Effect of varying external pneumatic pressure on hemolysis and red blood cell elongation index in fresh and aged blood
Randomized laboratory research

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Abstract

Background: External applied pneumatic pressure is usually used for rapid transfusion of red blood cells (RBCs). However, increased shear stress can cause increased hemolysis and decreased RBC elongation indices. Therefore, the purpose of this study was to measure the degree of hemolysis and the alteration of RBC elongation indices under varying external pressure in fresh and aged blood.

Methods: Venous blood samples were obtained from 20 healthy human volunteers. Each blood bag was divided into 2 subgroups (5 or 35 days of storage), and 5 levels of pressure were applied: 0, 150, 200, 250, and 300 mmHg. After infusion, a laboratory study was conducted. The percentages of irreversibly changed cells were evaluated using Bessis classification. RBC elongation indices were measured using a microfluidic ektacytometer.

Results: There were no significant differences in the percentage of irreversibly changed RBCs between the pressures of 0 and 300 mmHg. Moreover, there were no significant differences in laboratory test results or elongation indices among all levels of pressure. Irreversibly changed RBCs and hemolysis were increased depending on the storage period.

Conclusion: Irreversible changes in RBCs did not occur as a result of external pressure. The hemolysis and elongation indices of fresh RBCs were not influenced by external pneumatic pressure up to 300 mmHg. Only the storage period affected the irreversible changes in RBCs and hemolysis. Therefore, the application of external pressure to RBCs in variously aged blood is likely to be a safe procedure.

Abbreviations: EI = elongation indices, RBCs = red blood cells, SEM = scanning electron microscopy.

Keywords: blood, elongation indices, hemolysis, pressure, red blood cells, transfusion

1. Introduction

Rapid transfusion of red blood cells (RBCs) is often necessary in cases of massive hemorrhage, and for such a procedure, the use of an externally applied pneumatic pressure device can be helpful.

External pneumatic pressure devices can achieve flow rates of 3 to 27 mL/min depending upon external pressure ranging from 0 to 300 mmHg using a 20-gauge needle without dilution.[1] However, potential disadvantages of externally applied pressure include decreased deformability of RBCs and hemolysis caused by greater shear stress on erythrocytes. The shear stress is generated by the turbulent blood flow passing through the transfusion set or by the direct pressure applied to the cells in the blood bag.[2]

Indeed, several studies indicated that the application of higher external pressure results in greater hemolysis of transfused blood.[2–5] Shear stress may induce hemolysis. According to previous reports, external pneumatic pressure on a blood bag up to 300 mmHg may increase plasma-free hemoglobin, which is evidence of hemolysis.[2,3] The RBC deformability may also be reduced by shear stress exceeding physiological levels.[6] With appropriate external forces, RBCs undergo large mechanical deformation without rupture and then are restored to their original shape when released.[7] Furthermore, the duration of storage can affect the function of RBCs. Berezina et al[8] reported that the deformability of RBCs was normal on the fifth and seventh days of storage, but the deformability was significantly decreased on the 14th day of storage and remained low throughout the remainder of the storage period.

Thus, externally applied high pneumatic pressure on RBCs and the storage period may affect RBC deformability and hemolysis due to increased shear stress, especially in aged blood. Therefore,

the purpose of this study was to measure the degree of hemolysis and the alteration of RBC deformability using the elongation index under varying external pressure in fresh and aged blood.

2. Methods

2.1. Blood samples

After approval from the Korea University Anam Hospital Ethics Committee (IRB No. MD13015), informed consent was obtained from all volunteers. Venous blood samples were obtained from 40 healthy human volunteers with American Society of Anesthesiologists physical status I or II, aged between 20 and 50 years. Exclusion criteria included a history of abortion or delivery within the past 6 months, previous hemoglobin level <13 g/dL, blood pressure over 140/90 mmHg, bradycardia (under 50 beats/min), tachycardia (over 100 beats/min), and body temperature over 37.5 °C. The sampling was performed from the antecubital vein using a 16-gauge needle. Two units (total 600 mL) of whole blood were obtained from each participant and stored in a blood bag (Worldmedipharm, Uiwang-si, Korea) containing 56 mL of citrate phosphate dextrose adenine-1. The blood was preserved in a refrigerator maintained at 4 °C. Two blood samples were tested on day 5 after sampling, and another sample was tested on day 35.

2.2. Application of pressure

A CONSORT flow diagram of the experimental protocol is shown in Fig. 1. The collected blood was divided into 2 groups: one group with blood evaluated on day 5 after blood sampling and another with blood evaluated on day 35 after blood sampling. These 2 groups were divided into 5 subgroups: no external pressure (0), 150, 200, 250, and 300 mmHg. Most

commercially available external pneumatic devices are designed to apply a maximum pressure of 300 mmHg. Four different types of pressure were applied using an external pneumatic pressure device (Auto PC; Acemedical, Goyang-si, Korea). The blood bag was connected to an infusion set with a length of 20 cm and an internal diameter of 0.8 cm. Each unit was then run through a 20-gauge catheter without dilution and collected in a beaker. Blood samples for measuring deformability were collected 5 minutes after the initiation of pressurized RBC infusion.

2.3. Laboratory studies

Laboratory analysis of blood samples was performed as a control experiment to determine the effect of the duration of storage on the sampling day. After infusion under varying external pressure (150, 200, 250, and 300 mmHg), the blood samples were evaluated for hemoglobin, serum potassium, hematocrit, and RBC counts. The laboratory analysis of each blood sample was performed within 5 minutes after the initiation of the pressurized RBC infusion.

2.4. Scanning electron microscopy

The RBCs in the blood samples from the different subgroups of applied external pressure were observed through scanning electron microscopy (SEM) using an S4700 FE-SEM (Hitachi, Tokyo, Japan) electron microscope. Centrifuged blood cells were fixed in phosphate-buffered (pH 7.2–7.4) 2.5% glutaraldehyde for 2 hours, washed twice in 0.1 M phosphate buffer (pH 7.2–7.4), and mounted on poly-L-lysine-coated glass slides. The glass slides were kept in a moist atmosphere for 1 hour, washed in phosphate buffer, post-fixed in 1% osmium tetroxide for 1 hour, rinsed in distilled water, and dehydrated in graded ethanol (50%, 70%, 90%, and 100%). After drying in air and covering
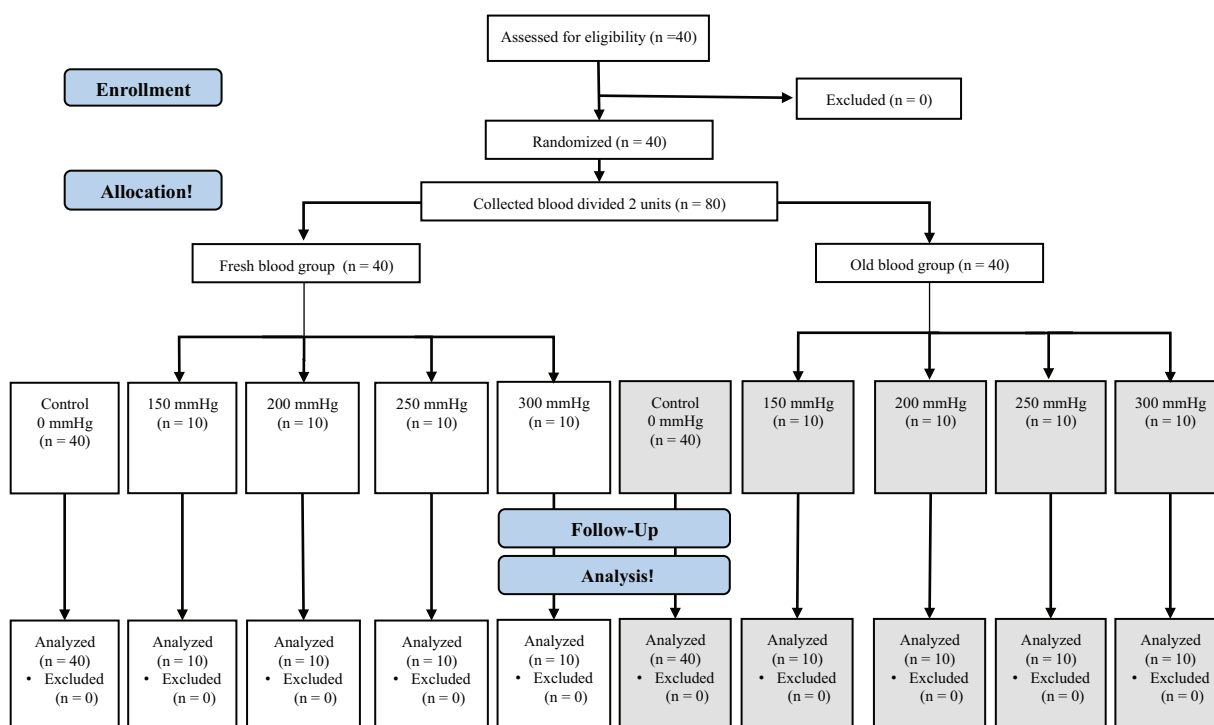

Figure 1. Diagram of experimental protocol.

with a gold layer by ion transfer, the samples underwent SEM analysis. The percentages of irreversibly changed cells were evaluated by counting 245 to 1455 cells in randomly chosen fields. The different cell shapes were identified using Bessis classification.[9]

2.5. Assessment of deformability using the elongation index

On completion of blood infusion, the tested blood remained in the beaker for 30 seconds until testing for deformability. To measure deformability of RBCs, a microfluidic ektacytometer (Rheoscan-D; Rheo Meditech, Seoul, Korea) was used. A suspension of RBCs was made by mixing 5 μL of whole blood and 5 mL of 0.14 mM polyvinylpyrrolidone (molecular weight 360,000). The suspension was then made to flow between a rectangle slit with a 3 Pa shear stress, which is similar to that of the microcirculation. The diffraction pattern of the RBCs was analyzed every 0.5 seconds and data were recorded using a charge-coupled device camera connected to a frame grabber equipped with a computer. RBC elongation indices (EI) were monitored and calculated using the long and short axes of the elliptical diffraction patterns (a and b, respectively) as:

$$EI = \frac{(a - b)}{(a + b)}$$

2.6. Statistical analysis

Data are presented as the mean ± SD. Data were analyzed using standard computer software (PASW Statistics version 18.0.0; SPSS Inc., Chicago, IL). To determine the effect of varying external pressure on irreversible changes in RBCs, laboratory findings and elongation index data were assessed for normality

and then compared using one-way analysis of variance or Kruskal-Wallis testing as appropriate. Post hoc comparisons were made using the Tukey test. The results of SEM, which was performed under pressures of 0 and 300 mmHg, were analyzed using the t test. Statistical significance was defined as $P < .05$.

3. Results

3.1. Scanning electron microscopy

SEM images of RBCs on days 5 and 35 of storage are shown in Fig. 2. The numbers of RBCs according to shape criteria were counted using SEM. Discocytes dominated among the cell population, and only a few irreversibly changed RBCs could be seen on day 5 of storage. The proportion of irreversibly changed RBCs on day 5 of storage was 11.6% ± 6.9% under 0 mmHg and 11.1% ± 6.4% under 300 mmHg. Spheroechinocytes and degenerated forms dominated among irreversibly changed RBCs on day 35 of storage. The proportion of irreversibly changed RBCs on day 35 of storage was 56.7% ± 20.6% under 0 mmHg and 58.0% ± 20.7% under 300 mmHg. The proportion of irreversibly changed RBCs varied significantly depending on the length of the storage period, 5 or 35 days ($P = .02$ under 0 mmHg and $P = .02$ under 300 mmHg). However, the proportion of irreversibly changed RBCs did not differ significantly according to the shear stress of 0 or 300 mmHg on day 5 of storage ($P = .41$) or on day 35 of storage ($P = .86$).

3.2. Laboratory analysis

The baseline laboratory data for fresh blood is shown in Table 1. There were no significant differences in hemoglobin levels, hematocrit, RBC counts, platelet counts, or serum potassium levels among blood samples subjected to all levels of external pressure (Table 2). In both groups of blood stored for 5 and
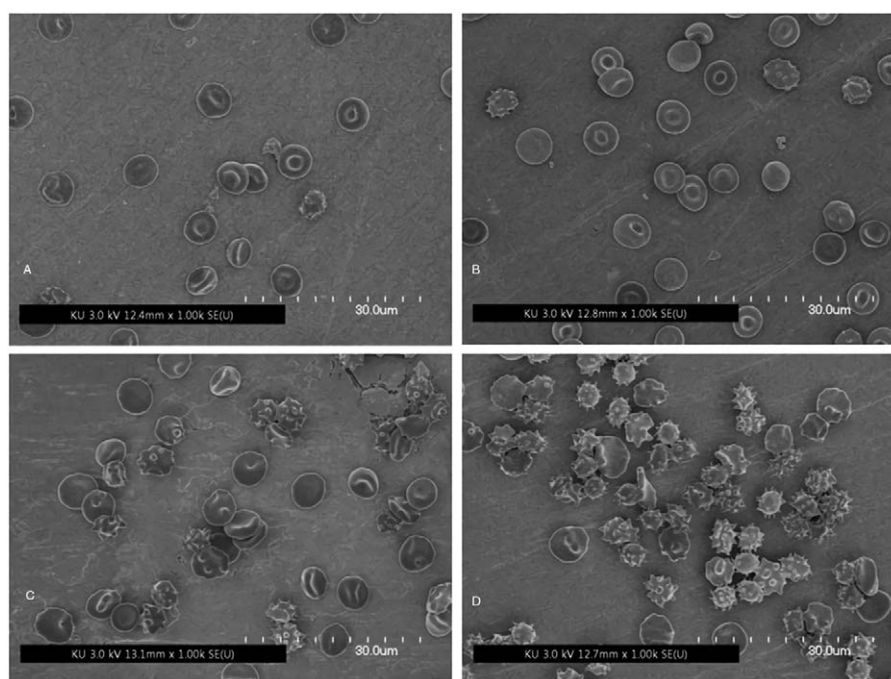

Figure 2. Scanning electron microscopy images of red blood cells (RBCs). (A) RBCs under 0 mmHg on day 5 of storage, (B) RBCs under 300 mmHg on day 5 of storage. (C) RBCs under 0 mmHg on day 35 of storage, (D) RBCs under 300 mmHg on day 5 of storage. Original magnification is ×1000.

Table 1

Laboratory studies of fresh blood.

	5 days (n = 10)	**35 days (n = 10)**
Hb, g/dL	15.15 ± 1.10	14.39 ± 1.90
Hct (%)	43.82 ± 3.33	43.04 ± 5.57
RBC count, ×10⁶/μL	5.03 ± 0.35	4.71 ± 0.57
Platelet, ×10³/μL	243.90 ± 35.40	246.80 ± 57.36
Potassium, mEq/L	4.05 ± 0.24	3.91 ± 0.21

The data are expressed as mean ± SD, with 5 days indicating the group in which RBCs were analyzed on day 5 of storage, and 35 days indicating the group in which RBCs were analyzed on day 35 of storage. Hb = hemoglobin, Hct = hematocrit, RBC = red blood cell.

35 days, the serum potassium level was increased relative to the baseline laboratory data. The hematocrit and RBC counts were decreased in blood stored for 5 and 35 days relative to the baseline laboratory data ($P < .05$). Moreover, the increase in potassium was higher, and the platelet count was lower on day 35 of storage than on day 5 of storage ($P < .05$).

3.3. Deformability using the elongation index

The mean values of the elongation indices of fresh and aged blood at the different levels of pressure are summarized in Table 3. There was no significant difference in the blood samples among all levels of external pressure. Moreover, there was no significant difference between the blood samples stored for 5 or 35 days, with values within normal range.

4. Discussion

Our results showed that the proportion of irreversibly changed RBCs increased depending on the storage period; however, we did not observe any differences based on the varying external pressure. As the length of the storage period increased, the potassium level increased and the platelet count decreased. However, hemolysis was not influenced by the level of external pneumatic pressure up to 300 mmHg applied to blood stored for 5 or 35 days. The elongation index of RBCs also was not influenced by external pneumatic pressure up to 300 mmHg in blood stored for up to 35 days.

RBCs manifesting into echinocyte and stomatocyte shapes can return to the discocyte shape under certain conditions. Thus, these RBC shape changes are considered potentially reversible transformations. In contrast, RBCs assuming spheroechinocyte, spherostomatocyte, spherocyte, ovalocyte, and degenerated

shapes are irreversibly changed.[8] A previous study[8] showed that mainly discocytes appeared, and there were only a few irreversibly changed RBCs on days 5 and 7 of storage. However, as the storage period increased, echinocytes and spheroechinocytes dominated and the proportion of irreversibly changed RBCs increased. Our results were similar. However, the proportion of irreversibly changed RBCs did not increase according to external pneumatic pressure.

The viability of RBCs is influenced by biochemical and mechanical factors. Mechanical factors can affect the destruction of erythrocytes when transfused and can be affected by the anesthesiologist. Cellular destruction by blood flow systems occurs as hemolysis, and the degree of hemolysis is directly related to the magnitude of cellular–solid surface interactions and shear stress.[10] Hemolysis can increase with increased infusion pressure and erythrocyte age. Hemolysis of erythrocytes increases directly with driving pressure,[2,3,11] but some studies reported that transfusion up to 300 mmHg did not cause significant hemolysis.[1,10,12] In our study, hemolysis also was not affected by external pressure up to 300 mmHg but was affected by the duration of storage. Similar to our report, previous studies[13] showed that hemolysis is maximum during the first week and increases during storage. Another study reported that during RBC storage, there were gradual increases in the levels of plasma potassium and hemoglobin,[14] which are plasma markers of cellular damage.

When RBCs are pressurized, their shapes change. RBCs at rest have an average diameter of 7.8 μm and must deform markedly to pass through the smallest capillaries of the microcirculation (3–7 μm). Therefore, the deformability of RBCs is important because it determines their orientation within laminar flow streams and their ability to traverse the microcirculation. This deformation of RBCs is reversible, and the biconcave-discoid shape of the RBC is maintained after the removal of the deforming forces.[15] Shear forces exceeding physiological levels may induce RBC damage ranging from alterations in cellular properties to hemolysis.[16] Baskurt et al[17] and Mohandas et al[18] reported that shear stress in the range of 100 to 300 Pa (0.75–2.25 mmHg) can induce a decrease in RBC deformability. Furthermore, RBCs can withstand shear stress up to 300 Pa for only short periods (<1 second) without hemolysis.[11] These alterations in membrane properties were attributed to mechanical breakdown of the membrane skeletal network due to prolonged near-maximal linear extension.[19] Thus, previous studies suggest that more hemolysis occurrs in RBCs subjected to more shear stress under higher external pressure.[2,3] However, in our study, despite

Table 2

Laboratory studies on 5- and 35-day aged blood under varying external pressure.

		0 mmHg	**150 mmHg**	**200 mmHg**	**250 mmHg**	**300 mmHg**	**P value**
Hb, g/dL	5 days (n = 10)	15.05 ± 4.56	12.48 ± 1.22	12.47 ± 1.22	12.51 ± 1.20	12.70 ± 1.10	.21
	35 days (n = 10)	13.57 ± 7.20	14.27 ± 4.90	13.93 ± 4.46	13.17 ± 3.42	12.27 ± 2.10	.98
Hct (%)	5 days (n = 10)	44.50 ± 12.82	37.02 ± 3.42	37.01 ± 3.41	37.13 ± 3.22	37.69 ± 3.11	.27
	35 days (n = 10)	44.08 ± 21.85	44.82 ± 14.68	43.94 ± 13.24	41.71 ± 10.47	38.49 ± 6.41	.94
RBC count, ×10⁶/μL	5 days (n = 10)	5.04 ± 1.51	4.18 ± 0.38	4.18 ± 0.38	4.20 ± 0.36	4.25 ± 0.35	.40
	35 days (n = 10)	4.53 ± 2.23	4.62 ± 1.43	4.54 ± 1.29	4.30 ± 1.01	4.03 ± 0.62	.99
Platelet (×10³/μL)	5 days (n = 10)	164.50 ± 44.67	158.60 ± 42.94	151.40 ± 44.94	146.80 ± 41.90	138.60 ± 43.73	.48
	35 days (n = 10)	113.60 ± 26.95	104.20 ± 37.02	103.10 ± 32.26	102.50 ± 32.53	102.70 ± 27.85	.92
Potassium, mEq/L	5 days (n = 10)	12.52 ± 3.22	10.91 ± 1.64	11.07 ± 1.69	11.01 ± 1.74	10.99 ± 1.68	.68
	35 days (n = 10)	21.79 ± 3.95	21.50 ± 3.22	21.32 ± 3.21	21.14 ± 3.12	21.19 ± 3.15	.99

The data are expressed as mean ± SD, with 5 days indicating the group in which RBCs were analyzed on day 5 of storage and 35 days indicating the group in which RBCs were analyzed on day 35 of storage. H = hemoglobin, Hct = hematocrit, RBC = red blood cell.

Table 3

Elongation index.

Storage	0 mmHg	150 mmHg	200 mmHg	250 mmHg	300 mmHg	*P* value
5 days (n = 10)	0.33 ± 0.02	0.33 ± 0.02	0.33 ± 0.02	0.33 ± 0.02	0.33 ± 0.02	.99
35 days (n = 10)	0.33 ± 0.02	0.33 ± 0.02	0.33 ± 0.02	0.33 ± 0.01	0.32 ± 0.02	.75

The data are expressed as mean ± SD, with 5 days indicating the group in which RBCs were analyzed on day 5 of storage and 35 days indicating the group in which RBCs were analyzed on day 35 of storage.

increasing shear stress, there was no significant difference in the elongation index.

Our study had some limitations. First, we used a 20-gauge catheter to infuse blood. Because smaller-gauge catheters cause more hemolysis,[2,4] using a smaller-gauge catheter may have shown a difference in deformability according to pressure. However, the most common catheter size in procedures and operations in adults is 20 gauge. In addition, in situations in which a massive transfusion is needed, medical staff insert a peripheral intravenous or central line with a catheter that is >20 gauge. Therefore, the result of this study will be helpful in ordinary clinical settings. Second, the sample size we analyzed was small. Additional research with more blood samples is needed to confirm our results.

In conclusion, deformability of RBCs was not influenced by external pneumatic pressures of up to 300 mmHg. However, the duration of blood storage significantly affected hemolysis.

Acknowledgments

Author contributions

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References

[1] de la Roche MR, Gauthier L. Rapid transfusion of packed red blood cells: effects of dilution, pressure, and catheter size. Ann Emerg Med 1993; 22:1551–5.

[2] Frelich R, Ellis MH. The effect of external pressure, catheter gauge, and storage time on hemolysis in RBC transfusion. Transfusion 2001; 41:799–802.

[3] Eurenius S, Smith RM. Hemolysis in blood infused under pressure. Anesthesiology 1973;39:650–1.

[4] Wilcox GJ, Barnes A, Modanlou H. Does transfusion using a syringe infusion pump and small-gauge needle cause hemolysis? Transfusion 1981;21:750–1.

[5] Miller MA, Schlueter AJ. Transfusions via hand-held syringes and small-gauge needles as risk factors for hyperkalemia. Transfusion 2004;44: 373–81.

[6] Meram E, Yilmaz BD, Bas C, et al. Shear stress-induced improvement of red blood cell deformability. Biorheology 2013;50:165–76.

[7] Dobbe JG, Hardeman MR, Streekstra GJ, et al. Analyzing red blood cell-deformability distributions. Blood Cells Mol Dis 2002;28: 373–84.

[8] Berezina TL, Zaets SB, Morgan C, et al. Influence of storage on red blood cell rheological properties. J Surg Res 2002;102:6–12.

[9] Bessis M. Red cell shapes. An illustrated classification and its rationale. Nouv Rev Fr Hematol 1972;12:721–45.

[10] Calkins JM, Vaughan RW, Cork RC, et al. Effects of dilution, pressure, and apparatus on hemolysis and flow rate in transfusion of packed erythrocytes. Anesth Analg 1982;61:776–80.

[11] Leverett LB, Hellums JD, Alfrey CP, et al. Red blood cell damage by shear stress. Biophys J 1972;12:257–73.

[12] Mateer JR, Perry BW, Thompson BM, et al. Effects of rapid infusion with high pressure and large-bore i.v. tubing on red blood cell lysis and warming. Ann Emerg Med 1985;14:966–9.

[13] Sawant RB, Jathar SK, Rajadhyaksha SB, et al. Red cell hemolysis during processing and storage. Asian J Transfus Sci 2007;1:47–51.

[14] Saini N, Basu S, Kaur R, et al. Assessment of changes in plasma hemoglobin and potassium levels in red cell units during processing and storage. Transfus Apher Sci 2015;52:319–25.

[15] Baskurt OK, Meiselman HJ. Blood rheology and hemodynamics. Semin Thromb Hemost 2003;29:435–50.

[16] Kameneva MV, Antaki JF. Handbook of Hemorheology and Hemodynamics. Amsterdam; 2007: 206–227.

[17] Baskurt OK, Uyuklu M, Meiselman HJ. Protection of erythrocytes from sub-hemolytic mechanical damage by nitric oxide mediated inhibition of potassium leakage. Biorheology 2004;41:79–89.

[18] Mohandas N, Clark MR, Health BP, et al. A technique to detect reduced mechanical stability of red cell membranes: relevance to elliptocytic disorders. Blood 1982;59:768–74.

[19] Chasis JA, Mohandas N. Erythrocyte membrane deformability and stability: two distinct membrane properties that are independently regulated by skeletal protein associations. J Cell Biol 1986;103: 343–50.