Human Erythrocyte Filterability at Low Driving Pressure

Shay Ginsbourg[1,2], Shlomo Levin[1], Shmuel Einav[2]* and Rafi Korenstein[1]

[1]Department of Physiology and Pharmacology and [2]Department of Bioengineering
Tel-Aviv University, Tel Aviv 69978 ISRAEL

*To whom correspondence should be addressed:

Prof. Shmuel Einav

Dept. of Biomedical Engineering

Tel Aviv University

Tel Aviv 69978 ISRAEL

Tel: +972-3-6409418

Fax: +972-3-6409448

Cell: +972-544-750433

E-mail: einav@eng.tau.ac.il

URL: http://www.tau.ac.il/~einav

Abstract

In this study, the human RBC capillary flow has been modeled by passing 11 μl of RBC suspension (Hematocrit = 6%) in phosphate buffer solution (PBS) of a viscosity of 1 and 2.6 cP (in the presence of 2% Dextran) through 5 μm pore diameter polycarbonate Nuclepore filters. We've developed a digitally controlled experimental system for measuring the RBC filterability at a constant driving pressure, in the range of 10 to 400 Pa, producing a wall shear stress range of 1 to 50 Pa. The RBC filterability was evaluated by measuring the cell suspension flow rate normalized by the PBS flow rate. The RBC filterability has been found to be a nonlinear function of the driving pressure, having a single minimum locus at 25 Pa. Lowering the driving pressure below 25 Pa revealed an unexpected increase of the RBC filterability.

The maximal RBC filterability (near unity) was detected at the lowest driving pressure (10 Pa) and the corresponding estimated RBC linear velocity while traveling through the capillary pore was as high as 800 μm/sec. Increasing the driving pressure above 25 Pa confirmed previous results, where RBC filterability is monotonically and asymptotically increasing. Increasing the PBS medium viscosity from 1 to 2.26 cP significantly attenuated the RBC filterability and led to the anomalous increase of RBC deformability at the 10Pa pressure range. We propose that the anomalous increase in RBC deformability was caused by RBCs undergoing spontaneous mechanical fluctuations.

Keywords: RBC deformability, low Reynolds number, spontaneous mechanical fluctuations, Dextran solution viscosity, polycarbonate Nuclepore filters, wall shear stress.

Symbols

τ	Shear stress (Pa)
τ_s	Wall shear stress (Pa)
τ_{yield}	Yield shear stress (Pa)
μ	Dynamics viscosity (Pa sec)
C	Constant
d	Diameter (mm)
$\dot{\gamma}, \frac{du}{dy}$	Shear strain rate (sec^{-1})
$\frac{\Delta p}{\Delta x}$	Pressure gradient (Pa/mm)
$\dot{Q}$	Volumetric flow rate (mm^3/sec)
$\vec{V}_{RBC}$	Linear velocity of RBC flow (μm/sec)

1. Introduction

A mature red blood cell (RBC) must survive a very wide range of fluid flow regimes. From near turbulence in the aorta, to the low Reynolds (Re << 1) and high shear strain rate ($\dot{\gamma} \leq$ 1,000 s-1) flow in the arterioles, each RBC has to change its biconcave discocyte shape (7.5 μm diameter) in order to pass through the narrowest capillaries (2.9 μm). The intense hydrodynamic forces must not tear the cell. While streaming at high Reynolds numbers, the membrane compliance is the dominant factor. In the capillaries, the conditions are contrasting, and it is important that the RBC maintains a highly deformable membrane in order to pass through blood capillaries.

Since the average circulation time of a RBC in the body is about 1 minute, it would imply that frequent significant changes in the mechanical properties of the membrane occur during the circulation, as the Reynolds number of the flow regime intensely varies. Several competing models for the underlying basis of the erythrocyte transformations have been suggested, but the general agreement is that the cytoskeleton plays a crucial role [2].

The microcirculation flow regime is characterized by extremely low Reynolds number (Re << 1) such that the inertial factors are essentially negligible in comparison with the viscous drag. The cell membrane elasticity becomes the dominant mobility factor in the microcirculation, rather than other factors, for example the internal viscosity of the hemoglobin solution [15]. Red blood cells undergo two different fluidization processes that are at the heart of the non-Newtonian behavior of the blood, namely, aggregate dispersion and deformation plus orientation of the dispersed RBC [4]. These two phenomena are associated with a progressive, shear-dependent decrease in the coefficient of viscosity, which is calculated from the ratio of the shear stress and the shear rate [13].

The macroscopic rheological properties of RBC suspensions are influenced by the shear stress, hematocrit, cell aggregation, and the mechanical properties of the cells [5]. Any rheological model describing the relation between the shear stress to the

shear strain rate (Casson's Hemorheological Model;[15]; see Equation 1) has first to express a **distinctive yield shear stress** stemming from the reversible aggregation of static RBC, known as "rouleaux".

$$\sqrt{\tau} = \sqrt{\tau_{yield}} + \sqrt{C\dot{\gamma}}$$

Equation 1

Casson's Hemorheological Model

Hemorheological factors set the rate of blood circulation by the blood viscosity coefficient μ, as described in the Hagen-Poiseuille law (see Equation 2 in [7]). The proper term describing the viscous behavior of fluid suspensions such as blood is "apparent viscosity", meaning that the viscosity coefficient is not a simple fluid property but rather a particular outcome of the specific measurement procedure, taking into account the various interactions of blood cells, the plasma, and the blood vessel wall [1].

$$\dot{Q} = \frac{\pi}{128} \frac{d^4}{\mu_{app}} \frac{\Delta p}{\Delta x}$$

Equation 2

Hagen-Poiseuille law

Filtration experiments though 3 or 5 micrometer Nuclepore membranes are often performed in order to assess the so-called "RBC deformability**"**. Its relationship with the "RBC filterability" has already been determined [10]. The calculated average RBC transit time through a membrane is comparable with directly measured values of RBC transit time through a single pore at 50 Pa driving pressure [9]. Therefore, the experimental data produced by applying the initial-flow filtration method is considered equivalent to the data resulting from measurements of the average RBC transit time through a porous membrane.

A recent review about RBC deformability measurements discussed the distinction between instrument sensitivity to various RBC-rheological features as well as the influence of temperature on the measurement, and the possibilities of those techniques with their medical applications, since the RBC-deformability has a key position in the etiology of a wide range of conditions [18].

The aim of this study was to examine the filterability characteristics, especially under low driving force, while employing a digitally controlled experimental system for measuring the RBC filterability at a constant driving pressure. The preparation of the RBC samples was performed according to standards and guidelines for rheological measurements [19]. The assumptions that underlie this work were that the RBC filterability would be attenuated by elevating the viscosity of the surrounding medium and that the measured filterability would be correlated with the deformability and fluctuations of the RBC [16].

2. Materials and Methods

2.1. *RBC preparation*

Venous blood *was* obtained on the day of the experiment from a healthy donor. 4ml of venous blood was introduced into a test tube prewashed with 50 mM EDTA. The blood was centrifuged at 1,500 RPM for 5 minutes and then the plasma and buffy coat were removed. The remaining RBCs were washed with 50 ml PBS and centrifuged again at 3,000 RPM for 10 minutes. The washout and centrifugation procedure were repeated for the second time and the RBC were diluted with PBS containing 10mM glucose, 1mg/ml BSA, pH 7.4, according to the required hematocrit. In long term experiments 0.5 unit/ml of PSN (Penicillin Streptomycin Neomycin) was added to the medium. The increase of medium viscosity was achieved by adding 2% Dextran (500kD) to the PBS medium. During the experimental sessions, the RBC suspension was slowly mixed at room temperature of 24 to 26 ^{0}C.

2.2 *Filterability set-up*

The digitally controlled experimental system was designed and built for conducting in-vitro measurements of RBC filterability at two medium viscosities under low driving pressure (range of 1.25 - 40 mm H_2O). The main physical dimensions are specified in figure 1 below and as follows: the tube internal diameter was 3.8 mm, the distance between slits was 1.0 mm, the volume between slits was 11.3 mm^3, the slit width was 0.1 mm, and the sample volume was 11.3 μl. The system elements were covered with common aluminum foil sheets in order to minimize the thermal radiation effects and the experiments were conducted in a closed room space to minimize heat convection effects.

A vertical glass tube was especially manufactured for performing the actual filterability measurements. The external tube walls were blackened to block passage of visible light from a tungsten source, and coated with a preservative hydrophobic layer. A set of 2 parallel pairs of slits allowed the simultaneous passage of two thin horizontal and parallel light rays through the otherwise blackened glass tube.

The transmitted light was detected by a photocell, positioned on the opposite side to light source.

In each experiment, a sample of either medium solution or RBC suspension was injected into this glass tube. The sample flow direction was vertically downwards; ultimately reaching the filter housing that was tightly positioned below the tube. The electronically monitored driving pressure suctioned the sample through the housed Nuclepore filter positioned at the bottom of the tube. While the sample was flowing down, the parallel rays were reflected twice: once, while the concaved fluid-air interface (i.e. the upper sample surface) crossed the upper pair of parallel slits, and then while that interface crossed the lower pair. Both events reflected the corresponding ray and resulted in a sharp transient decrease of the monitored transmitted light detected by the photocell. The time duration between these two events was detected and calculated by the software application.

The software application was programmed within the following 4 development environments: MS-Visual Studio, Matlab, 4NT Shell, and Daxpert 910 scripting environment (accompanying the Daxpert 818 A/D converter board, manufactured by Advantech PC Lab Cards). The hardware was installed onto a 133 MHz Intel Pentium processor based workstation running a Microsoft Windows 32-bit operating system. The software application was developed according to industry-standard programming principles [11]. It performed data sampling and archiving, online monitoring and offline graphic presentations. The software application also performed automatic signal processing, standard analysis report creation and all textual or graphic displays. While the physical system was being calibrated, the source code was fully tested and debugged according to industry-standard software quality assurance methodology and techniques [12].

The electronic pressure meter used for monitoring the driving pressure was based on a 0-1” H_2O range differential transmitter model 616-00 by Dwyer Instruments, Inc. The 5-micron pore filter simulating the capillary vessels was a 13 mm diameter sterile hydrophilic Nuclepore (PC) Polycarbonate filter manufactured by Costar Scientific.

The photocell collecting the light was a 13 mm^2 Silicon photodiode model S1223-01 manufactured by Hamamatsu.

3. Results

Several experimental sets were carried out. The first being a calibration step of the filterability set-up, the second was filterability measurement of the solution in which RBCs were suspended and the rest being filterability measurements of RBCs suspended in media of low and high viscosity.

The first experimental set was performed with deionized water used as the system's calibration medium. Once this was completed, the second (PBS) cycle was run. In this cycle, PBS having a viscosity of 1 cP was streamed trough the nuclepore membrane filter, according to the above-mentioned procedure, where the differential driving pressure was varied from 10 to 400 Pa. The PBS cycle consisted of two parallel measurements; each repeated twice, once by filtering the PBS solvent alone and then by filtering a suspension of red blood cells (HCT = 6%) in PBS. From the combined data, the relative filtration time ratio (filterability) was calculated as the time required for filtering of 11 μl PBS divided by the time required for filtering 11 μl RBC suspension in the same buffer (Figure 2).

The filterability graph (Figure 2) shows a monotonous decrease upon attenuating the driving force. However, at the low end of the tested pressure range a surprising major increase of RBC mobility was observed. At approximately 10 Pa, the measured filterability was 0.91 (see Table 1); meaning that although RBCs were suspended in the medium's solution, the total effect of their presence in the filtered fluid on the filtration time was negligible. At a higher driving force of 25 Pa the filterability dropped to a minimum of 0.5 while at higher driving force filterability increased towards the value of 0.6 ($p = 0.05$) (see Figure 2 and Table 1).

Similar experiments were repeated in a PBS solution containing 2% Dextran, yielding a medium of a higher viscosity of 2.26 cP. Again, two measurements were carried out at each driving force. One included filtering the high viscosity medium, devoid of cells while the other one consisted of filtering the red blood cells (HCT = 6%) in the same viscous fluid solution. The flow rates of the RBC suspensions in the media of 1 and 2.26 cP viscosities as a function of the driving force

are given in Figure 3, showing that the flow rates of the two RBC suspensions are obeying the Newtonian fluid model, i.e. linear with respect to the driving pressure at the relevant range of up to 400 Pa, except for the 1cP PBS suspension at the lowest driving force region.

From the above data, the relative filtration time ratio (filterability) of the medium alone and of the RBC suspension was calculated for the viscous Dextran solution. The Dextran cycle produced additional results, which have been found to be statistically significant (see Table 1). The filterability graph shows an attenuated increase of the RBC mobility at the low end of the tested pressure range. At approximately 10 Pa, the filterability was near 0.2, meaning that the mobility of the cells was being compromised due to the increased viscosity of the medium. Afterwards, the filterability further dropped to a minimum of 0.17 at 25 Pa, and then significantly increased towards the value of 0.6 as happened in the PBS cycle, but at a steeper slope (displayed in Figure 2).

To summarize, the results obtained from this experimental research are:

1. The RBC filterability is a nonlinear function of the driving pressure, having a single minimum locus at 25 Pa (see Figure 2).
2. Lowering the driving pressure below 25 Pa reveals an unexpected increase of the RBC filterability. The suspension flow rate roughly equals that of a physiological solution (PBS) (see Figure 2).
3. Increasing the driving pressure above 25 Pa confirms previous results. The RBC filterability is monotonically and asymptotically increasing (see Figure 2).
4. Increasing the PBS medium viscosity from 1 to 2.26 cP significantly attenuates the RBC filterability (see Figure 2).
5. Above a driving pressure of 50 Pa, the RBC flow rate is directly proportional to the PBS medium viscosity (see Figure 3).
6. Below a driving pressure of 50 Pa, increasing the PBS medium viscosity from 1 to 2.26 cP significantly attenuates the RBC flow rate (see Figure 3).
7. The maximal RBC filterability measured was near unity in PBS (at the lowest driving-pressure, i.e. 10 Pa) and the corresponding estimated linear velocity of the cells, while traveling through the capillary pores, was as high as 800 μm/sec (see Equation 8 and Figure 4).
8. Above a driving pressure of 50 Pa, the apparent viscosity of the suspension is constant and directly proportional to the PBS medium viscosity (see Figure 5).
9. Below a driving pressure of 50 Pa, the resistance to flow of the viscous suspension is sharply increased, whereas that of the thin suspension is decreased (see Figure 5).
10. The relative filterability ratio of RBC in a Dextran solution steeply increases as the driving pressure is increased.

5. Discussion

During the course of an average circulation time of one minute, the RBC experiences a broad range of fluid flow-regimes. Exposed to high Reynolds numbers at the aorta (Re = 1,780), the RBC membrane must be rigid enough to avert tearing by the intense hydrodynamic forces. Yet at the capillary extreme shear flow (Re << 1), the cell membrane must maintain high deformability in order to pass through blood vessel diameters as narrow as 4 μm [15].

The present study explored RBC filterability at the low pressure range, under normal and high medium viscosity. The human erythrocyte capillary flow was modeled by passing 11 μl of RBC (HCT = 6%) in phosphate buffer solution (PBS) at a viscosity of 1 and 2.26 cP through a 5 μm Polycarbonate Nuclepore filter. The suspension volume contained a total of $7x10^6$ cells (as estimated in Equation 4), of which 40 cells on average were filtered per one pore during each measurement (see Equation 5).

$$N_{RBC} = \frac{V_{Sample}}{V_{RBC}} \times Hct = \frac{11.34\,\mathrm{mm}^3}{94\,\mu\mathrm{m}^3} \times 6\% = 7.24 \times 10^6 \qquad \text{Equation 4}$$

Where N_{RBC} is the estimated total number of RBC in the suspension volume, V_{Sample} is the suspension volume, V_{RBC} is the mean RBC volume, and *Hct* is the hematocrit.

$$N_{RBC/pore} = \frac{N_{RBC}}{N_{pores}} = \frac{7.24 \times 10^6}{1.8 \times 10^5} \sim 40 \qquad \text{Equation 5}$$

Where $N_{RBC/pore}$ is the estimated number of RBC passing through a single pore, N_{RBC} is the estimated total number of RBC in the suspension volume, and N_{pores} is the effective number of pores for RBC filtration.

The average thickness length of the Nuclepore membrane used is 11 μm. However, due to entrance phenomena, the equivalent hydrodynamic length is considered to be 17 μm. The average pore volume is 340 μm^3, which is approximately 4 times larger than the average RBC volume of 94 μm^3 [14, 10]. A driving pressure of 10 to 400 Pa (1 to 40 mm H_2O) produced a wall shear stress in the range of 1 to 50 Pa (Equations 6 and 7).

$$\sum F = \Delta P \cdot \pi \frac{d^2}{4} - \tau_s \cdot \pi d \Delta L = 0 \qquad \text{Equation 6}$$

Newton's Second Law

$$\tau_s = \frac{d}{4} \times \frac{\Delta P}{\Delta L} \qquad \text{Equation 7}$$

The Wall Shear Stress

The filterability was evaluated by measuring the transit time of the RBC suspension and normalizing it by the transit time of the PBS solution through that filter. The maximal RBC filterability measured was near unity in PBS (at the lowest driving-pressure, i.e. 10 Pa) and the corresponding estimated linear velocity of the cells, while traveling through the capillary pores, was as high as 800 μm/sec (see Equation 8 and Figure 4).

$$\vec{V}_{\substack{RBC \\ filterability \approx 1}} = \frac{V_{Sample}}{\frac{\pi}{4} d_{pore}^2 \times N_{pores} \times t_{sample}} = \frac{11.34\,\mu l}{\frac{\pi}{4} 25\,\mu m^2 \times 1.8 \times 10^5 \times 4\,sec} = 800\,\mu m/sec$$

Equation 8

Estimated cell linear velocity

The above is about twice faster than the extrapolated cell velocity through the filters. We observed a significant decrease of the filterability (approximately 5 times) in the

presence of 2% Dextran (Molecular Weight = 500 kD; η = 2.26 cP). This effect might be explained by the assumption that RBC rouleaux are being formed in the presence of Dextran. At low driving pressure (10 Pa) and this concentration of Dextran, the microscopic aggregation index is increased by 2.7 to 2.8 times [3].

Continuing this notion, the observed increase of the filterability at the higher driving pressure range (25 to 400 Pa) is the result of de-aggregation of RBC rouleaux due to the corresponding shearing forces. This phenomenological behavior is supported by the observation that as the driving pressure was elevated, the decrease of the RBC filterability at the presence of 2% Dextran was significantly attenuated. However one still needs to explain the apparent anomaly of the observed decrease in RBC flow resistance (increase of RBC filterability) at the low driving force region of 10 Pa.

This phenomenon can be explained by taking into account that fact that RBCs undergo spontaneous mechanical fluctuations. A direct correlation between the filterability and the amplitude of the cell membrane fluctuations has previously been established [17]. The power spectrum of these fluctuations shows a frequency range of 0.3-30 Hz demonstrating the $1/f^{\alpha}$ dependence. Thus the highest amplitudes occur in the time range of ~0.2 to 3 sec, which is 10 to 20 times greater than the transit time of a single RBC through a pore at the high driving pressure range. However, at the low driving pressure range, the transit time is in the range of 0.2 to 1 sec, in which one observes the fluctuations of the highest amplitude.

One may envision that such fluctuations may increase the penetration of the RBC into the pore. Furthermore, once the RBC is in the pore, the membrane fluctuations yield repulsion forces within the pore's wall due to the existence of "Helfrich forces" [8, 6]. The combination of these two effects is expected to lead to increased RBC filterability at the lowest range of the driving pressure. This observation is supported by RBC filterability when suspended in a medium of high viscosity. Our group has previously shown that cell membrane fluctuations are attenuated in the presence of high viscosity medium [16]. Thus the disappearance of this anomalous increased filterability in the

presence of high medium viscosity may origin from the fact that cell membrane fluctuations have been decreased.

References

[1] R. M. Berne and M. N. Levy, *Physiology*, 4th edition, Mosby, 1998.

[2] J. P. Brody, Y. Han, R. H. Austin and M. Bitensky, Deformation and Flow of Red Blood Cells in a Synthetic Lattice: Evidence for an Active Cytoskeleton, *Biophysical J.* **68** (1995), 2224-2232.

[3] S. Chien, Biophysical Behavior of Red Blood Cells in Suspensions, in: *The Red Blood Cell*, D. Mac Surgenor, ed, Vol. 1, 1975, pp. 1031-1135.

[4] D. Elad and S. Einav, Physical and flow properties of blood, in: *Standard Handbook of Biomedical Engineering*, M. Kutz, ed, Chapter 3, McGraw Hill Pub., NY, 2002.

[5] J. Enderle, S. Blanchard and J. Bronzino, *Introduction to Biomedical Engineering*, Academic Press, 2000.

[6] E.A. Evans and V. A. Parsegian, Thermal mechanical fluctuations enhance repulsion between bimolecular layers, *Proc. Natl. Acad. Sci. USA* **83** (1986), 7132-7136.

[7] Y. C. Fung, *Biomechanics - Circulation*, 2nd edition, Springer Publishers, 1996.

[8] W. Helfrich, Steric interaction of fluid membranes in multilayer systems, *Z. f. Naturforchung* **33a** (1978), 303-305.

[9] H. Kiesewetter, V. Dauer, M. Gesh, D. Seiffge, B. Angelkort, and H. Schmid-Schonbein, A Method for the Measurement of the Red Blood Cell Deformability, *Scand. J. Clin. Invest.* **41** Suppl. (1981), 229-232.

[10] D. Koutsouris, M. Hanss and R. Skalak, Determination of Erythrocytes Transit Times Through a 5μ "Nuclepore" filter, *Biorheology* **20** (1983), 779-787.

[11] S. McConnell, *Code Complete*, Microsoft Press, 1993.

[12] R. S. Pressman, *Software Engineering*, 5th edition, McGraw-Hill Publishing Company, 2000.

[13] E. A. Schmalzer, R. Skalak, S. Usami, M. R. Vayo and S. Chien, Influence of Red Cell Concentration on Filtration of Blood Cell Suspensions, *Biorheology* **20** (1983), 29-40.

[14] R. Skalak, Theoretical Models of deformability in Blood Flow, *scand. J. Clin. Lab. Invest.* **41** Suppl. (1981), 55-58.

[15] B. Bo Sramek, J. Valenta and F. Klimes, *Biomechanics of the Cardiovascular System*, Czech Technical University Press, 1995.

[16] S. Tuvia, A. Almagor, A. Bitler, S. Levin, R. Korenstein and S. Yedgar, Cell membrane fluctuations are regulated by medium macroviscosity: Evidence for a metabolic driving force, *Pro. Natl. Acad. Sc. USA* **94** (1997), 5045-5049.

[17] S. Tuvia, A. Moses, N. Guljaev, S. Levin and R. Korenstein, β-Adrenergic agonists regulate cell membrane fluctuations of human erythrocytes, *J. Physiology* **516** (1999), 781-792.

[18] M. Musielak, Red blood cell-deformability measurement: Review of techniques, *Clin. Hemorheol. Microcirc.* 2009; 42(1):47-64.

[19] Baskurt OK, Boynard M, Cokelet GC, Connes P, Cooke BM, Forconi S, Liao F, Hardeman MR, Jung F, Meiselman HJ, Nash G, Nemeth N, Neu B, Sandhagen B, Shin S, Thurston G, Wautier JL, New guidelines for hemorheological laboratory techniques, *Clin. Hemorheol. Microcirc.* 2009; 42(2):75-97.

Tables

Table 1a - Statistical Analysis: Complete mean, standard deviation, variability values, and student T-Test results

Mean Driving Pressure [mmH2O]	Mean control filterability [s/s]	STD control filterability [s/s]	VAR control filterability	# tests control filterability	Mean viscous filterability [s/s]	STD viscous filterability [s/s]	VAR viscous filterability	# tests viscous filterability	T-test P-value filterability
1.25	0.91	0.13	0.14	5	0.22	0.06	0.26	4	0.00004
2.50	0.48	0.17	0.35	8	0.17	0.04	0.25	4	0.00089
5.00	0.52	0.10	0.20	11	0.30	0.08	0.27	7	0.00013
10.00	0.52	0.09	0.17	8					
20.00	0.58	0.07	0.13	9	0.48	0.07	0.14	7	0.014
40.00	0.60	0.07	0.11	7	0.53	0.09	0.17	4	0.240

Table 1b - Statistical Analysis: P-value T-test of filterability (Control)

[mmH2O]	40.00	20.00	10.00	5.00	2.50
1.25	0.0029	0.0021	0.0006	0.0006	0.0003
2.50	0.09	0.15	0.58	0.58	
5.00	0.05	0.13	1.00		
10.00	0.06	0.15			
20.00	0.54				

Table 1c - Statistical Analysis: P-value T-test of filterability (Dextran)

[mmH2O]	40.00	20.00	5.00	2.50
1.25	0.0019	0.0002	0.08	0.21
2.50	0.0015	0.00001	0.01	
5.00	0.01	0.00074		
20.00	0.35			

Figure captions

Figure 1 - Experimental system overview

Figure 2 - The dependence of RBC filterability on the driving force and the viscosity of the medium. The filtration of RBC suspended (6% HCT) in PBS (1cP, black line) and in PBS solution to which 2% w/v Dextran was added (2.26 cP, light black line) were measured at different driving pressures. Each data point represents a mean ± SD of n independent measurements as specified in Table 1.

Figure 3 - Rate of flow of RBC suspended (6% HCT) in PBS (1cP, black line) and in PBS solution to which 2% w/v Dextran was added (2.26 cP, light black line) measured at different driving pressures. Each data point represents a mean ± SD of n independent measurements as specified in Table 1.

Figure 4 - The dependence of the estimated linear velocity of the cells while traveling through the capillary pore on the driving pressure.

Figure 5 - Dependence of 6% RBC + 2% Dextran in PBS solution resistance to flow on the driving pressure compared to the dependence of 6% RBC in PBS solution resistance to flow on the driving pressure (PBS solution viscosity = 1 cP; 2% Dextran in PBS solution viscosity = 2.26 cP).

Figures

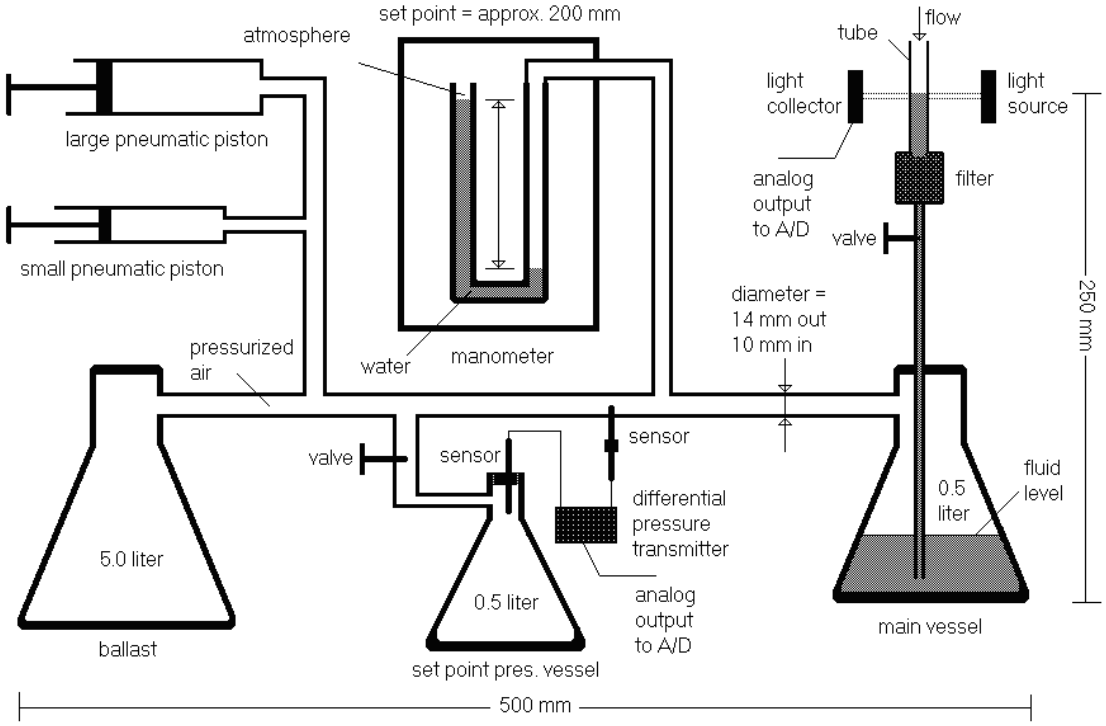

Figure 1

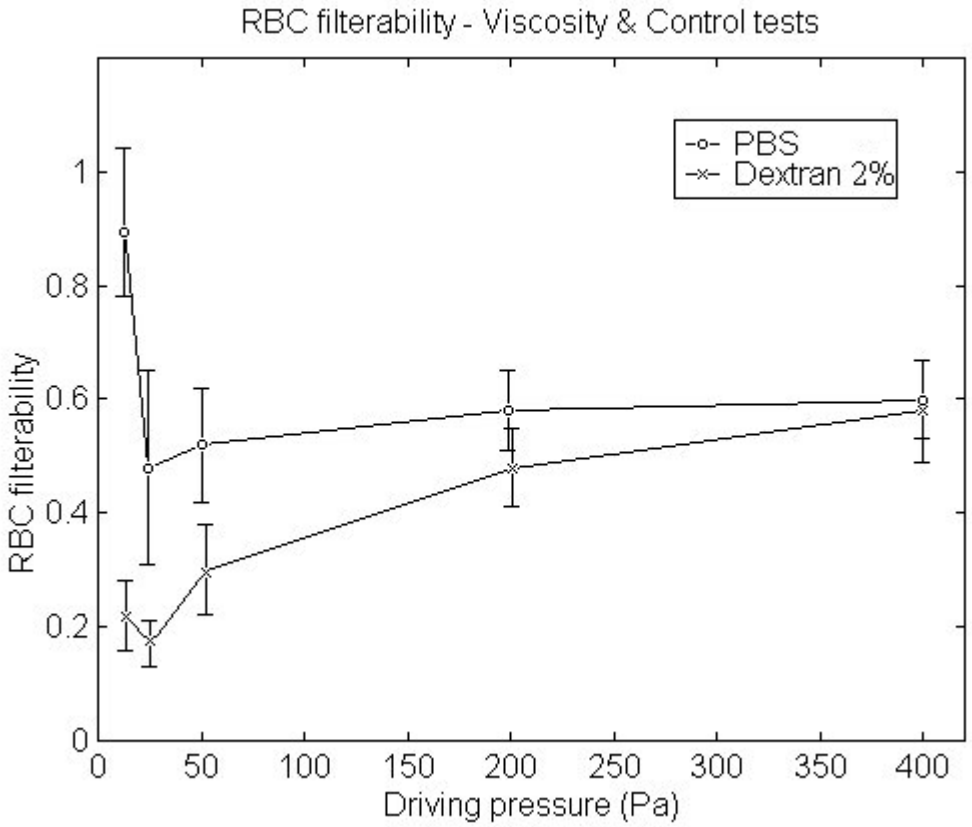
RBC filterability - Viscosity & Control tests
PBS
Dextran 2%
RBC filterability
Driving pressure (Pa)
0
0.2
0.4
0.6
0.8
1
0
50
100
150
200
250
300
350
400

Figure 2

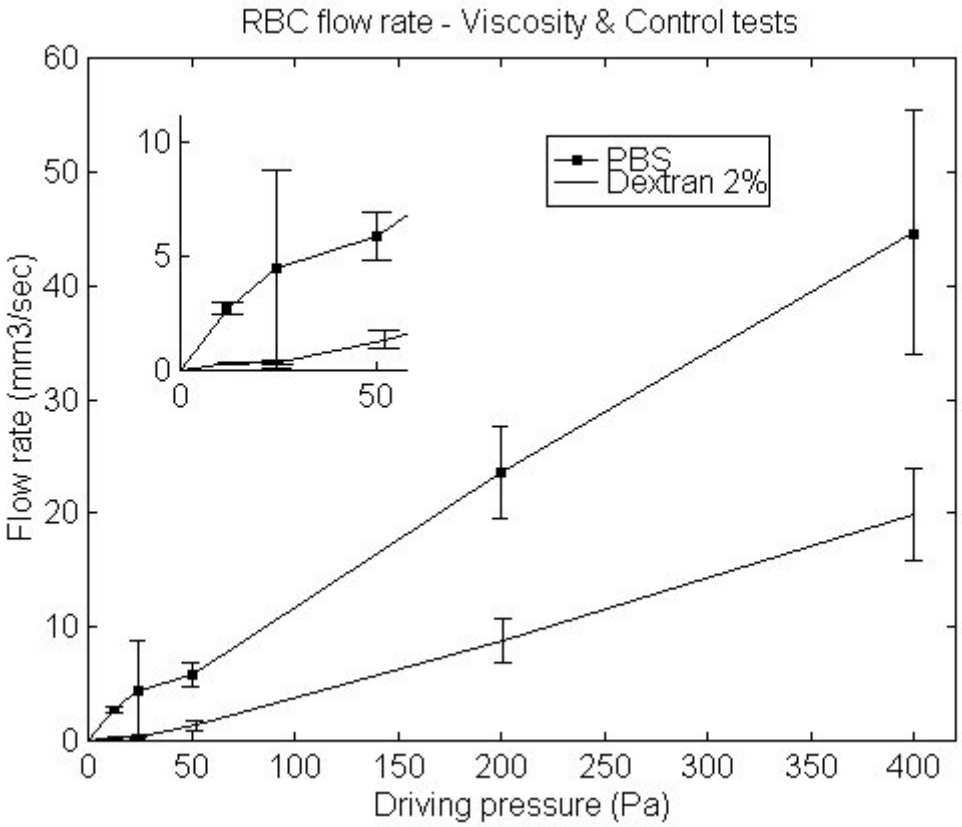
RBC flow rate - Viscosity & Control tests
PBS
Dextran 2%
Flow rate (mm3/sec)
Driving pressure (Pa)
0
10
20
30
40
50
60
0
50
100
150
200
250
300
350
400
0
5
10
0
50

Figure 3

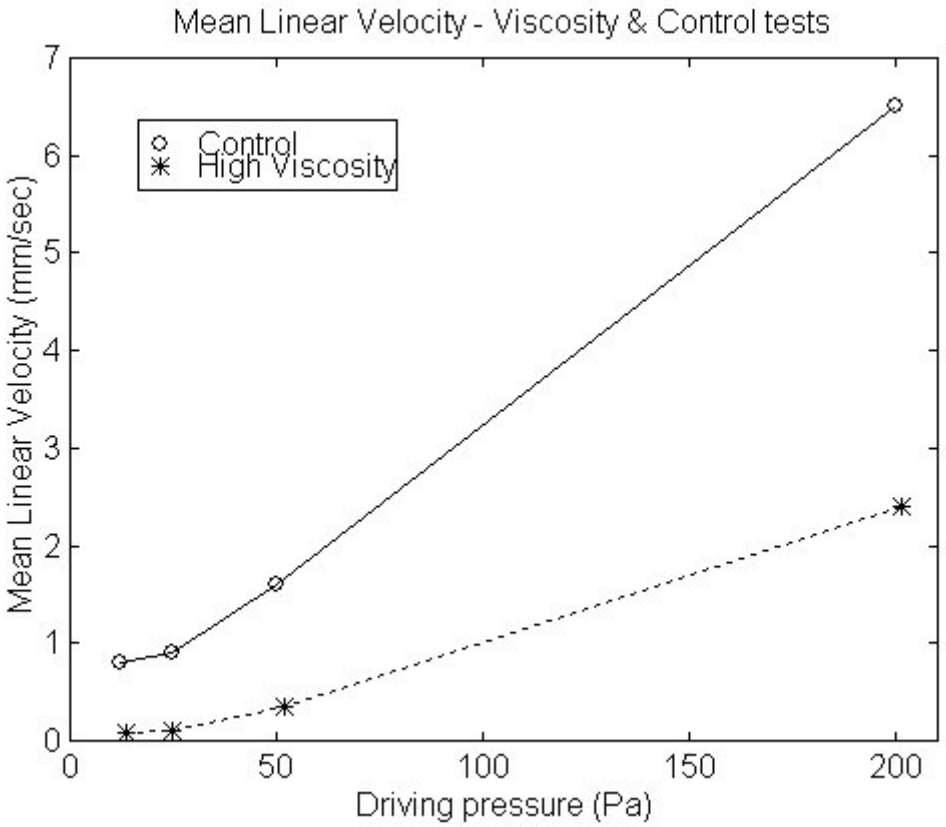
Mean Linear Velocity - Viscosity & Control tests
Control
High Viscosity
Mean Linear Velocity (mm/sec)
Driving pressure (Pa)
0
1
2
3
4
5
6
7
0
50
100
150
200

Figure 4

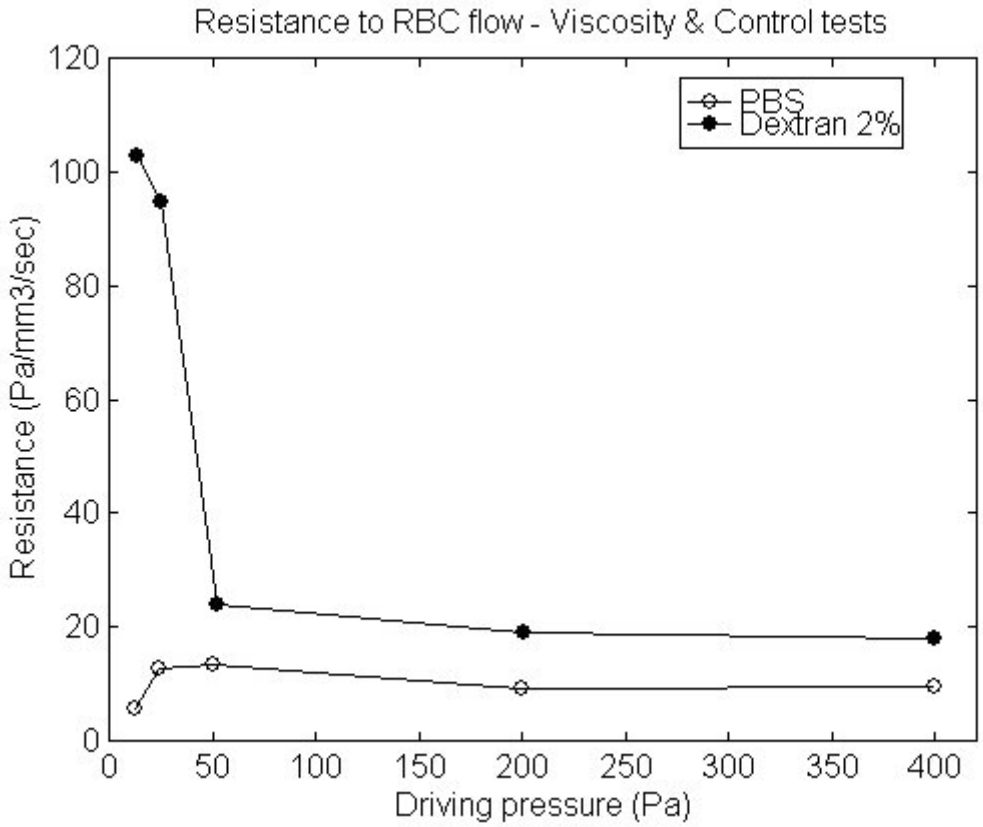
Resistance to RBC flow - Viscosity & Control tests
PBS
Dextran 2%
Resistance (Pa/mm3/sec)
Driving pressure (Pa)
0
20
40
60
80
100
120
0
50
100
150
200
250
300
350
400

Figure 5