

Effects of storage duration and temperature of human blood on red cell deformability and aggregation

Mehmet Uyuklu^a, Melike Cengiz^a, Pinar Ulker^a, Timea Hever^b, Julien Tripette^c, Philippe Connes^c, Norbert Nemeth^b, Herbert J. Meiselman^d and Oguz K. Baskurt^{a,*}

^a *Department of Physiology, Akdeniz University Faculty of Medicine, Antalya, Turkey*

^b *Department of Operative Techniques and Surgical Research, Auguszta Surgical Center, Institute of Surgery, Medical and Health Science Centre, University of Debrecen, Hungary*

^c *Laboratoire ACTES (EA 3596), Département de Physiologie, Université des Antilles et de la Guyane, 97159 Pointe à Pitre, Guadeloupe, French West Indies*

^d *Department of Physiology and Biophysics, Keck School of Medicine, Los Angeles, CA, USA*

Abstract. Blood samples used in hemorheological studies may be stored for a period of time, the effects of storage have yet to be fully explored. This study evaluated the effects of storage temperature (i.e., 4°C or 25°C) and duration on RBC deformability and aggregation for blood from healthy controls and from septic patients. Our results indicate that for normal blood, RBC deformability over 0.3–50 Pa is stable up to six hours regardless of storage temperature; at eight hours there were no significant differences in EI but $SS_{1/2}$ calculated via a Lineweaver–Burk method indicated impaired deformability. Storage temperature affected the stable period for RBC aggregation: the safe time was shorter at 25°C whereas at 4°C aggregation was stable up to 12 hours. Interestingly, blood samples from septic patients were *less* affected by storage. Blood can thus be stored at 25°C for up to six hours for deformability studies, but should be limited to four hours for RBC aggregation; storage at 4°C may prolong the storage period up to 12 hours for aggregation but not deformability measurements. Therefore, the time period between sampling and measurement should be as short as possible and reported together with results.

Keywords: Aggregation, blood storage, deformability, temperature

1. Introduction

Clinical and basic studies in the field of hemorheology include the measurement of blood and plasma viscosities as well as red blood cell (RBC) deformability and aggregation. The results of the measurements of such hemorheological parameters are known to be affected by a variety of factors, including sampling technique, storage of the samples, pre-analytical handling and the measurement conditions, as well as the instrument specifications. These factors have been the subject of several investigations [2,8, 14,19,23] and, in part, were the basis for guidelines for good laboratory practice (GLP) in hemorheology made by the Expert Committee on Blood Rheology [13]. However, there have been significant technical developments in the field of hemorheology since the 1986 date of these guidelines, and the newer

*Corresponding author: Oguz K. Baskurt, MD, PhD, Professor and Chairman, Department of Physiology, Akdeniz University Faculty of Medicine, Antalya, Turkey. Tel.: +90 242 310 1560; Fax: +90 242 310 1561; E-mail: baskurt@akdeniz.edu.tr.

methods and instruments have improved sensitivities for alterations in hemorheological parameters (e.g., RBC deformability and aggregation), which necessitated the revision of the above mentioned guidelines. Hence a new expert panel is currently working on such a revision [3] and various laboratory studies are also being conducted for determining the best laboratory practice in hemorheology.

Blood samples used in hemorheological studies may need to be stored for a period of time prior to being measured. This period may sometimes include shipping to a remote laboratory and thus the storage might be prolonged for 24 hours or longer. It is well known that RBC properties are affected by prolonged storage, with these effects potentially related to metabolic depletion, disturbed ion homeostasis, protein and lipid modifications (e.g., oxidation, degradation, cross-linking), and volume changes accompanied by alterations in intracellular hemoglobin concentrations [12,21]. These alterations may have different time courses at cooler temperatures compared to room or body temperatures, and the time course of these alterations may also be altered for RBC obtained from patients with various diseases. RBC mechanical properties have also been shown to be altered during the time period between the sampling and measurement [2,23]. While it was previously recommended that the storage duration for the blood samples should be less than four hours [13], these recommendations were mostly related to previous hemorheological methods which may not be relevant to current technology.

The present study was designed to explore the effects of storage temperature and storage duration on RBC deformability and aggregation parameters for human blood samples obtained from healthy individuals and septic patients. Its overall objective was to determine the time period after the blood sampling that could be allowed without significant hemorheological alterations due to storage; mechanisms of alterations during the storage period were not considered.

2. Materials and methods

2.1. Blood samples

Venous blood samples were obtained from 10 healthy human male volunteers, aged between 25 to 52 years. A tourniquet was briefly applied to locate the antecubital vein and 50 ml blood samples were obtained and anticoagulated with sodium heparin (15 IU/ml). Additionally, blood samples from 10 patients hospitalized in the intensive care unit of Akdeniz University Hospital with the diagnosis of severe sepsis (5 male, 5 female; aged between 18 to 55 years) were obtained using the same sampling technique. Note that all septic patients had respiratory insufficiency, with the presumed sources of sepsis being the lower respiratory tract, blood or urinary tract. Vasoactive drugs were used in six of the patients for maintenance of hemodynamic stability, and only one patient died because of adult respiratory distress syndrome before discharge from ICU.

After saving a 2 ml aliquot for immediate measurements, the remaining blood was divided into 20 aliquots of 2 ml each. Ten of these aliquots were stored in a refrigerator at $4 \pm 2^\circ\text{C}$ and the other 10 aliquots were kept at room temperature ($25 \pm 2^\circ\text{C}$). RBC deformability and aggregation were measured for both healthy and septic samples immediately following blood sampling and at 1, 2, 3, 4, 6, 8, 12 and 24 hours after sampling for the two series of aliquots kept at 4 or 25°C .

In a separate series of experiments to test the effects of re-warming duration, aliquots of blood samples from the group of healthy individuals described above were stored at $4 \pm 2^\circ\text{C}$ for 4 hours and then re-warmed at 37°C for periods between 0–30 min prior to measuring RBC deformability and aggregation.

2.2. RBC deformability measurements

RBC deformability was determined at various fluid shear stresses by laser diffraction analysis using an ektacytometer (LORCA, RR Mechatronics, Hoorn, The Netherlands). The system has been described elsewhere in detail [10]. Briefly, a low hematocrit suspension of RBC in an isotonic viscous medium (4% polyvinylpyrrolidone 360 solution, MW 360 kD) is sheared in a Couette system composed of a glass cup and a precisely fitting bob, with a gap of 0.3 mm between the cylinders. A laser beam is directed through the sheared sample and the diffraction pattern produced by the deformed cells is analyzed by a microcomputer. Based upon the geometry of the elliptical diffraction pattern, an elongation index (EI) is calculated as: $EI = (L - W)/(L + W)$, where L and W are the length and width of the diffraction pattern. An increased EI at a given shear stress indicates greater cell deformation and hence greater RBC deformability. All measurements were carried out at 37°C.

EI values were obtained for nine separate shear stresses between 0.3–50 Pascal (Pa). Additionally, the maximum EI at infinite shear stress (EI_{max}) and the shear stress required for half-maximal deformation ($SS_{1/2}$) were calculated using this data set for each measurement using a Lineweaver–Burk analysis as described elsewhere [4]. Briefly, the shear stress–EI curve was linearized by plotting the reciprocal of EI versus the reciprocal of shear stress. The x-intercept of the resulting line corresponds to the negative reciprocal value of shear stress causing half-maximal deformation ($SS_{1/2}$). Obviously, impairment in RBC deformability leads to increased $SS_{1/2}$ values.

2.3. Determination of RBC aggregation

RBC aggregation was assessed using a custom-built photometric aggregometer interfaced to a digital computer via monitoring light transmittance through a blood sample during the aggregation process [5]. The shearing portion of the system consists of two parallel glass plates with a gap of 0.3 mm between them; a stepper motor, controlled by the computer, rotates one of these plates. The blood sample under investigation is placed between the glass plates, and is first sheared at 500 s^{-1} for 10 s to disperse RBC aggregates. After a sudden stop of the motor, the infrared light transmission through the blood sample is monitored for 10 s and recorded by the computer. The computer then calculates the area under the light transmission curve and reports a dimensionless index (M) which increases with the extent of RBC aggregation. Measurements were done in triplicate for each sample and the mean of the three measurements assigned to that sample. Measurements were carried out at 37°C.

2.4. Red blood cell properties and morphology

Mean RBC Volume (MCV) for the blood aliquots was determined using an electronic hematology analyzer (Cell-Dyn 3500R, Abbott Diagnostic Division, Illinois, USA). RBC shape was examined in wet-mount, diluted and unstained preparations under light microscopy.

2.5. Statistics

Results are expressed as mean \pm standard error (SE). Statistical comparisons between groups were done by “repeated measures ANOVA” followed by “Newman–Keuls post test”, with p values <0.05 accepted as statistically significant.

3. Results

3.1. Samples from healthy donors

MCV values were 82–88 fl in the samples immediately after the sampling and were not changed during first 12 hours of storage regardless of the temperature. However, after 24 hour storage at room temperature, MCV had slightly but significantly increased to 86.2 ± 0.9 fl compared to the control value measured immediately after sampling (85.3 ± 0.7). MCV remained unchanged in the samples stored at 4°C. No significant changes in RBC morphology were detected.

3.1.1. Red blood cell deformability

There were no significant changes of red cell EI values over the entire range of shear stress (i.e., 0.3–50 Pa) for blood samples from healthy donors stored for up to 6 hours at either room temperature or 4°C. However, at eight hours and beyond, some EI values differed from that for the initial measurement: (1) for room temperature storage at eight hours, only EI values measured at shear stresses between 1.1 and 3.9 were significantly decreased (i.e., –22% at 1.1 Pa, –6% at 3.9 Pa); (2) at 12 hours, EI for room temperature stored blood was 5% lower over a stress range of 7.4–50 Pa and about 7% lower over 7.4–26 Pa for blood stored at 4°C; (3) at 24 hours only blood stored at 4°C exhibited decreased EI values (i.e., 7% decrease at 26.4 and 50 Pa).

Figure 1 presents EI_{\max} values calculated using the data obtained at the nine levels of shear stress. EI_{\max} values remained unchanged during eight hours of storage at room temperature and were significantly decreased at 12 hours. However, if the blood was stored at 4°C, this significant decrement in EI_{\max} was only seen at 24 hours. $SS_{1/2}$ values were found to be significantly increased following eight hours of storage for both room temperature and 4°C (Fig. 2). Interestingly, $SS_{1/2}$ returned to control level after 12 hours of storage at room temperature, while the return of $SS_{1/2}$ to control was delayed to 24 hours in the samples stored at 4°C.

3.1.2. Red blood cell aggregation

Values for the RBC aggregation index (M) measured during the 24 hour period after sampling are presented in Fig. 3. At room temperature, RBC aggregation remained stable for at least four hours;

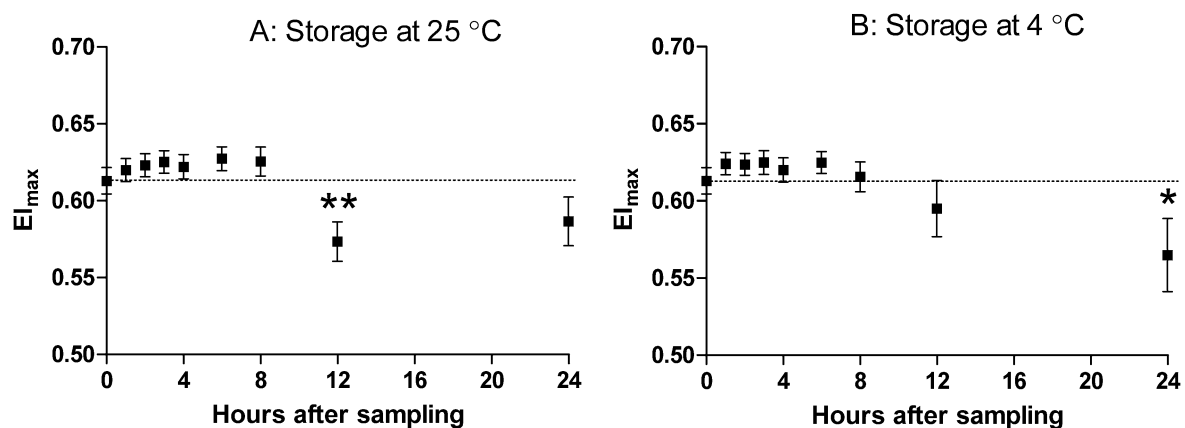


Fig. 1. Maximum EI at infinite shear stress (EI_{\max}) calculated using EI measured at nine shear stresses between 0.3–50 Pa for normal blood samples stored up to 24 hours after sampling. (A) At room temperature ($25 \pm 2^\circ\text{C}$). (B) At $4 \pm 2^\circ\text{C}$. Data are presented as mean \pm standard error. Difference from control (0 time), * $p < 0.05$.

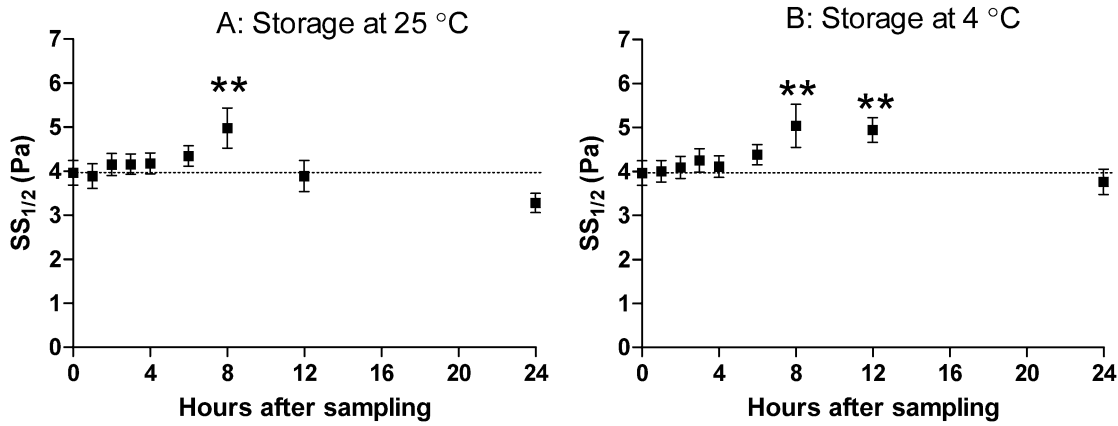


Fig. 2. Shear stress for half-maximal deformation ($SS_{1/2}$) calculated using EI measured at nine shear stresses between 0.3–50 Pa for normal samples stored up to 24 hours after sampling. (A) At room temperature ($25 \pm 2^\circ\text{C}$). (B) At $4 \pm 2^\circ\text{C}$. Data are presented as mean \pm standard error. Difference from control (0 time), * $p < 0.05$; ** $p < 0.01$.

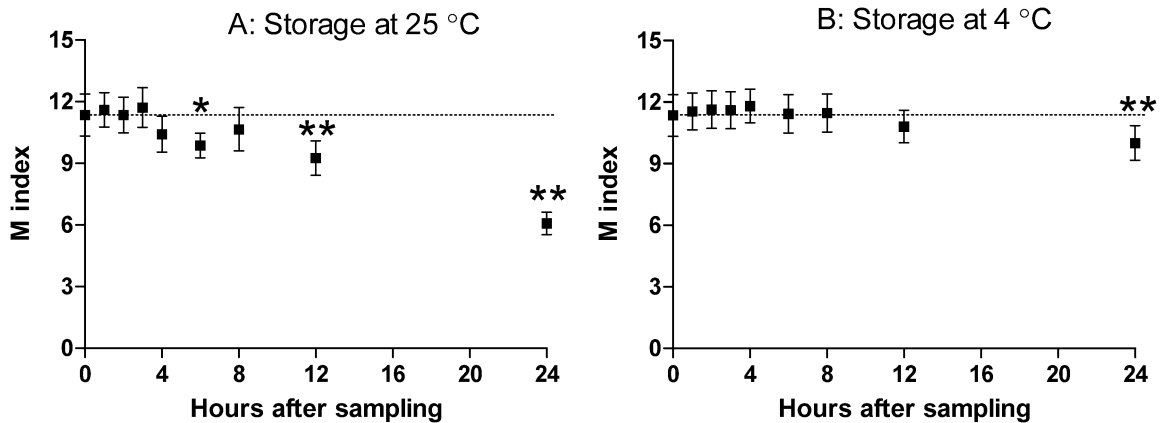


Fig. 3. Red blood cell aggregation indexes for normal blood samples stored up to 24 hours after sampling. (A) At room temperature ($25 \pm 2^\circ\text{C}$). (B) At $4 \pm 2^\circ\text{C}$. Data are presented as mean \pm standard error. Difference from control (0 time), ** $p < 0.01$.

aggregation started to decrease after six hours and was only 50% of control after 24 hours (Fig. 3A). In contrast, aggregation values were more stable if the blood was stored at 4 °C, with a significant decrement only detected after 24 hours (Fig. 3B).

3.1.3. Effect of 37 °C re-warming time after storage at 4 °C

Figure 4 presents the RBC deformability parameters ($SS_{1/2}$ and EI_{\max}) and the aggregation index M measured after various periods of re-warming at 37 °C following four hours of storage at 4 °C. Note that: (1) the parameters measured immediately after removing the sample from the storage at 4 °C (i.e., 0 minutes re-warming) were essentially identical to those obtained prior to cooling; (2) there was no effect of re-warming over the entire 5–30 min periods (Fig. 4).

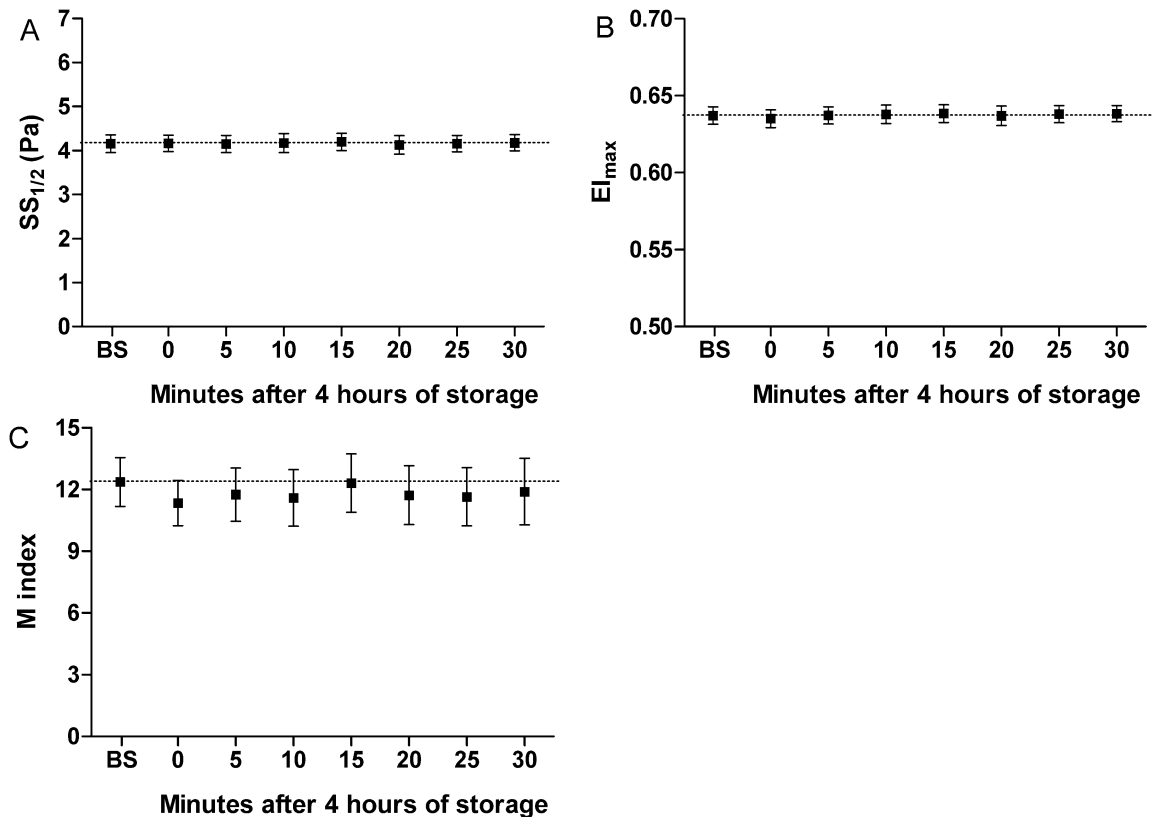


Fig. 4. Shear stress for half-maximal deformation ($SS_{1/2}$), maximum EI at infinite shear stress (EI_{max}) and RBC aggregation indexes for normal blood samples measured before storage (BS) and after various periods of pre-warming at 37°C following storage at 4°C for four hours. No significant alterations were observed for any of the parameters.

3.2. Samples from septic patients

3.2.1. Red blood cell deformability

Unlike the data obtained for samples from healthy subjects that showed some decreases of EI with storage time at both room temperature and 4°C , EI for septic subjects tended to *increase* with storage at room temperature. The most prominent increases were observed at 24 hours: the significant increases of EI at 24 hours were inversely related to shear stress and, on average, ranged from 35% at 0.6 Pa to 2% at 50 Pa. Storage at 4°C essentially eliminated alterations of EI. There were no significant alterations of EI_{max} values during the 24 hour storage period regardless of the storage temperature (data not shown). $SS_{1/2}$ in septic patients was found to be decreased significantly only after 24 hours of storage at room temperature, with no alterations observed if the kept at 4°C (Fig. 5).

3.2.2. Red blood cell aggregation

RBC aggregation for blood from septic patients remained stable at room temperature for up to 12 hours after the sampling, with a significant decrement in aggregation index observed only after 24 hours (Fig. 6A). Storage at 4°C prevented this alteration (Fig. 6B).

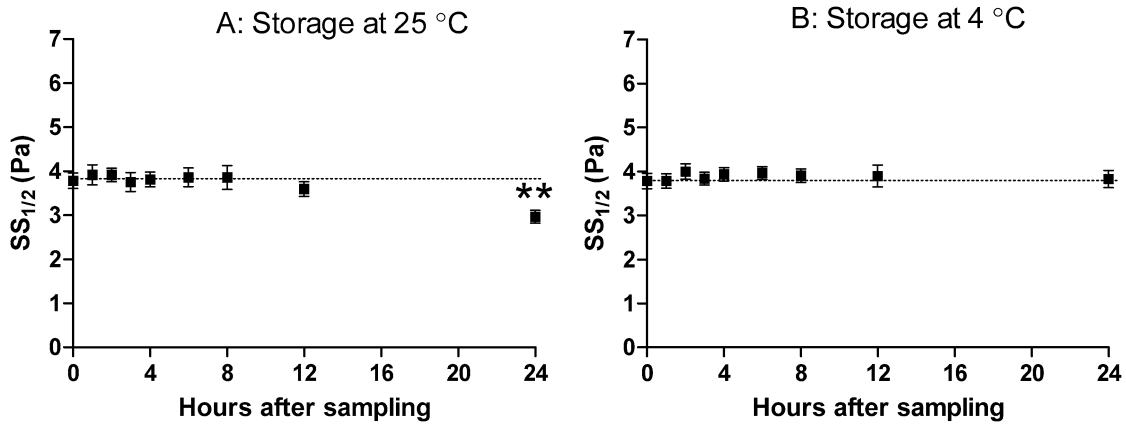


Fig. 5. Shear stress for half-maximal deformation ($SS_{1/2}$) calculated using EI measured at nine shear stresses between 0.3–50 Pa for samples from septic patients stored up to 24 hours after sampling. (A) At room temperature ($25 \pm 2^\circ\text{C}$). (B) At $4 \pm 2^\circ\text{C}$. Data are presented as mean \pm standard error. Difference from control (0 time), * $p < 0.05$; ** $p < 0.01$.

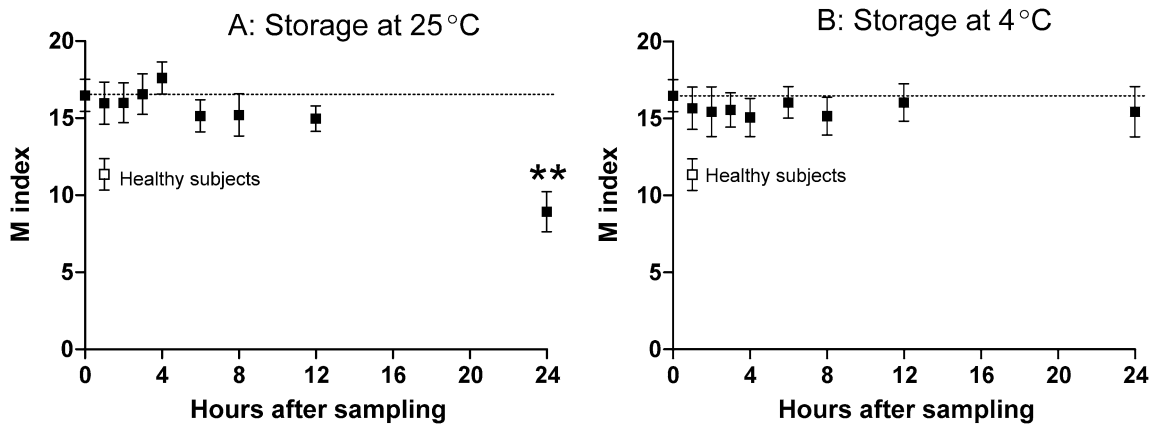


Fig. 6. Red blood cell aggregation indexes for blood samples from septic patients stored up to 24 hours after sampling. (A) At room temperature ($25 \pm 2^\circ\text{C}$). (B) At $4 \pm 2^\circ\text{C}$. Data are presented as mean \pm standard error. Difference from control (0 time), ** $p < 0.01$. The open squares in Fig. 5A, B indicate M values measured for healthy subjects immediately following sampling (i.e., no storage at either temperature).

4. Discussion

Our results indicate that the deformability of RBC from normal human blood, as measured by an ektacytometer over a shear stress range of 0.3–50 Pa, is stable up to six hours after sampling regardless of the storage temperature (i.e., 4 or 25°C). Although at 8 hours there were no significant differences in EI as tested by “repeated measure ANOVA”, $SS_{1/2}$ values calculated via a Lineweaver–Burk method were significantly increased at this time point, indicating impaired deformability; EI_{\max} values were stable up to 12 hours.

The ektacytometer used in this study is a sensitive device with good reproducibility [11]. Therefore, any deterioration in RBC deformability during the six hours after the sampling should have been detected using this device. Based on the findings summarized above, it can be argued that RBC in whole blood can be safely stored up to 6 hours either at room temperature or at 4°C for the measurement of

deformability. However, RBC deformability, indicated as an elevated $SS_{1/2}$, was found to be impaired in samples stored for eight hours at either room temperature or 4°C. Interestingly, RBC deformability returned to values for fresh samples with further storage at room temperature, and at 24 hours at room temperature had a $SS_{1/2}$ lower than control value, thus indicating an improvement of deformability. The small but significant increase of the average MCV in the room temperature samples stored for 24 hours (i.e., 85.3–86.2 fl) may provide a partial explanation for this improved RBC deformability. However, measurements made 12 hours after sampling indicated significantly decreased EI_{max} , probably indicating a structural change in the RBC membrane: these alterations were slower (i.e., no change in EI_{max} after 12 hours) and milder (Fig. 1) if the samples were stored at 4°C. Note that as a sub-study, some of the blood samples from healthy donors were stored up to 72 hours at room temperature: $SS_{1/2}$ values were very significantly decreased (1.92 ± 0.09 Pa versus 3.96 ± 0.28 Pa measured immediately after the sampling); storage at 4°C partly prevented this decrement in 72 hours (data not shown). These observations at 72 hours can be interpreted as reflecting changes in membrane mechanical behavior, probably due to disintegration of the membrane skeletal network [18]. Therefore, the biphasic pattern of RBC deformability changes might reflect two separate processes: the first phase yielding impairment of RBC deformability due to altered metabolic activity and/or disturbed ion homeostasis, and the second which becomes effective later interfering with membrane mechanical properties. It appears that the second effect becomes operative sooner if the blood samples are stored at room temperature.

In contrast to the behavior of samples from normal individuals, no significant alterations of EI were found during the first 12 hours in the samples from septic patients. It can be speculated that the above mentioned second phase of the storage effect might be shifted to earlier periods for septic blood, thus offsetting the mechanisms that might have caused impairment of RBC deformability.

Storage temperature affected the stable period for RBC aggregation in normal healthy samples: the safe storage time was shorter if the samples were kept at room temperature (Fig. 3), with aggregation indexes stable at least up to 12 hours if the samples were stored at 4°C. It was again interesting to observe that the samples from septic patients seemed to have better stability compared to the samples from normal subjects (Fig. 3 versus Fig. 5). Both plasmatic and cellular factors affect RBC aggregation [17]. Alterations in plasmatic factors play a significant role in the enhanced aggregation seen in sepsis [6], whereas RBC properties should be more sensitive to prolonged storage and hence the contribution of cellular factors seem to be more prominent in the normal samples versus the septic samples. At room temperature, RBC aggregation tended to decrease after 12 hours of storage, with aggregation indexes approaching very low values in the samples stored for 72 hours (1.32 ± 0.32 versus 11.35 ± 1.02 measured immediately after the sampling). Conversely, aggregation indexes decreased to only 8.24 ± 1.28 if the samples were stored for 72 hours at 4°C. These findings are in agreement with the previously published effects of prolonged storage on RBC aggregation [16] and the greater stability of low shear blood viscosity with storage at 4°C [1].

In this study, we have also evaluated whether a pre-warming time is required for the samples stored for four hours at 4°C. It seems reasonable that all RBC metabolism-related mechanisms (e.g., ion pumps) might be suppressed by cooling, and that a certain time period at 37°C prior to the measurement might be needed to restore disturbed cellular homeostasis. However, re-warming at 37°C up to 30 min had no effect on the measured RBC deformability and aggregation parameters, indicating that such a re-warming may not be necessary. This finding is in contrast with the report by Bartoli et al., which indicated a significant effect of re-warming time on whole blood filtration [2]. However, whole blood filtration is known to be very sensitive to leukocyte alterations and the dependence on re-warming time might have reflected the influence on leukocyte function [22].

This study did not include any measured parameters specifically designed to investigate the mechanisms of alterations during the storage. However, the biphasic nature of the effects of storage on RBC deformability (Fig. 2) was unexpected and clearly warrants further studies. It can be hypothesized that the first phase of this response to prolonged storage (i.e., increased $SS_{1/2}$ indicating impaired deformability) likely reflects metabolic depletion that results in: (1) slowing of ion pumps [15]; (2) lipid and protein oxidation and hence increased oxidant damage due to insufficient generation of antioxidant cofactors (NADH and NADPH) leading to [7]; (3) alterations of RBC membrane skeleton structure (e.g., increased protein crosslinking) [20]. The later phase of the storage-induced changes is characterized by very significant decrements in $SS_{1/2}$. This decrease may reflect degradation of oxidized-damaged proteins [9], resulting in abnormalities of the viscous and/or elastic rheologic behavior of the cell membrane rather than an improvement of RBC function.

In summary, blood samples can be stored at room temperature for up to six hours for the measurement of RBC deformability, but this period should be limited to four hours if the study also includes RBC aggregation. Storage at 4°C may prolong the storage period up to 12 hours for aggregation measurements but not for deformability measurements. However, it should be kept in mind that these “safe storage periods” apply to samples obtained from normal, healthy individuals. The time course of changes in RBC deformability and aggregation might be significantly different in the blood samples from patients with various pathologies (e.g., hemoglobinopathies) as observed herein for the samples from septic patients. Therefore, the time period between sampling and measurement should be as short as possible and, together with storage temperature, should be reported with results.

Acknowledgements

This study was supported by NIH Research Grants HL15722, HL 70595 and FIRCA IR03 TW01295, and by the Akdeniz University Research Projects Unit.

References

- [1] T. Alexy, R. Wenby, E. Pais, L.J. Goldstein, W. Hogenauer and H.J. Meiselman, An automated tube-type blood viscometer: validation studies, *Biorheology* **42** (2005), 237–247.
- [2] V. Bartoli, B. Albanese, P.G. Manescalchi, L. Mannini and G. Pasquini, Influence of blood storage conditions and anticoagulants on results of blood cell filtration test, *Clin. Hemorheol.* **6** (1986), 137–149.
- [3] O.K. Baskurt, M. Boynard, G.R. Cokelet, P. Connes, B.M. Cooke, M.R. Hardeman, F. Liao, H.J. Meiselman, G.B. Nash, N. Nemeth, B. Sandhagen, S. Shin, G.B. Thurston, J.L. Wautier and S. Yedgar, New guidelines for the assessment of hemorheological parameters, *Biorheology* **45** (2008), 82.
- [4] O.K. Baskurt and H.J. Meiselman, Analyzing shear stress-elongation index curves: comparison of two approaches to simplify data presentation, *Clin. Hemorheol. Microcirc.* **31** (2004), 23–30.
- [5] O.K. Baskurt, H.J. Meiselman and E. Kayar, Measurement of red blood cell aggregation in a “plate–plate” shearing system by analysis of light transmission, *Clin. Hemorheol. Microcirc.* **19** (1998), 307–314.
- [6] O.K. Baskurt, A. Temiz and H.J. Meiselman, Red blood cell aggregation in experimental sepsis, *J. Lab. Clin. Med.* **130** (1997), 183–190.
- [7] O.K. Baskurt and S. Yavuzer, Some hematological effects of oxidants, in: *Environmental Oxidants*, J.O. Nriagu and M.S. Simmons, eds, Wiley, New York, 1994, pp. 405–423.
- [8] P. Connes, M. Uyklu, J. Triplette, J.H. Boucher, E. Beltan, T. Chalabi, O. Yalcin, R. Chout, O. Hue, M.D. Hardy-Dessources and O.K. Baskurt, Sampling time after tourniquet removal affects erythrocyte deformability and aggregation measurements, *Clin. Hemorheol. Microcirc.* **41** (2009), 9–15.
- [9] K.J.A. Davies and A.L. Goldberg, Proteins damaged by oxygen radicals are rapidly degraded in extracts of red blood cells, *J. Biol. Chem.* **262** (1987), 8227–8234.

- [10] M.R. Hardeman, P.T. Goedhart, J.G.G. Dobbe and K.P. Lettinga, Laser-assisted optical rotational cell analyzer (LORCA). I. A new instrument for measurement of various structural hemorheological parameters, *Clin. Hemorheol.* **14** (1994), 605–618.
- [11] M.R. Hardeman, P.T. Goedhart and N.H. Schut, Laser-assisted optical rotational cell analyzer (LORCA): II. Red blood cell deformability elongation index versus cell transit time, *Clin. Hemorheol.* **14** (1994), 619–630.
- [12] J. Ho, W.J. Sibbald and I.H. Chin-Yee, Effects of storage on efficacy of red cell transfusion: When is it not safe?, *Critical Care Medicine* **31** (2003), S687–S697.
- [13] ICSH Expert Panel on Blood Rheology, Guidelines for measurement of blood viscosity and erythrocyte deformability, *Clin. Hemorheol.* **6** (1986), 439–453.
- [14] A.J. Keidan, S.S. Marwah and J. Stuart, Evaluation of phosphate and hepes buffers for study of erythrocyte rheology, *Clin. Hemorheol.* **7** (1987), 627–635.
- [15] N. Mohandas and S.B. Shohet, The role of membrane-associated enzymes in regulation of erythrocyte shape and deformability, *Clin. Hematol.* **10** (1981), 223–237.
- [16] V. Nagaprasad and M. Singh, Sequential analysis of the influence of blood storage on aggregation, deformability and shape parameters of erythrocytes, *Clin. Hemorheol. Microcirc.* **18** (1998), 273–284.
- [17] M.W. Rampling, H.J. Meiselman, B. Neu and O.K. Baskurt, Influence of cell-specific factors on red blood cell aggregation, *Biorheology* **41** (2004), 91–112.
- [18] B.D. Riquelme, P.G. Foresto, J.R. Valverde and R.J. Rasia, Alterations to complex viscoelasticity of erythrocytes during storage, *Clin. Hemorheol. Microcirc.* **22** (2000), 181–188.
- [19] R.S. Rosenson and C.C. Tangney, Effects of tourniquet application on plasma viscosity measurement, *Clin. Hemorheol. Microcirc.* **18** (1998), 191–194.
- [20] N. Uyesaka, S. Hasegawa, N. Ishioka, R. Ishioka, H. Shio and A.N. Schechter, Effect of superoxide anions on red cell deformability and membrane proteins, *Biorheology* **29** (1992), 217–229.
- [21] R. van Wijk and W.W. van Solinge, The energy-less red blood cell is lost: erythrocyte enzyme abnormalities of glycolysis, *Blood* **106** (2005), 4034–4042.
- [22] J.L. Wautier, G.W. Schmid-Schonbein and G.B. Nash, Measurement of leukocyte rheology in vascular disease: clinical rationale and methodology, *Clin. Hemorheol. Microcirc.* **21** (1999), 7–24.
- [23] J. Zhang, X. Zhang, N. Wang, Y. Fan, H. Ju, J. Yang, J. Wen and X. Qu, What is the maximum duration to perform the hemorheological measurement for the human and mammals, *Clin. Hemorheol. Microcirc.* **31** (2004), 157–160.