suggest that RBC aggregation may blunt to some extent the Fahraeus effect (i.e., the lowering of hematocrit during the passage of capillaries [22,53]).

The AMVN experimental setup has inherent limitations. It is an *in vitro* study on an idealized, artificial microvasculature comprised of rectangular microchannels 5 to 70 μm wide, but only 5 μm high. Thus, our device simulates the *in vivo* dimensions of arterioles and venules less well, allowing only the formation of two-dimensional, but not 3-dimensional aggregates, which occur in such vessels [8]. Furthermore, the AMVN lacks endothelial lining. *In* vivo, endothelial cells, coated by a glycocalix, interact with flowing RBCs and produce the vasodilatory active nitric oxide (NO). It has been recently shown that RBC aggregation reduces NO production [17]. Additionally, the AMVN device is constructed of PDMS, which is stiff and inert, unlike microvessels *in vivo* which are capable of dynamically altering vessel diameter in order to modulate blood flow [13,24]. However, spontaneous self-sustained oscillations of capillary blood flow, resulting solely from the non-linear rheological behavior of blood at the microscale, have previously been demonstrated in the AMVN system despite the lack of active regulatory input, such as vasomotion [24]. Last but not least, we studied only samples with a single standardized hematocrit and suspending medium viscosity. Our data, therefore, should only be used to draw conclusions about the hemodynamic effects of RBC aggregation induced by dextran 70 *in vitro*. They should not be directly and uncritically extrapolated to the microcirculation *in vivo* since dextran solutions, although studied intensively, do not represent physiological suspensions. Nevertheless, the AMVN has been rigorously validated in several studies

[13,14,44,50,51,54,55] and it is arguably the best available model system for measuring the influence of various properties of blood on microvascular perfusion *in vivo*.

Limitations notwithstanding, our data are not in favor of therapeutic reduction of RBC aggregation in patients, which has been attempted by lowering fibrinogen with the snake venom ancrod in acute stroke [27,36], by infusing low molecular weight dextran (dextran 40) in critically ill patients [37], hydroxyethyl starch (6% HAES 130/0.4) in severe sepsis [20,43] and during cardiopulmonary bypass operations [39]. It is noteworthy that the latter study showed that reduced RBC aggregation induced by HAES 130/0.4 resulted in an undesirable activation of endothelial cells [39]. One of these clinical studies on patients receiving either HAES 130/0.4 or saline also analyzed sublingual microcirculation and found an increased capillary flow index, percentage of perfused capillaries and perfused capillary density after HAES 130/0.4 [20]. However, these therapeutic approaches have not been consistently successful, and are no longer recommended [29,40,46,52,64,71]. Our data show that the negative results of these clinical trials could be explained, at least in part, by RBC aggregation having no negative influence on tissue perfusion, and therefore lowering of RBC aggregation could not be expected – from this purely hemorheological point of view – to improve the clinical outcome of a disease.

RBC oxygen transport capacity, and thus tissue oxygenation, depends on both capillary hematocrit and blood flow velocity [45]. Our data of a higher “capillary” hematocrit and unchanged flow velocity for dextran 70 suspensions suggests that increased RBC aggregation during an acute phase reaction could be beneficial for tissue oxygenation. This is an intriguing finding, which, if confirmed, could contribute significantly to a better understanding of pathophysiological mechanisms.

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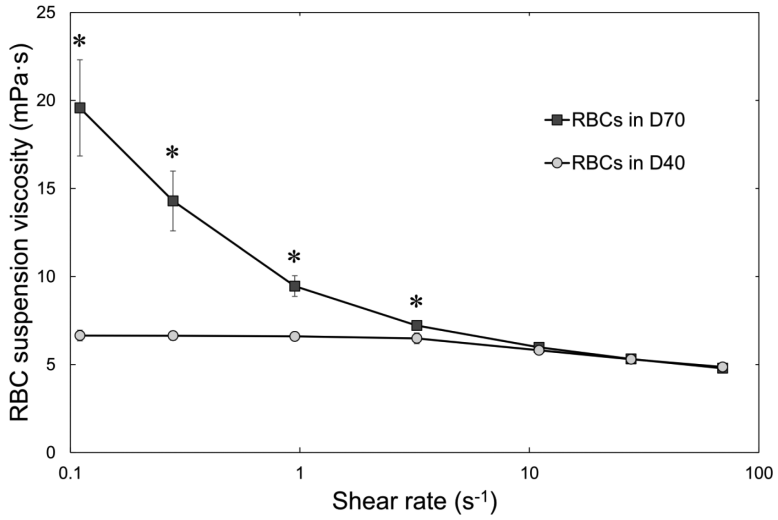

Figure 1.

Viscosity of 30% hematocrit suspensions of RBCs in a 46.5 g/L solution of dextran 40 in normal saline (D40; circles) or in a 35.0 g/L solution of dextran 70 in normal saline (D70; squares). Measurements were performed at room temperature for shear rates ranging from 0.11 to 69.5 s^{-1}. Asterisks denote a statistically significant difference (p-value < 0.01) in RBC suspension viscosity between RBCs in D40 and D70. Mean values ± standard deviation, n = 10.

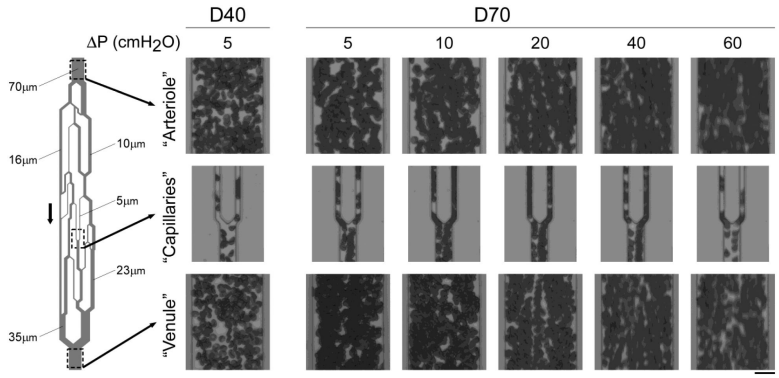

Figure 2.

Photographs taken at the level of "arterioles" (top row), "capillaries" (middle row) and "venules" (bottom row) of RBC suspensions flowing through the AMVN at increasing driving pressures ranging from 5 to 60 cm H_2O (0.49 to 5.884 kPa). RBCs were suspended in a 46.5 g/L solution of dextran 40 in GASP buffer (D40; far left) or in a 35.0 g/L solution of dextran 70 in GASP buffer (D70; right). Arrow indicates direction of flow. Scale bar is 20 μm.

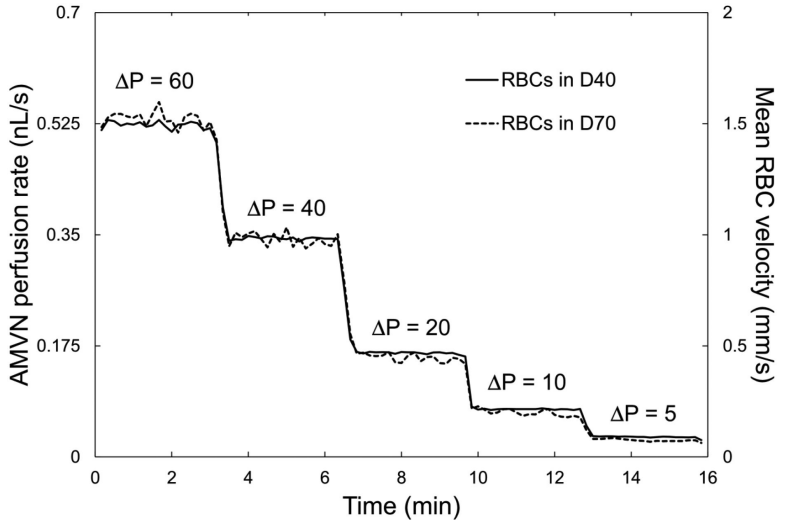

Figure 3.

Typical traces of AMVN perfusion rate (left scale) and mean RBC velocity (right scale) obtained at the "venular" level for 30% hematocrit suspensions of RBCs in a 46.5 g/L solution of dextran 40 in GASP buffer (D40; solid line) or in a 35.0 g/L solution of dextran 70 in GASP buffer (D70; dashed line) at decreasing driving pressures (ΔP) ranging from 60 to 5 cm H_2O (5.88 to 0.49 kPa).

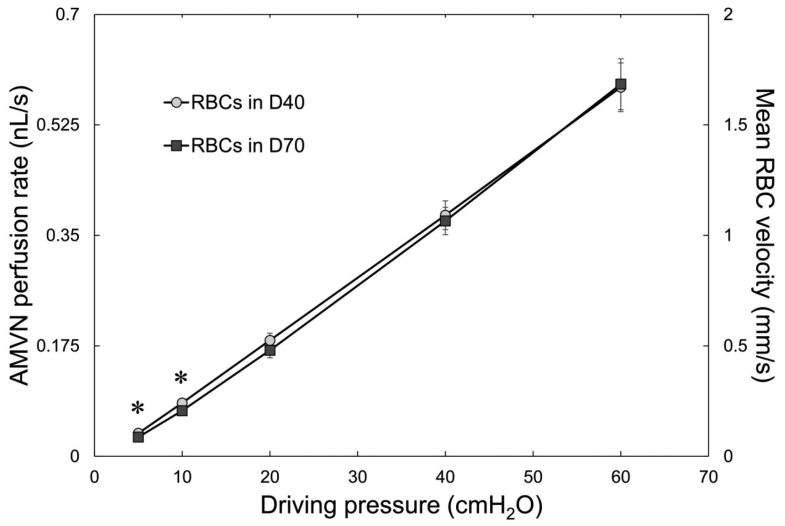

Figure 4.

AMVN perfusion rate and mean RBC velocity measured in the "venular" microchannels for 30% hematocrit suspensions of RBCs in a 46.5 g/L solution of dextran 40 in GASP buffer (D40; circles) or in a 35.0 g/L dextran 70 solution in GASP buffer (D70; squares) for driving pressures ranging from 5 to 60 cm H_2O (0.49 to 5.88 kPa). Asterisks denote a statistically significant difference (p-value < 0.01) in AMVN perfusion rate and mean RBC velocity between RBCs in D40 and D70. Mean values ± standard deviation, n = 7.

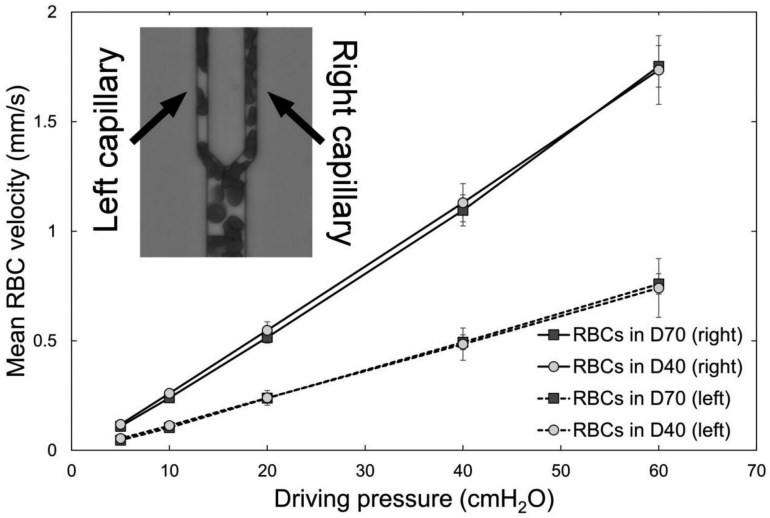

Figure 5.

Mean RBC velocity measured in “capillary” microchannels – corresponding to the left capillary (dashed line) and right capillary (solid line) – for RBCs suspended at 30% hematocrit in a 46.5 g/L solution of dextran 40 in GASP buffer (D40; circles) or in a 35.0 g/L solution of dextran 70 solution in GASP buffer (D70; squares) for driving pressures ranging from 5 to 60 cm H_2O (0.49 to 5.88 kPa). Asterisks denote a statistically significant difference (p-value < 0.01) in mean RBC velocity between RBCs in D40 and D70 in both the left and right “capillaries”. Mean values ± standard deviation, n = 7.

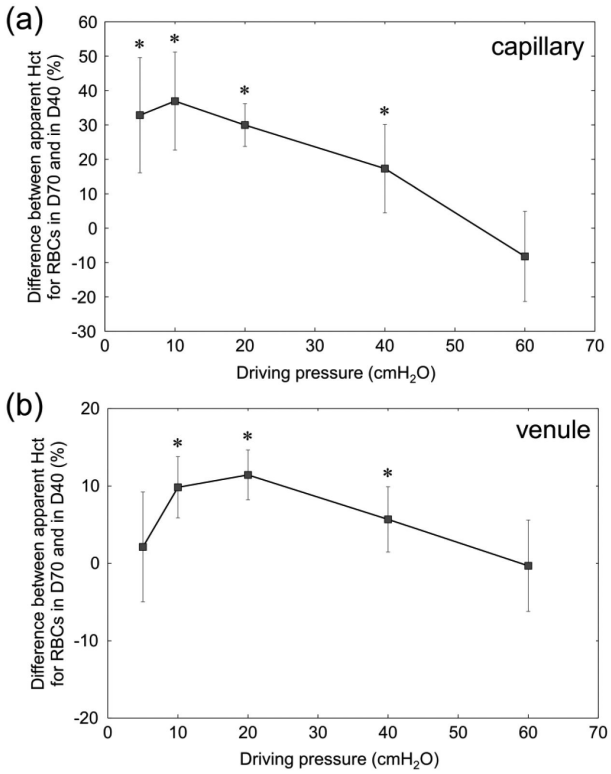

Figure 6.

Difference between estimated hematocrit for RBCs suspended in a 46.5 g/L solution of dextran 40 in GASP buffer (D40) and those suspended in a 35.0 g/L solution of dextran 70 in GASP buffer (D70) for (**a**) “capillaries” and (**b**) “venules” of the AMVN, for driving pressures ranging from 5 to 60 cm H_2O (0.49 to 5.88 kPa). Asterisks denote a statistically significant difference (p-value < 0.01) in estimated hematocrit between RBCs in D40 and D70. Mean values ± standard deviation, n = 7.

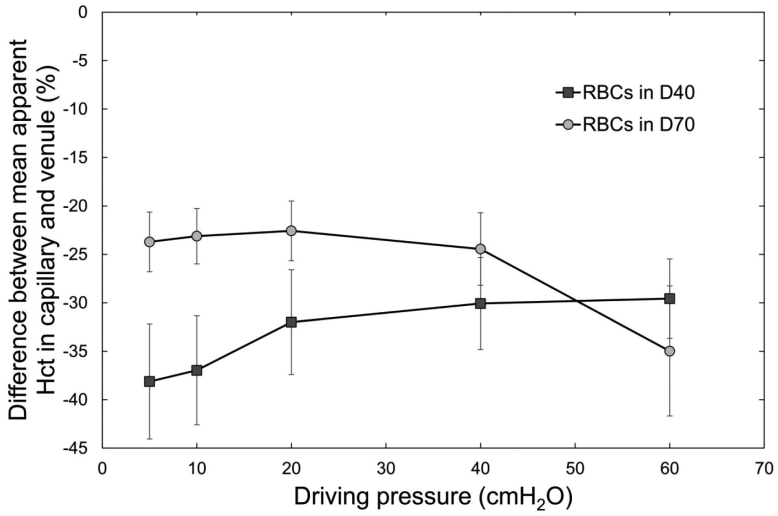

Figure 7.

Difference between mean estimated hematocrit in “capillaries” and “venules” of the AMVN for RBCs suspended in a 46.5 g/L solution of dextran 40 in GASP buffer (D40) and those suspended in a 35.0 g/L solution of dextran 70 in GASP buffer (D70), for driving pressures ranging from 5 to 60 cm H_2O (0.49 to 5.88 kPa).

Table 1

Viscosities of 46.5 g/L dextran 40 and 35.0 g/L dextran 70 solutions in saline, and their effect on RBC aggregation in suspensions with a hematocrit of 30%. RBC aggregation was measured using Myrenne aggregometer either at stasis (M mode) or at low shear rate (M1 mode). Mean values ± standard deviation, n = 5.

	46.5 g/L dextran 40	**35 g/L dextran 70**
Solution viscosity (mPa·s)	2.13 ± 0.24	2.08 ± 0.20
RBC aggregation at stasis (M mode)	7.9 ± 2.6	23.9 ± 5.4
RBC aggregation at 3 s^{-1} (M1 mode)	15.2 ± 3.8	28.4 ± 7.0