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Experimental evaluation of mechanical and electrical properties of RBC suspensions in Dextran and PEG under flow II. Role of RBC deformability and morphology

N. Antonova[a,*], P. Riha[b], I. Ivanov[a] and Y. Gluhcheva[c]
[a]*Institute of Mechanics and Biomechanics, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[b]*Institute of Hydrodynamics, Academy of Sciences of the Czech Republic, Prague, Czech Republic*
[c]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*

Abstract. Mechanical and electrical properties of the normal RBCs suspensions and of hardened after treatment with glutaraldehyde (0.01–2.5%) RBCs in isotonic physiological solution and Dextran 70 000 (Dextran 70) and Polyethylene glycol 35 000 (PEG) and adjusted to hematocrit of 40%, were evaluated. Apparent viscosity and conductivity were measured under steady and transient flow regimes at low shear rates and at different local structure of the flow at 37°C. A time course of conductivity was recorded in parallel with the rheological properties of the RBC suspensions and conductivity and apparent viscosity dependences on shear rates were studied and compared at different concentrations of Dextran 70, PEG and glutaraldehyde. Low shear viscosity decreased after RBCs treatment with glutaraldehyde and at 0.5–2.5% it is constant. Echinocytes are observed at low Dextran 70 and PEG concentrations while spherocytes are found mainly in smears treated with higher concentrations. The results show that the apparent viscosity and conductivity of RBCs suspensions in Dextran 70 and PEG are strongly influenced by flow, shear rates, concentration, cell deformability and morphology and the method is sensitive to study the mechanical and electrical properties of RBC suspension and to provide experimental description of RBCs and other cell-to-cell interactions.

Keywords: RBC suspensions, viscosity, conductivity, morphology, glutaraldehyde, Dextran 70 000, Polyethylene glycol 35 000 (PEG)

1. Introduction

Different methods and parameters are used to quantify RBC deformability as viscometry, micropipette aspiration, filtration technique, ektacytometry etc. [6–11, 13–23]. High-shear viscosity is determinant of RBC deformability and erythrocyte rigidity index is derived from viscosity measurements. Ektacytometry quantifies erythrocyte deformability by measuring the elongation of suspended red blood cells subjected

*Corresponding author: Nadia Antonova, Institute of Mechanics and Biomechanics, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 4, 1113 Sofia, Bulgaria. Tel.: +359 2 9796413; Fax: +359 2 8707498; E-mail: antonova@imbm.bas.bg.

to a range of shear stresses. A cell-deformability characterization involves general measurement of highly complex relationships between the cell biology and physical forces to which the cell is subjected. The modern technical solutions exist simulating the action of the force applied to the red blood cell in macro- and microcirculation [18].

Ginsbourg et al. [11] examined 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 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.

Kenyeres et al. [15] analysed two curve fitting formulas, used widely in the literature for interpretation of shear stress-elongation index plots obtained by measuring the elongation of suspended red blood cells subjected to a range of shear stresses by ektacytometry – of Lineweaver-Burke and of Streekstra-Bronkhorst. The Lineweaver-Burke method overestimates maximal deformability if shear stresses below 1 Pa are applied. The Streekstra-Bronkhorst method provides efficient data reduction though the theoretical background of the formula. The parameters have expressive meaning; however, both maximal and minimal deformations are slightly underestimated.

Stadnick et al. [22] introduced the Eadie-Hofstee transformation as an alternative linearization method to simplify the analysis of RBC deformability curves, obtained by ektacytometry for RBCs treated with hydrogen peroxide (H_2O_2), *tert*-butyl-hydroperoxide (*t*-BuOOH), or methyl-cyclodextrin (M_CD) and analyzed via ektacytometry (LORCA). RBC hemopathological clinical isolates (hereditary spherocytosis and thalassemia) were also analyzed by LORCA. Following ektacytometry, Eadie-Hofstee linearization was performed to obtain the maximum deformability (EImax) and shear stress at half maximal deformation (KEI) parameters. Significant changes in deformability parameters were observed with all agents tested. The ability of Eadie-Hofstee linearization to detect and resolve changes in RBC deformability induced *in vitro* as well as deformability changes associated with *in vivo* hematological disorders are demonstrated in the study.

Erythrocyte deformability, expressed by the deformability index, was determined by shear stress laser diffractometer (Rheodyne SSD) [10, 23]. It was established that erythrocyte deformability as well as left ventricular geometry and performance was improved during therapies aimed at reducing cardiovascular disease (CVD) risk [10]. According to the results obtained by A. Vayá et al. [23] a decreased erythrocyte deformability related to insulin resistance and inflammation is highly associated with abdominal fat in morbidly obese subjects. Waist circumference, which reflects abdominal fat, is from all the variables analyzed, the one that shows the highest association with decreased erythrocyte deformability.

In our previous investigations using a novel electrorheological technique we quantified mechanical and electrical properties of blood and RBC suspensions [1–5]. The apparent viscosity and conductivity of the samples were measured under different flow conditions: steady and non-steady flow and different shear rates. It was found that conductivity of blood and RBC suspensions is shear rate-, time- and temperature-dependent. We suggested that monitoring the electrical properties of red cell suspensions during aggregation can provide information about the time course of this process. The effect of aggregating agents on the ability of RBCs to aggregate was also estimated [3]. We quantified these changes by means of electrorheological technique and observed their effect on the apparent viscosity over a wide range of shear rates as well as on the conductivity of RBC suspensions in phosphate-buffered saline (PBS), containing dextran with different molecular weight: 70 000, 150 000 and 500 000 and polyethylene glycol with molecular weight 35 000 (PEG) using the Low Shear 30 Contraves viscometer and Data acquisition system [2].

The aim of the present study is to examine the influence of changes of RBC deformability, induced by incubation of normal human RBCs in glutaraldehyde (GA) from 0.01% to 2.5%, on rheological and electrical properties of RBC suspensions in Dextran 70 and PEG in PBS under steady and non-steady flow conditions. The study aims to evaluate in parallel apparent viscosity, conductivity of RBCs suspensions in Dextran 70 and PEG and the influence of flow, shear rates, concentration, cell deformability and morphological transformations of RBCs.

2. Materials and methods

2.1. RBC suspension samples

Whole human conserved blood with CPDA-1 (63 ml CPDA-1 in 450 ml blood) from the National Center for Hematology and Transfusiology in Sofia, stored at 4°C was used for the experiments.

2.1.1. Modification of RBC aggregation with Dextran 70 and PEG

RBC suspensions in Dextran 70 000 (Dextran 70) and polyethylene glycol 35 000 (PEG) were prepared by the following procedure: blood was centrifuged at 1500 × g for 10 min and plasma and conserving solution separated from erythrocytes. Erythrocytes were washed twice in phosphate-buffered saline PBS: (NaCl, KCl, Na_2HPO_4, KH_2PO_4 and distilled water, pH 7.4). For the preparation of the RBCs suspensions the twice washed in PBS RBCs were resuspended in PBS and the hematocrit was adjusted to H = 60%. Erythrocytes were then re-suspended either in PBS (control) or in PBS with Dextran 70 and polyethylene glycol 35 000 (PEG) with final concentration of dextran or PEG between 0.5 and 3.5 g/dl. The samples used for the experiments have been adjusted to hematocrit H = 40%:

1. Dextran 70 000 (Dextran 70) in PBS with initial concentration of 10 g/dl to the final concentration in the RBCs suspension of 1, 2, 3 and 3.5 g/dl, H = 40%.
2. Polyethylene glycol 35 000 (PEG) in PBS with initial concentration of 10 g/dl to the final concentration in the RBCs suspension of 0.5, 1.5 and 2.5 g/dl, H = 40%.

2.1.2. Modification of RBC deformability with glutaraldehyde

Washed RBCs were incubated at room temperature (20°C) for 30 min in solutions of glutaraldehyde (GA) in PBS with different concentrations: 0.01; 0.02; 0.03; 0.05; 0.1; 0.2; 0.5; 1 and 2.5%. Treated in GA RBCs were then washed three times with PBS after the incubation and re-suspended in Dextran 70 and in Polyethylene glycol 35 000 (PEG) at H = 40%. The measurements of the suspensions of RBCs in Dextran and PEG have been carried out immediately after the sample preparation at 37°C.

2.1.3. Morhological observations of RBCs

Blood smears were prepared, fixed and stained with May-Grünwald-Giemsa (Sigma). Morphological characteristics were observed on a light microscope Opton, at magnification × 630. Measurements were carried out within 2 h after suspensions were prepared.

2.2. Rheological and electrorheological measurements

The apparent viscosity of RBC suspensions in PBS with Dextran 70 and PEG and control were measured using a rotational viscometer Contraves Low Shear 30 at a steady and non-steady flow at shear rates

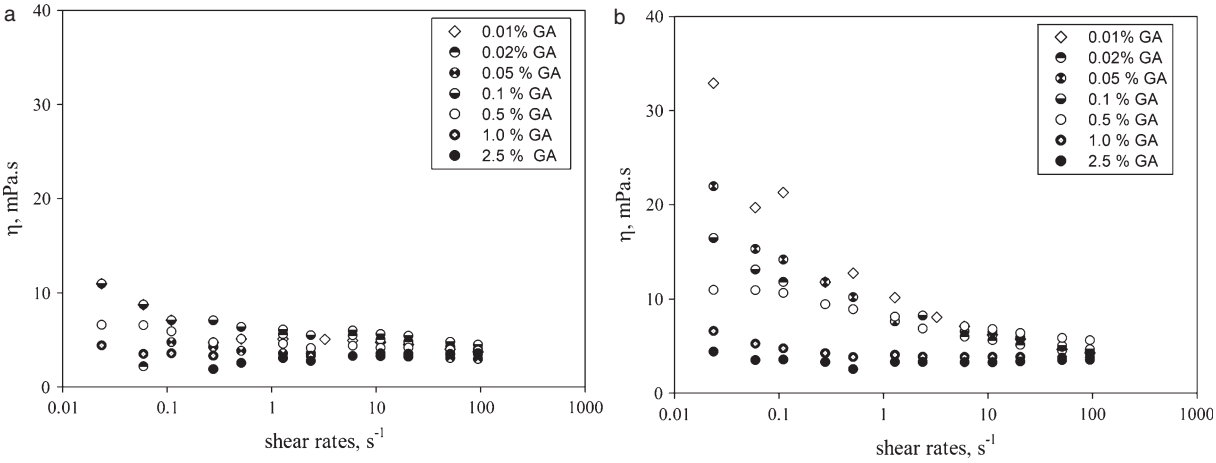

Fig. 1. Apparent viscosity η versus shear rate for the RBCs treated with GA with different concentrations (from 0.01% to 2.5% GA) and suspended in a) Dextran 70 (1 g/dl) and b) Dextran 70 (3 g/dl) solutions in PBS, Contraves LS 30, T = 37°C, $n = 3$, H = 40%, shear rate from 0.0237 s^{-1} to 94.5 s^{-1}.

from 0.0237 s^{-1} to 128.5 s^{-1}. The procedure of the preparation of the RBC samples and measurements were performed according to standards and guidelines for hemorheological experiments of Baskurt et al. [6]. RBC suspensions conductivity is quantified at non-equilibrium flow conditions by means of electro-rheological techniques. It includes also a resin replica of the Couette type measuring system MS 1/1 of the rheometer with a pair of platinum electrodes embedded into the wall; a device, constructed by the conductometric method and software (Data acquisition system) [2]. A method, based on dielectric properties of dispersed systems in Couette viscometric blood flow was applied to investigate RBC deformability and morphology [1, 4, 5] and the kinetics of RBC aggregation and the formation and break-up of the aggregates [3]. The main advantage of this technique is that blood is subjected to a uniform shearing field in a Couette rheometric cell, and information about the mechanical and electrical properties of the fluid is obtained in parallel. Shear rate changes were programmed and controlled by Rheoscan 100.

3. Results

The experimental dependences of the apparent viscosity versus shear rate for the RBCs incubated in GA with concentrations from 0.01% to 2.5% GA for Dextran 70 in PBS with final dextran concentration in the suspensions 1 g/dl and 3 g/dl, are shown on Fig. 1 – panel a and b; H = 40%, T = 37°C. The results show that the apparent viscosity is more influenced by GA at low shear rates than at high shear rates, which depends also on the concentration of GA and Dextran 70. The RBC suspension's apparent viscosity decreases with increasing RBC rigidity or decreasing RBC deformability, varying GA concentration from 0.01% to 2.5%. The apparent viscosity dependences on shear rate for RBC suspensions in Dextran 70 (3 g/dl) at 0.01% to 0.5% GA exhibit non-newtonian behaviour but at GA with concentration higher than 0.5% to 2.5% they are newtonian (Fig. 1b). The apparent viscosity of RBC suspensions in Dextran 70 (1 g/dl) at 0.05% to 2.5% GA (Fig. 1a) does not depend on shear rate. The last finding confirms the earlier data in the literature [7].

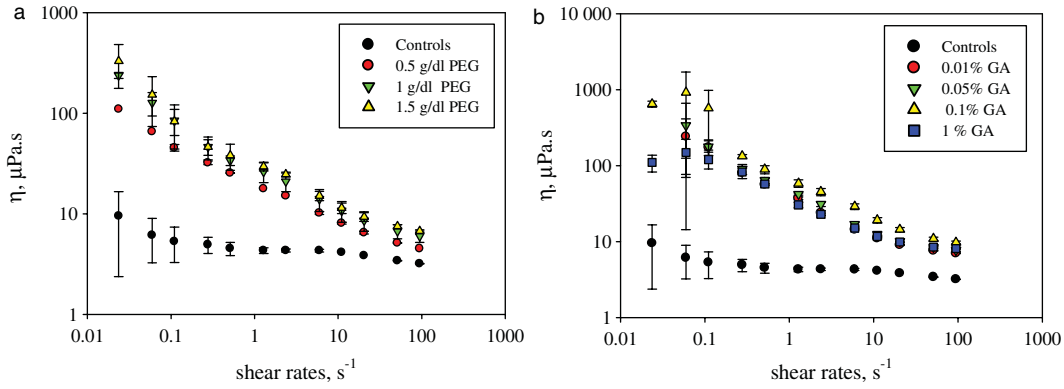

Fig. 2. Apparent viscosity η versus shear rate for: a) Suspensions of normal RBCs in PEG (c = 0.5 g/dl;1 g/dl and 1.5 g/dl) and control in PBS and b) RBCs treated with GA with concentrations from 0.01% to 2.5% GA and suspended in PEG (1.5 g/dl) solutions in PBS, Contraves LS 30, T = 37°C, $n = 3$, H = 40%, shear rate from 0.0237 s^{-1} to 94.5 s^{-1}.

The experimental dependences of the apparent viscosity on shear rate for normal RBCs in PEG in PBS, with final PEG concentration in the RBC suspensions 0.5 g/dl, 1 g/dl and 1.5 g/dl and RBCs treated with GA with concentrations from 0.01% to 1% GA in PEG (1.5 g/dl) in PBS, are shown on Fig. 2 – panel a and b; H = 40%, ($n = 3$), T = 37°C. Both RBC suspensions in PEG of normal and of treated RBCs with GA from 0.01% to 1% in PEG (1.5 g/dl), exhibit non-newtonian behaviour within the whole shear rate range from 0.0237 s^{-1} to 94.5 s^{-1} in comparison with the control sample of normal non-treated RBCs in PBS.

The results show that the apparent viscosity of RBC suspension's in Dextran 70 and in PEG depends on GA concentration (Fig. 3a, b). The apparent viscosity-GA concentration dependence gradually decreases with increasing GA concentration more than 0.01% for RBC suspensions in Dextran70 (Fig. 3a) and reaches a maximum at 0.1% GA (Fig. 3b) for RBC suspensions in PEG (1.5 g/dl). The apparent viscosity dependences on shear rate are decreasing too with decreasing RBC deformability or increasing cell rigidity: at 0.5% to 1% GA the apparent viscosity does not depend on shear rate at high shear rate (94.5 s^{-1}).

It was found that the apparent viscosity of RBC suspensions in Dextran 70 and PEG in PBS depends on Dextran, PEG and glutaraldehyde concentration, especially at low shear rates. The influence of the decreased RBC deformability is more pronounced for RBC suspensions in Dextran 70 than in PEG. Obviously, the aggregation effect of PEG prevail the desaggregation effect of decreased deformability of RBCs. The variation of the low-shear viscosity is not negligible. The low-shear viscosity gradually decreases for RBC suspensions in Dextran 70 (Fig. 3a) and has a peak at 0.01% GA for RBC suspensions in PEG (Fig. 3b), but their high shear rate-viscosity (94.5 s^{-1}) is almost constant. The residual effect of shear rate on viscosity in the absence of aggregation is due to the flexibility of the red blood cell. At very low shear rates, the orientation of the red blood cell relative to the direction of flow is random, but with increasing shear rate the flexibility of the red cell allows them to orient in a manner that presents a minimal cross-section to the flow stream, effectively reducing particle size. If the red blood cell is hardened by incubation in glutaraldehyde, the viscosity becomes almost independent of shear rate [1, 4, 5]. In fact, it is commonly known that the low shear viscosity is strongly dependent on RBC aggregation, which is also affected by cellular properties including RBC deformability [6, 8].

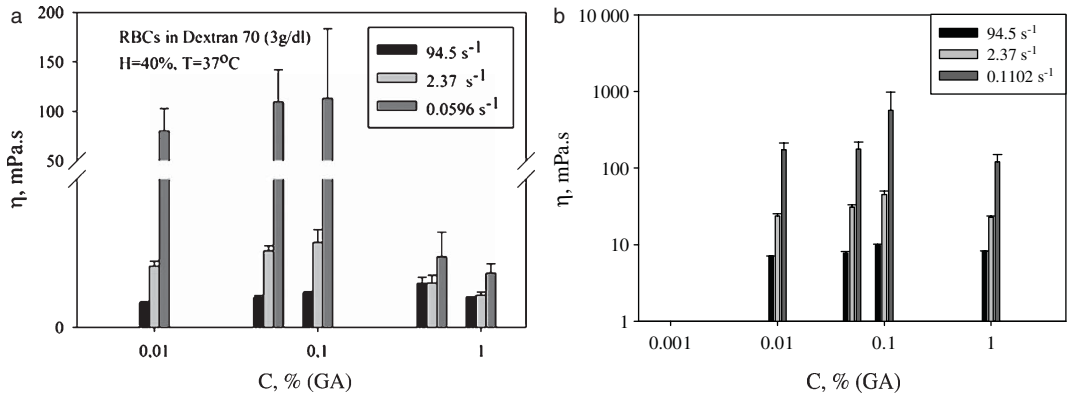

Fig. 3. Influence of glutaraldehyde (GA) concentration on the apparent viscosity of treated with GA RBCs in: a) Dextran 70 (3 g/dl) solution in PBS at shear rates 0.0596 s^{-1}, 2.37 s^{-1} and 94.5 s^{-1}; and b) PEG 35000 (1.5 g/dl) solution in PBS at shear rates - 0.1102 s^{-1}, 2.37 s^{-1} and 94.5 s^{-1}; T = 37°C, H = 40%.

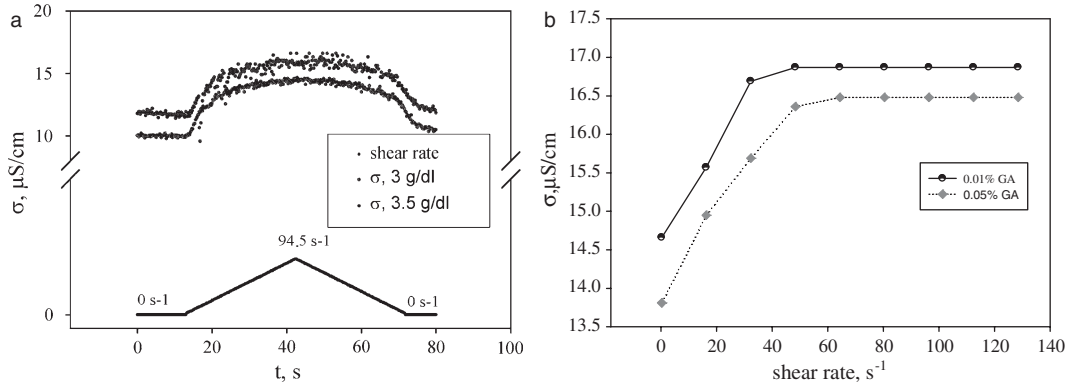

Fig. 4. Experimental conductivity dependences at triangular regime of shear rates from: 0 s^{-1} - 94.5 s^{-1} - 0 s^{-1} with duration 60 s. a)Time-dependences of RBC suspensions in Dextran 70 (3 and 3.5 g/dl) and b) Shear rate-dependences of treated with GA RBCs in Dextran 70 (3 g/dl); H = 40%, T = 37°C.

It is observed that deformability affects conductivity of the studied RBCs suspensions which is also dependent on shear rate and on the concentration of GA. More deformable RBCs, treated with 0.01% GA and suspended in Dextran70 (3 g/dl) in PBS, T = 37°C, H = 40% exhibit higher conductivity than more rigid RBCs treated with 0.05% GA (Fig. 4a). RBCs suspension conductivity-shear rate dependence exponentially increases with shear rate up to 40–50 s^{-1} and then reaches a plateau. Further increase of the shear rate from 50 to 128.5 s^{-1} does not influence RBC suspension conductivity. In fact, it is commonly known that the low shear viscosity as well as conductivity [3] is strongly dependent on RBC aggregation, which is also affected by cellular properties including RBC deformability.

It was shown in [1, 4, 5] that dextrans induced morphological changes in the shape and arrangement of RBCs in suspensions. Microscopic observations performed by us revealed changes in RBC morphology under *in vitro* influence of Dextran 70 at different concentrations (1–3.5 g/dl) [5]. Morphological studies on blood smears showed (Fig. 5a) dextran concentration dependence of the transition in the erythrocyte

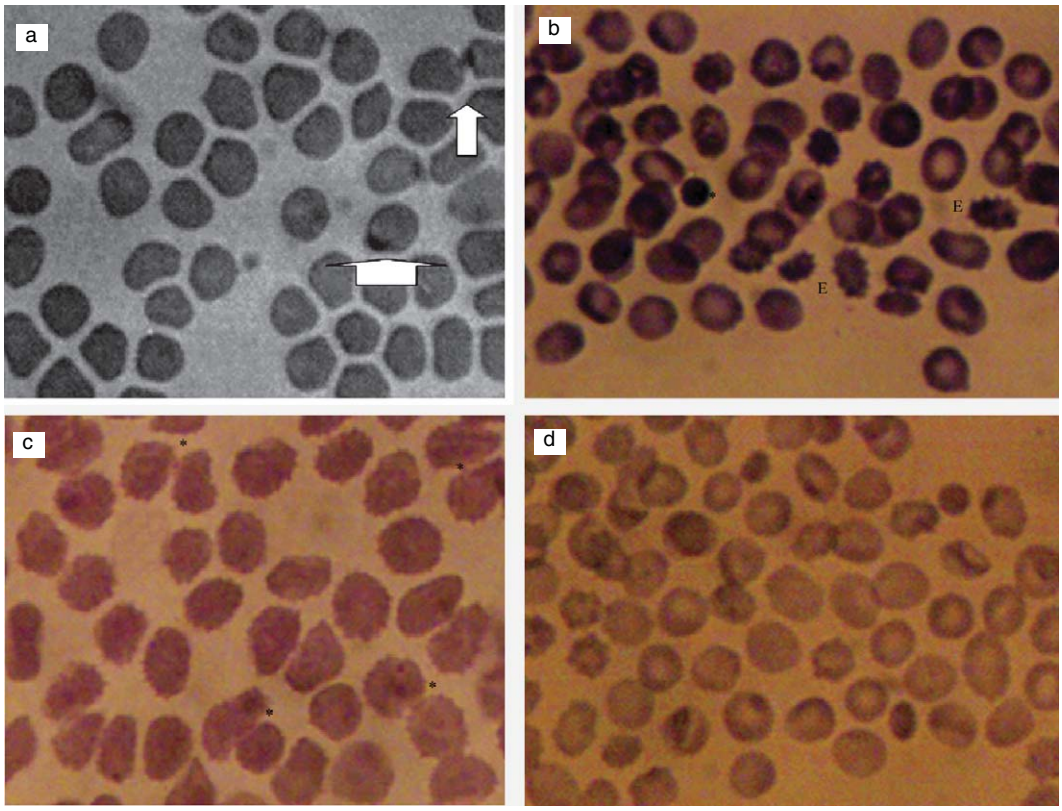

Fig. 5. (a) Echinocytes, two stomatocytes (arrows) and discocyte (arrowhead), after treatment with high Dextran 70 (3.5 g/dl). May-Grünwald-Giemsa staining, × 630. (b) A lot of echinocytes (E) and microspherocytes (*) are found in blood smears of RBCs incubated in 1% glutaraldehyde. May-Grünwald-Giemsa staining, × 630. (c) Echinospherocytes forming cell-to-cell contacts (*) prevail in smears of RBCs treated with 1.5 g/dl PEG. May-Grünwald-Giemsa staining, × 630. (d) Spherocytes and echinocytes forming cell-to-cell contacts are observed in smears of RBCs incubated in 1% glutaraldehyde and treated with 1.5 g/dl PEG. May-Grunwald-Giemsa staining, × 630.

shape and final transformation of normal RBCs (discocytes) into echinocytes. Echinocytes were mainly observed in suspensions treated with different Dextran 70 concentrations. Stage one echinocytes were irregularly contoured RBCs - with a few sharp superficial angulations (small cytoplasmic protrusions). We have observed these cellular type after the treatment of RBC suspensions with low Dextran 70 concentrations (1-2 g/dl) [5]. Stage one echinocytes and especially stomatocytes could form intercellular aggregates. Spherocytes (spheroechinocytes; stage 2 echinocytes) are flat ovoid red blood cells, arranged in a continuous network - observed by us in suspensions/smears treated with high Dextran 70 concentrations (3 g/dl and 3.5 g/dl) (Fig. 5a). In the same blood smears one could see also rare discocytes and stomatocytes (see arrows and big arrowheads).

Microscopic observations performed by us revealed changes in RBC morphology under *in vitro* influence of glutaraldehyde GA (1%): a lot of echinocytes (E) and microspherocytes (*) are found in blood smears of RBCs (Fig. 5b). We observed that echinospherocytes forming cell-to-cell contacts (*) prevail in smears of RBCs treated with 1.5 g/dl PEG (Fig. 5c). The results are in agreement with data of Hashemi-Najafabadi et al. [12]. The cell-to-cell interactions increase with the increase of PEG

concentration. Spherocytes contacting with each other are most often observed in smears of RBCs incubated with 0.01% and 0.05% glutaraldehyde and treated 1.5 g/dl PEG. Spherocytes and echinocytes forming cell-to-cell contacts are observed in smears of RBCs incubated in 1% glutaraldehyde and treated with 1.5 g/dl PEG (Fig. 5d).

4. Discussion

The glutaraldehyde treatment of RBCs results in intra- and intermolecular cross-linking membrane and cytosol constituents with drastic alteration of membrane viscoelastic and cytosol viscous properties and a reduction in cell deformability. It has been known that the higher the concentration of GA use, the less deformable RBCs become [20]. Evans and Parsegian [9] formulated theoretical consideration about the importance of cell deformability for RBC aggregation. The work of deformation that has to be carried out by the actual aggregation forces to form a stable adhesive contact between two cell surfaces includes the work required to deform cell interior (change cell volume) and cell membrane. Additionally, the authors observed the aggregation behaviour of the control and treated RBC in phosphate-buffered saline, containing dextran 70 000 as aggregating macromolecules using the zeta sedimentation technique [14]. The correlations between cell electrical (membrane sialic acid content, electrophoretic mobility, electrical polarizability) and mechanical (cell deformability) properties and the parameters of erythrocyte aggregation are investigated and discussed [14].

During the adhesion process, the viscous properties (which include the membrane permeability) only limit the rate of contact formation but not the extent. In case of stationary aggregation (time-independent), the reversible work of deformation competes with the adhesive forces (chemical affinity) determining the extent of aggregation. Following this argument, one should expect that a decrease in cell deformability on the basis of altered membrane and cytosol mechanical properties will result in slowing down the aggregation process and reduce the extent of stationary cell aggregation. In fact, this is found experimentally in this work and by other research groups [9]. On the other hand, glutaraldehyde treatment removes the positive charges originating from the amino groups of the surface glycoproteins. This is reflected by altered electrokinetic behaviour of GA-RBC at low and high pH-values, low and high ionic strengths and with increasing dextran concentrations [9]. However, these changes at physiological pH and ionic strengths and for Dextran 70 000 concentrations in the range 0–3 g/dl as in the present investigation are relatively small [9]. Fixation with high GA concentration (3%) at physiological pH and ionic strength increased of RBC with 14%. Based on the two layers distribution of sialic acid residues, we could speculate for this particular case, that due to increased electrostatic repulsion between the two negative surface layers (absence of positive charges) that the glycocalyx of GA-RBC may expand. The steric repulsion will increase and hence together with the increased electrostatic repulsion will decrease the net attractive energy between cells. Therefore, we conclude from our results that altered RBC deformability is the major factor for decreased aggregation of RBC.

Reinhart et al. [20] supposed that the viscoelasticity of RBCs membrane gradually decreases with increasing the concentration of GA. They also suggested that the low concentrations of GA (lower than 0.03%) increases ESR. A time course of blood conductivity recorded under different flow conditions provides experimental description of RBC aggregation-desaggregation processes and other cell-to-cell interactions. The microscopy observations performed within this time, reveal changes in RBCs morphology after treatment with different concentrations of dextran 70 and PEG [1–5]. One could say that there is a transition from the biconcave shape of red blood cells to elongated, almost polygonal shaped cells.

A concentration of dextran 1 g/dl induces changes in the membrane of RBCs which were observed as folding-like spines, which disappear at higher concentrations of dextran and PEG.

5. Conclusion

The results show that the changes in the membrane elasticity of normal human RBCs induced by incubation in glutaladehyde solutions with concentrations from 0.01% to 2.5% influence the apparent viscosity and conductivity of RBC suspensions in Dextran 70 and PEG 35 000. The blood conductivity is strongly dependent on the considered blood factors and is influenced by flow, shear rates, concentration and cell deformability. The apparent viscosity becomes almost independent of shear rate after treatment of RBCs with glutaraldehyde (0.5–2.5%).

The present investigation shows the possibilities, which offers the simultaneous study of electrical and mechanical properties of RBC with regard to their aggregation behaviour and deformability of RBCs. This technique may be very useful in combination with the viscometric technique and other techniques as well as for the clinical practice.

Understanding the morphological, mechanical, electrical properties of cells as well as elucidating the complex cell-to-cell and cell-polymer interactions of RBC suspensions in Dextran 70 and PEG gives instrument for understanding the mechanism of RBC aggregation and deformability and offers a better tool for diagnostics, therapeutics and effective drug assays.

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References

[1] N. Antonova, I. Ivanov, Y. Gluhcheva and E. Zvetkova, Rheological and Electrical Properties of RBC suspensions in Dextran 70. Changes in RBC Morphology, in: *IFMBE Proceedings Series,* D. Panagiotis Bamidis, Nicolas Pallikaris Eds, XII Mediterranean Conference on Medical and Biological Engineering and Computing 2010, Springer, Chalkidiki, Greece, 2010, **29** pp. 647–650.

[2] N. Antonova and P. Riha, Studies of blood electrorheological properties, *Clin Hemorheol Microcirc* **35** (2006), 19–29.

[3] N. Antonova, P. Riha and I. Ivanov, Experimental evaluation of mechanical and electrical properties of RBC suspensions under flow. Role of RBC aggregating agent, *Clin Hemorheol Microcirc* **45** (2010), 253–261.

[4] N. Antonova, P. Riha and I. Ivanov, Experimental evaluation of mechanical and electrical properties of RBC suspensions under flow. Role of RBC deformability, J Series on Biomechanics **25**(3-4) (2010), 45–52.

[5] N. Antonova, P. Riha, I. Ivanov and Y. Gluhcheva, Study of Rheological and Electrical Behaviour of RBC Suspensions in Dextran and PEG under Non-steady Flow. Role of RBC Deformability and Morphology, in: *IFMBE Proceedings Series,* C.T. Lim and J.C.H. Goh, Eds, Springer, 2010, **31** pp. 330–333.

[6] O.K. Baskurt, M. Boynard, G.C. Cokelet, P. Connes, B.M. Cooke, S. Forconi, F. Liao, M.R. Hardeman, F. Jung, H.J. Meiselman, G. Nash, N. Nemeth, B. Neu, B. Sandhagen, S. Shin, G. Thurston and J.L. Wautier, New guidelines for hemorheological laboratory techniques. *Clin Hemorheol Microcirc* **42** (2009), 75–97.

[7] O.K. Baskurt and H.J. Meiselman, Blood rheology and hemodynamics. *Seminars in Thrombosis and Hemostasis* **29** (2003), 435–450.
[8] J.J. Bishop, A.S. Popel, M. Intaglietta and P.C. Johnson, Rheological effects of red blood cell aggregation in the venous network: A review of recent studies. *Biorheology* **38** (2001), 263–274.
[9] E.A. Evans and V.A. Parsegian, Energetic of membrane deformation and adhesion in cell and vesicle aggregation, *Annals of the New York Academy of Sciences* **416** (1983), 13–33.
[10] M. Fornal, R.A. Korbut, J. Królczyk and T. Grodzicki, Evolution of rheological properties of erythrocytes and left ventricular geometry in cardiovascular disease risk patients, *Clin Hemorheol Microcirc* **45**(2–4) (2010), 155–159.
[11] S. Ginsbourg, S. Levin, S. Einav and R. Korenstein, Human erythrocyte filterability at low driving pressure, *Clin Hemorheol Microcirc* **43**(4) (2009), 309–319.
[12] S. Hashemi-Najafabadi, E. Vasheghani-Farahani, S.A. Shojaosadati, M.J. Rasaee, M. Moin and Z. Pourpak, Factorial design optimization of red blood cell PEGylation with a low molecular weight polymer, *Iranian Polymer Journal* **15** (2006), 75–683
[13] C. Hirata, K. Hiromi, N. Mizuno, H. Kutsuna, K. Ishina and M. Ishii, Effect of normal human erythrocytes on blood rheology in microcirculation, *Osaka City Med J* **53** (2007), 73–85.
[14] S. Jovtchev, I. Djenev, S. Stoeff and S. Stoyilov, Role of electrical and mechanical properties of red blood cells for their aggregation, *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **164** (2000), 95–104.
[15] P. Kenyeres, M. Rabai, A. Toth, G. Kesmarky, Z. Marton, T. Alexy and K. Toth, Reviewing data reduction methods for ektacytometry, *Clin Hemorheol Microcirc* **47**(2) (2011), 143–150.
[16] N. Maeda, Erythrocyte rheology in microcirculation, *Japan J Physiol* **46** (1996), 1–14.
[17] L. Mirossay, J. Mojžiš, M. Jandošeková, Š. Lukačn and A. Niáak, Comparison of two methods in erythrocyte microrheology determination using glutaraldehyde-treated cells, *Clin Hemorheol Microcirc* **17** (1997) 187–192
[18] M. Musielak, Red blood cell-deformability measurement: Review of techniques, *Clin Hemorheol Microcirc* **42** (2009), 47–64.
[19] A.R. Pries, T.W. Secomb and P. Gaehtgens, Biophysical aspect of blood flow in the microvasculature, *Cardiovasc Res* **32** (1996), 654–667.
[20] W.H. Reinhart, M. Singh-Marchettin and P.W. Straub, The influence of erythrocyte shape on suspension viscosities, *Eur J Clin Invest* **22**(1) (2008), 38–44.
[21] S. Shin, Y. Ku, M.-S. Park and J.-S. Suh, Measurement of red cell deformability and whole blood viscosity using laser-diffraction slit rheometer, *Korea-Australia Rheology Journal* **16**(2) (2004), 85–90.
[22] H. Stadnick, R. Onell, J.P. Acker and J.L. Holovati, Eadie-Hofstee analysis of red blood cell deformability, *Clin Hemorheol Microcirc* **47**(3) (2011), 229–239.
[23] A. Vayá, R. Cámara, A. Hernádez-Mijares, M. Romagnoli, E. Solá, D. Corella and B. Laiz, Erythrocyte deformability in morbid obesity before bariatric surgery. Influence of abdominal obesity, *Clin Hemorheol Microcirc* **46**(4) (2010), 313–320.