Acta Scientific Biotechnology

Volume 5 Issue 1 February 2024

Research Article

Nanotechnological Innovations: A Modern Outlook on Solving Challenges in Erythrocyte Preservation During Storage

AN Belousov[1,2,3]*, EI Malygon[2,3], TO Kalynychenko[4], EY Belousova[1,2] and MV Yagovdik[4]

[1]*Laboratory of Applied Nanotechnology of Belousov, Ukraine*

[2]*Kharkiv National Medical University, Ukraine*

[3]*Kharkiv Regional Center of Blood Service, Ukraine*

[4]*Institute of Hematology and Transfusiology of the National Academy of Medical Sciences of Ukraine, Ukraine*

***Corresponding Author:** AN Belousov, Laboratory of Applied Nanotechnology of Belousov, Ukraine.

Received: January 21, 2024

Published: January 31, 2024

Abstract

At present, nanotechnology offers fresh possibilities for influencing anaerobic glycolysis processes and hexose monophosphate reactions in preserved red blood cells. We investigated components containing red blood cells donated with the CPDA-1 preservative. We utilized modified solutions consisting of 0.9% NaCl and 5% glucose for resuspension. The solutions underwent treatment with magnetite nanoparticles (specifically, ICNB brand) using the Belousov's method. Spectrophotometry was employed to determine the levels of 2,3-DPG, ATP, reduced glutathione, and glutathione peroxidase. This research presents promising avenues for extending the shelf life and maintaining the functional activity of preserved red blood cells. Our findings demonstrated a significant increase in ATP and reduced glutathione, accompanied by a reduction in 2,3-DPG and glutathione peroxidase. It was evident that the enhancement of anaerobic glycolysis was less pronounced in experiments involving modified physiological saline compared to those using a glucose solution. Instead, the pentose glucose oxidation cycle prevailed. A comprehensive analysis of the collected data suggests that the modified resuspension solutions possess membrane-protective properties, which can be attributed to the rise in ATP and reduced glutathione, maintaining the cell's redox potential in equilibrium. The introduction of magnetite nanoparticles (ICNB) induces changes in the mobility and orientation of hydrogen protons within the resuspension solutions. This results in polarization of the aqueous environment surrounding the erythrocytes due to van der Waals forces. This polarization constitutes the primary cause behind the activation of ATP phosphate residue hydrolysis and the activation of intracellular enzymes that regulate anaerobic glycolysis and the pentose phosphate cycle. As a consequence, transmembrane metabolism and metabolic processes are altered, leading to a shift in the energy state of erythrocytes and the activation of enzymes. All of these changes exert a substantial influence on the energy supply of preserved red blood cells, thus preserving their functional capabilities under storage conditions at temperatures ranging from 2 to 6°C.

Keywords: Magnetite Nanoparticles; Resuspending Solution; Preserved Erythrocytes; Membrane-Protective Effect; Anaerobic Glycolysis; Pentose Cycle

Introduction

Within the human body, aerobic cells derive their energy from molecular oxygen (O_2). However, oxygen also serves as the precursor for generating a limited quantity of harmful substances known as reactive oxygen species (ROS), as documented by Turrens in 2003 and Hayyan., *et al*. in 2016 [1,2]. These compounds are potent oxidizing agents or highly reactive free radicals that can inflict damage on cellular structures and essential molecules. Erythrocytes, due to their transport role, are especially vulnerable to the effects of reactive oxygen species, as they are exposed to elevated oxygen levels. When these reactive oxygen species come into contact with the unsaturated fatty acids in erythrocyte membranes, they trigger the formation of hydroperoxides.

To counteract the detrimental impact of these hydroxyl and lipid radicals, erythrocytes employ an antioxidant defense system, which includes superoxide dismutase, catalase, and reduced glutathione (GSH). This antioxidant system effectively neutralizes ROS and mitigates the harm they cause, as supported by research conducted by Devasagayam in 2004 and Waszczak in 2018 [3,4].

The efficient operation of this antioxidant system depends on substances that maintain a balanced metabolic state within erythrocytes. Erythrocyte metabolism is essentially limited to anaerobic glycolysis and the hexose monophosphate pathway (HMW), as outlined in studies by Jacobasch in 1982 and Guitton., *et al*. in 2003 [5,6].

Glutathione represents a crucial low-molecular thiol-containing compound within cells, primarily tasked with antioxidant duties. It actively contributes to preserving the cell's redox potential, engages in detoxification processes, aids in eicosanoid synthesis, and plays a regulatory role in various cellular signaling mechanisms. Its role involves countering the effects of free radicals, which are molecules with unpaired electrons that avidly snatch electrons from other compounds. Consequently, this leads to the disruption of molecular chains, structural alterations in substances, and impediments in chemical reactions, as reported in studies by Pastore., *et al*. in 2001 and Pompella., *et al*. in 2003 [7,8].

Molecules that lose an electron in this process become radicals themselves, referred to as oxidized molecules. This electron loss process is known as oxidation. An exceptional characteristic of glutathione, distinguishing it from other molecules in the human body, is its rapid ability to replenish lost electrons. By readily surrendering its electrons to "trap" free radicals, it safeguards cells against damage. It transforms from its oxidized form (GSSG) to its reduced form (GSH). The predominant form of glutathione in cells is the reduced form (GSH), while the oxidized form (glutathione disulfide - GSSG) typically constitutes less than 1% of the total intracellular glutathione content. A significant portion, approximately 85-90%, of GSH is found in the cytosol. Maintaining an optimal GSH/GSSG ratio within cells is imperative for their normal operation and survival. Hence, strict regulation of the system governing this ratio is of utmost importance. A deficiency in GSH leaves cells vulnerable to oxidative damage, as documented by Novello and McLean in 1968 and Lu., *et al*. in 2012 [9,10].

Furthermore, under the influence of glutathione reductase, NADPH+H+ provides hydrogen protons for the regeneration of reduced glutathione (GSH). GSH assumes a critical role as the most vital erythrocyte antioxidant and functions as a coenzyme in the reduction of methemoglobin to its functional hemoglobin state. Reduced glutathione is instrumental in detoxifying H_2O_2 as well as hydroperoxides, which arise during the reaction of reactive oxygen species (ROS) with the unsaturated fatty acids found in erythrocyte membranes, as elucidated by Halprin and Ohkawara in 1967 [11].

In addition to the enzyme glutathione reductase, there is another crucial protective enzyme known as selenium-containing glutathione peroxidase (GSH-Px), as described in studies by Murakami., *et al*. in 1989 and Scholz., *et al*. in 1989 [12,13]. This enzyme facilitates the conversion of lipid hydroperoxides into their respective alcohols and the reduction of hydrogen peroxide into water by utilizing reduced glutathione. The status of the glutathione system within erythrocytes has a substantial impact on hemoglobin function and the overall regulation of oxygen transport in the bloodstream, as highlighted in research by Mills., *et al*. in 1994, Richie., *et al*. in 1996, Schafer and Buettner GR. in 2001, and van't Erve., *et al*. in 2013 [14-17].

At present, nanotechnology provides novel avenues for influencing anaerobic glycolysis processes and the activity of hexose monophosphate reactions within erythrocytes. Utilizing nanotechnological preparations, we can now exert an effect on the functional capabilities of erythrocytes, alter their aggregation characteristics, and enhance oxygen delivery to tissues, as demonstrated by Belousov in 2011 [18]. Through the examination of human erythrocytes, it has been revealed that magnetite nanoparticles (NPs) induce significant alterations in the polarization structure of the cellular microenvironment's aqueous sector, thereby modulating erythrocyte membrane permeability. This effect has been extensively discussed in research by Belousov in 2013, 2014, Belousov., *et al*. in 2018, and Belousov., *et al*. in 2019 [19-22]. When physiological saline solutions are resuspended after being treated with magnetite nanoparticles (ICNB), it leads to a substantial reduction in the levels of primary and secondary lipid peroxidation products. Moreover, it inhibits the Schiff reaction involved in the peroxidation of neutral lipids during the storage phases of preserved donor erythrocytes, as elucidated in research by Belousov., *et al*. in 2021 [23].

Hence, the encouraging outcomes of previous research endeavors served as the primary motivation for further exploring the impact of nanotechnology techniques on the functional capabilities of erythrocytes.

The objective of this investigation is to assess how nanotechnological treatment of resuspension solutions affects the performance of anaerobic glycolysis and hexose monophosphate reactions in stored donor erythrocytes.

To fulfill this objective, the following tasks need to be addressed:

- Quantify the levels of ATP and 2,3-DPG within erythrocytes.
- Examine the activity of glutathione peroxidase (GSH-Px) and monitor alterations in the levels of reduced glutathione (GSH).

Materials and Methods

Improved resuspension solutions were created through the application of standardized magnetite nanoparticles (ICNB) to saline sodium chloride and a 5% glucose solution. This was achieved using Belousov's method, ensuring the complete removal of NPs from the solution. The magnetite NPs were synthesized via the co-precipitation method. ICNB exhibits several fundamental physical and chemical attributes, including: a magnetite NPs colloidal solution concentration in the NaCl physiological solution of 0.0225%; a theoretical osmolality of the colloid solution of 500 mOsm/L; magnetite nanoparticle size ranging from 6 to 12 nm; the total surface area of magnetite nanoparticles (Ss) measuring between 800-1200 m^2/g; a saturation magnetization (Is) of 2.15 kA/m; and a ζ-potential of -19 mV.

Erythrocyte-containing components (ECC) were derived from the peripheral blood of healthy adult donors using the standard CPDA-1 preservative (n=10, with a total of 560 studies). To investigate the influence of resuspension solutions on preserved erythrocytes, a 0.9% NaCl solution and a 5% glucose solution were utilized, both before and after treatment with magnetite nanoparticles (ICNB). The component containing red blood cells (RBCs) was divided into four equal portions. The erythrocytes from the first portion (SC) were resuspended in saline (control). In the second portion (ST), RBCs were resuspended in modified saline (test). The third portion (GC) of RBCs was resuspended in a 5% glucose solution (control). The fourth portion (GT) of RBCs was resuspended in modified 5% glucose solution (test).

The 2,3-DPG content (measured in μmol/ml) in erythrocytes was assessed using Mranov's spectrophotometric technique. This involved determining the 2,3-DPG content by measuring the difference in phosphorus concentration between the filtrate of erythrocyte lysate without protein and lysate adsorbed with activated carbon. Initially, the phosphorus concentration was determined in ash-free erythrocyte hemolysate (total phosphorus), and subsequently, in the lysate following activated charcoal treatment. The phosphorus concentration was determined employing the phosphorus-molybdenum method, as described by Mranova in 1975 [24].

The ATP content (measured in μmol/ml) was determined spectrophotometrically in erythrocyte lysate. ATP present in the samples phosphorylated glucose in the presence of hexokinase. The resulting glucose-6-phosphate served as a substrate for the glucose-6-phosphate dehydrogenase reaction. The quantity of ATP involved in the reaction equaled the amount of NADPH2 generated by the glucose-6-phosphate dehydrogenase reaction, and this was quantified spectrophotometrically at 340 nm, following the procedure described by Eschenko in 1982 [25].

The levels of reduced glutathione (measured in μmol/min per 1 g Hb) and glutathione peroxidase (measured in μmol/min per 1 g Hb) were determined using spectrophotometry based on a color reaction with Ellman's reagent at 412 nm, following the techniques outlined by Moin in 1986 and Severin in 1989 [26,27].

Resuspended red blood cells were kept under hypothermic conditions (at a temperature of 2-6°C) and were examined on days 2, 9, 16, 23, 30, 37, and 44 from the commencement of blood preservation. These time points correspond to different stages, labeled as I, II, III, IV, V, VI, and VII.

The data obtained were subjected to statistical analysis using STATISTICA 6.1 (StatSoft, USA) computer software. To determine the appropriate choice between parametric and nonparametric criteria for dependent or independent groups, the distribution of variables within the groups was evaluated. When the Shapiro-Wilk criterion (W) yielded $p > 0.05$, it indicated a normal distribution. Student's t-test was employed to compare the SC and GC groups with the ST and GT groups. For non-normally distributed data (Shapiro-Wilk criterion $p < 0.05$), the sign and Wilcoxon tests were ap-

plied. In cases where the two criteria yielded different results for the same variable, the Wilcoxon criterion was used for assessment. Comparisons between groups were conducted separately for each stage. If one of the variables being compared followed a normal distribution and the other did not, the nonparametric approach was adopted, and the Wilcoxon criterion was used to evaluate the results.

Results

In the initial phase of the investigation, noticeable indicators of anaerobic glycolysis and the pentose cycle activation were observed in the test conditions, where a modified physiological solution was employed as the resuspension medium for preserved donor erythrocytes. Notably, this activation exhibited a distinctive pattern characterized by a substantial surge in ATP levels along with an elevation in 2,3-DPG, all while witnessing a reduction in glutathione peroxidase (GSH-Px) levels (see Figure 1).

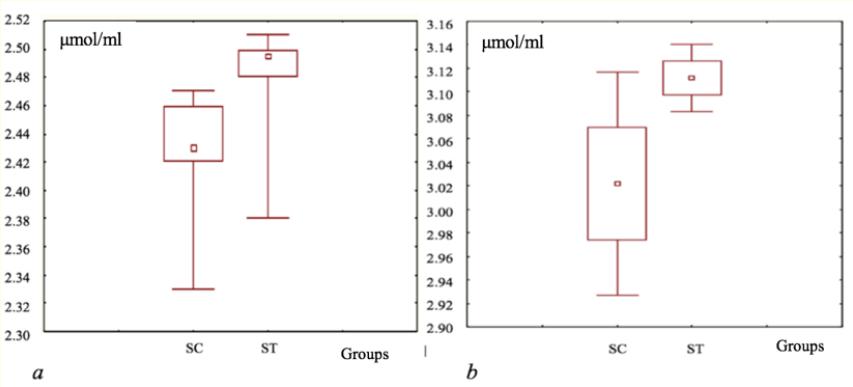

Figure 1: Alterations in the intergroup disparity of median ATP levels (measured in µmol/ml) and mean 2,3-DPG values (measured in µmol/ml) were analyzed in samples of the "Erythrocytes" component during Stage I of the investigation: (a) - The impact of resuspending physiological saline on ATP levels revealed a statistically significant difference (Wilcoxon test p = 0.028). In the graphic representation, a small square represents the median, while the upper and lower boundaries of the rectangle correspond to the 25% and 75% quartiles. The vertical line extends from the minimum to maximum values, based on data from n = 10 samples. (b) - When assessing the effect of resuspending saline on the levels of 2,3-DPG, a significant difference was observed (Student's t-test, p = 0.004). In this visual representation, the small square represents the median, and the upper and lower boundaries of the rectangle symbolize the mean standard deviation. The vertical line extends up to mean ± 1.96 times the standard deviation, based on data from n = 10 samples. The notations used are as follows: SC - saline (control), ST - modified saline (test).

Conversely, in the experimental setup employing a modified glucose solution as the resuspension medium, there was a notable rise in ATP levels, but the increase in 2,3-DPG was negligible. Similar to the observations in the tests involving the physiological solution, the quantity of glutathione peroxidase remained markedly lower than in the control group (refer to Figure 2).

The data gathered during the initial stages of the investigation revealed a noteworthy increase in ATP levels in erythrocytes for both test variants with modified solutions. This surge in ATP levels contributes to the stability of ion transport, maintenance of electrolyte balance, and the proper functioning of erythrocyte ATPase systems.

In instances where an elevation in 2,3-DPG, a critical allosteric regulator of hemoglobin's affinity for oxygen, was documented, the oxyhemoglobin dissociation curve exhibited a rightward shift. This shift potentially enhances the delivery of oxygen to tissues by erythrocytes, as previously indicated by Benesch and Benesch

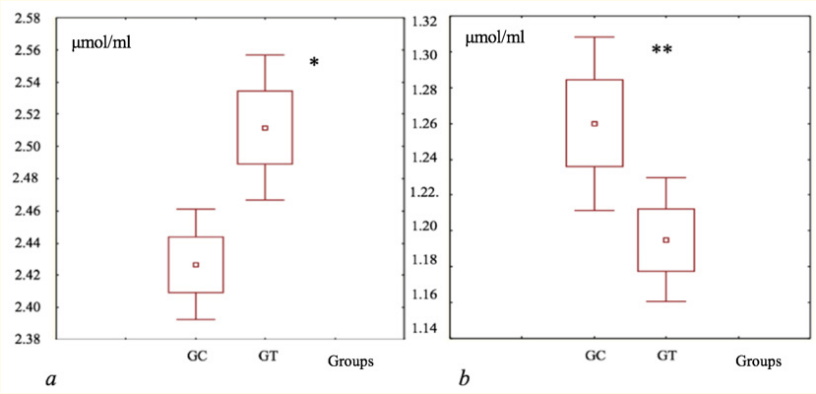

Figure 2: The variations in intergroup disparities of mean ATP levels (measured in μmol/ml) and mean GSH-Px values (measured in μmol/min per 1 g Hb) (b) were assessed in the samples of the "Erythrocytes" component during Stage I of the study: (a) - The influence of resuspending a 5% glucose solution on ATP levels. (b) - The effect of resuspending a 5% glucose solution on the quantity of GSH-Px. The notations used are as follows: GC - 5% glucose solution (control), GT - modified 5% glucose solution (test). Symbols "* - Student's t-test, p = 0.004 x 10-1," and "** - Student's t-test, p = 0.006" denote statistical significance. In the graphical representation, a small square signifies the median, the upper and lower boundaries of the rectangle represent the mean ± standard deviation, and the vertical line extends from mean ± 1.96 times the standard deviation, based on data from n = 10 samples.

(1967) and Brewer (1974) [28,29].

Conversely, when there was a decrease in 2,3-DPG, the oxyhemoglobin dissociation curve shifted to the left, amplifying the oxygen-binding capacity of preserved erythrocyte hemoglobin.

The observed variations in the 2,3-DPG levels across test variants are attributed to the unique primary effector impact of the polarized water environments within the erythrocyte microenvironment on the enzymes responsible for allosteric control.

During Stage II of the study, a brief inhibition of anaerobic glycolysis was evident in the test group with physiological solution in

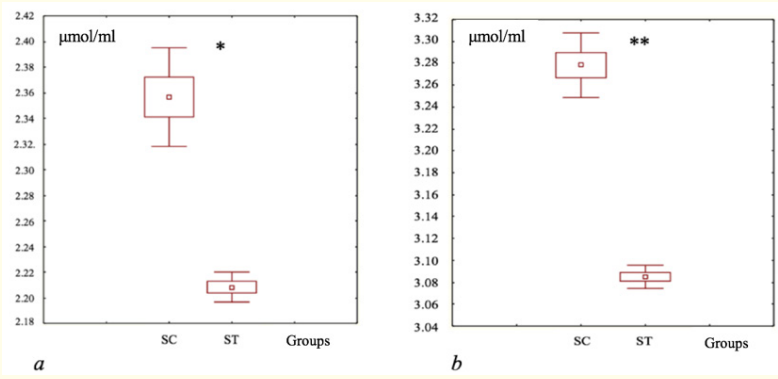

Figure 3: Modifications in the intergroup disparities of mean ATP levels (measured in μmol/ml) and mean 2,3-DPG values (measured in μmol/ml) were assessed within the samples of the "Erythrocytes" component during Stage II of the study: (a) - The impact of resuspending physiological saline on ATP levels. (b) - The effect of resuspending physiological saline on the levels of 2,3-DPG. The notations used in the study include: SC - saline (control), ST - modified saline (test). Symbols "* - Student's t-test, p = 0.001 x 10-1," and "** - Student's t-test, p = 0.0012 x 10-2" indicate statistically significant results. In the graphical representation, a small square signifies the median, the upper and lower boundaries of the rectangle represent the mean ± standard deviation, and the vertical line extends from mean ± 1.96 times the standard deviation, based on data from n = 10 samples.

comparison to the control (see Figure 3).

This was evident through a reduction in ATP and 2,3-DPG levels, occurring alongside the sustained activation of the pentose phosphate cycle (refer to Figure 4). These alterations result from the

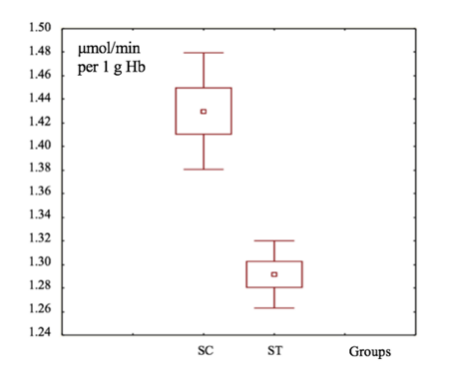

Figure 4: Variations in the intergroup disparities of mean GSH-Px values (measured in µmol/min per 1 g Hb) were analyzed within the samples of the "Erythrocytes" component during Stage II of the study. The notations employed include: SC - saline (control) and ST - modified saline (test). The analysis was conducted using Student's t-test, yielding a p-value of 0.0051 x 10-1. In the graphical representation, a small square designates the median, the upper and lower boundaries of the rectangle represent the mean ± standard deviation, and the vertical line extends from mean ± 1.96 times the standard deviation, based on data from n = 10 samples.

impact of specific inhibition of the Embden-Meyerhof pathway.

In the test scenario with glucose, the activation of anaerobic glycolysis, akin to the initial stage, was characterized by a reduction in 2,3-DPG levels and an increase in ATP (as depicted in Figure 5).

The quantity of reduced glutathione remained notably elevated in comparison to the control, while observing a decrease in glutathione peroxidase levels (refer to Figure 6).

In the subsequent phases of the investigation, there was a marked increase in ATP and reduced glutathione levels, along with a reduction in 2,3-DPG and glutathione peroxidase in all experimental variations compared to the control (as depicted in Figure 7).

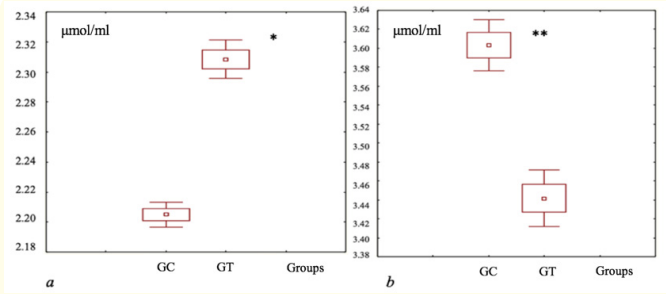

Figure 5: Alterations in the intergroup disparities of mean ATP levels (measured in µmol/ml) and mean 2,3-DPG values (measured in µmol/ml) were examined within the samples of the "Erythrocytes" component during Stage II of the study: (a) - The influence of resuspending a 5% glucose solution on ATP levels. (b) - The effect of resuspending a 5% glucose solution on the levels of 2,3-DPG. The notations used are: GC - 5% glucose solution (control) and GT - modified 5% glucose solution (test). Symbols "* - Student's t-test, p = 0.0014 x10-1," and "** - Student's t-test, p = 0.0014 x10-1" indicate statistical significance. In the graphical representation, a small square signifies the median, the upper and lower boundaries of the rectangle represent the mean ± standard deviation, and the vertical line extends from mean ± 1.96 times the standard deviation, based on data from n = 10 samples.

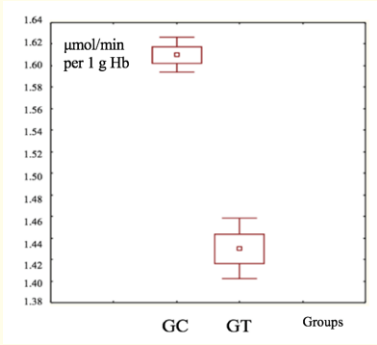

Figure 6: Alterations in the intergroup disparities of mean GSH-Px values (measured in µmol/min per 1 g Hb) were assessed within the samples of the "Erythrocytes" component during Stage II of the study. The notations used are GC - 5% glucose solution (control) and GT - modified 5% glucose solution (test). The analysis was conducted using Student's t-test, yielding a p-value of 0.002 x10-1. In the graphical representation, a small square designates the median, the upper and lower boundaries of the rectangle represent the mean ± standard deviation, and the vertical line extends from mean ± 1.96 times the standard deviation, based on data from n = 10 samples.

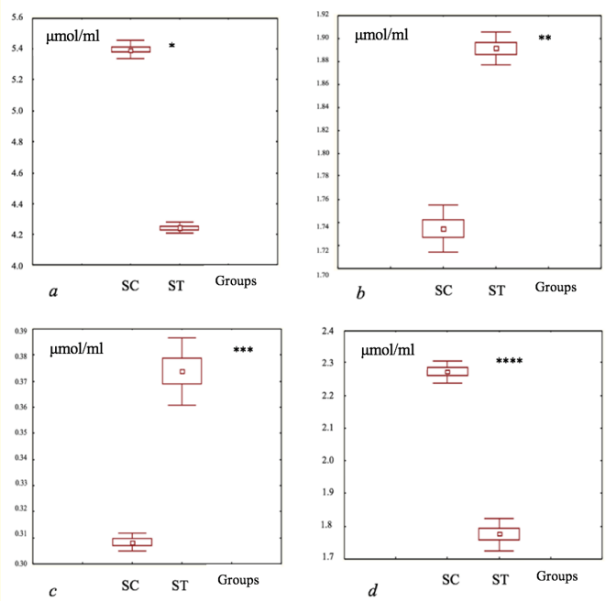

Figure 7: Alterations in the intergroup disparities of mean 2,3-DPG levels (measured in µmol/ml), ATP values (measured in µmol/ml), the range of mean GSH values (measured in µmol/min per 1 g Hb), and mean GSH-Px values (measured in µmol/min per 1 g Hb) were examined within the samples of the "Erythrocytes" component at Stage VII of the study: (a) - The impact of resuspending physiological saline on 2,3-DPG levels. (b) - The effect of resuspending physiological saline on ATP levels. (c) - The influence of resuspending physiological saline on GSH levels. (d) - The effect of resuspending physiological saline on GSH-Px values. The notations employed are SC - saline (control) and ST - modified saline (test). The statistical significance was assessed using Student's t-test, revealing p-values of 0.001 x10-3, 0.007 x10-2, 0.01 x10-2, and 0.001 x10-2 for the respective measurements. In the graphical representation, a small square designates the median, the upper and lower boundaries of the rectangle represent the mean ± standard deviation, and the vertical line extends from mean ± 1.96 times the standard deviation, based on data from n = 10 samples.

Discussion

The analysis of data obtained from the various test scenarios revealed significant differences in the extent of alterations in the examined parameters. In tests using the modified physiological saline, the activation of anaerobic glycolysis was notably less pronounced compared to tests with a glucose solution. Conversely, there was a more pronounced utilization of the pentose-phosphate cycle for glucose oxidation. This divergence is attributed to the absence of glucose in the resuspending solution, which serves as an external energy substrate to initiate the Embden-Meyerhof pathway. In this particular variant, the energy deficit and disrupted metabolism might be expected to reduce the erythrocyte's lifespan. However, in our study, the process of glucose oxidation prevailed through the activation of the pentose cycle, clearly surpassing anaerobic glycolysis.

The fate of pyruvate following the initial ten stages of glycolysis is influenced by the cell's microenvironment [30]. The application of nanotechnological treatments to resuspending solutions alters the state of the erythrocyte microenvironment, resulting in the correction of metabolic processes. This capacity for distinct impacts on metabolic processes introduces novel avenues for enhancing erythrocyte adaptation to changes in the external environment. Advancements in methods to elevate ATP levels in erythrocytes, which encompass the use of allosteric effectors and specialized solutions tailored to erythrocyte and transfusate metabolites, represent the primary focus of optimizing and adjusting erythrocyte function [31]. In our study, the modification of water's polarization structure activates a cascade of enzyme systems responsible for G-6PDH dehydrogenation. This, in turn, governs the adaptive reconfiguration of metabolic processes, primarily oriented toward preserving the structural integrity of erythrocyte membranes. This concept contributes to the development of innovative approaches for controlling metabolic processes in erythrocytes.

Glucose-6-phosphate dehydrogenase deficiency is recognized for its capacity to diminish the erythrocytes' resistance against oxidative stress [32]. Ion balance within erythrocytes is governed by energy-dependent mechanisms, which are, in turn, contingent on redox and energy metabolism. To counteract oxidative stress and minimize oxygen consumption, mature RBCs eliminate their mitochondria and bolster their antioxidant systems to specifically uphold the reduction state of hemoglobin iron, even in the presence of elevated oxygen levels [33]. Furthermore, storing erythrocytes under hypothermic conditions has an adverse impact on proton pumps and disrupts the regulation of ion homeostasis.

The acquired data enhance our comprehension of the mechanisms underpinning the membrane-protective effects of nanotech-

nologically modified resuspending solutions. This enhancement primarily results from an augmentation in the levels of high-energy molecules, such as ATP, and the activation of the pentose-phosphate pathway for glucose metabolism, leading to the generation of reduced nicotinamide adenine dinucleotide phosphate (NADPH2) and the conversion of oxidized glutathione into its reduced form. The hexose monophosphate pathway serves as the exclusive route for regenerating nicotinamide adenine dinucleotide phosphate (NADPH), which is essential for fueling the thiol-based antioxidant system crucial for maintaining cellular homeostasis. In oxygenated erythrocytes, the preference is for the hexose monophosphate pathway due to its ability to generate NADPH through interactions within the Embden–Meyerhof pathway, providing the necessary reducing equivalents for antioxidant systems [34]. In our particular case, the significant reduction in glutathione peroxidase levels and the simultaneous increase in reduced glutathione within the erythrocytes signify the transition of the antioxidant system into an active state, effectively safeguarding erythrocyte membrane lipids from free-radical oxidation [22].

Water possesses dielectric properties and can exhibit electric polarization, which reduces both its configurational and vibrational entropy. Experiments involving the transfer of quantum states have demonstrated that alterations in the electric polarization of water lead to an increase in the catalytic activity of an intracellular enzyme, enolase. Enolase plays a pivotal role in regulating the glycolysis pathway [35]. Magnetite nanoparticles induce a sustained magnetic field, which, in turn, influences the mobility and orientation of hydrogen protons within resuspending solutions. Importantly, magnetic nanoparticles have demonstrated a high level of biocompatibility with cell membranes [36]. The introduction of nanotechnologically treated solutions into preserved donor erythrocytes results in the polarization of the aqueous microenvironment sector due to Van der Waals forces. This polarization, in turn, triggers the hydrolysis of phosphate ATP residues, thereby enhancing the energy supply to erythrocytes. Additionally, it leads to a shift towards an active state of anaerobic glycolysis and the pentose phosphate cycle, thereby modulating intracellular enzyme activity and altering transmembrane exchange and metabolism. These changes ultimately translate into improved preservation and enhanced functional capacity of erythrocytes stored at temperatures ranging from 2 to 6°C.

Conclusion

The findings obtained have opened up new possibilities for the biological utilization of magnetic nanoparticles in various biointerfaces and within blood suspensions.

Influencing the activity of anaerobic glycolysis and the hexose monophosphate reaction in preserved donor erythrocytes was achieved through the application of nanotechnological treatment to the resuspending solution. This resulted in a consistent increase in ATP and reduced glutathione, coupled with reductions in 2,3-DPG and glutathione peroxidase. These cumulative effects stem from the use of modified resuspending solutions on preserved erythrocytes during storage at temperatures ranging from 2 to 6°C. Notably, it was observed that the activation of anaerobic glycolysis was less pronounced in tests involving modified physiological saline compared to those with glucose solutions. Conversely, the pen-

Bibliography

1. Turrens J F. "Mitochondrial formation of reactive oxygen species". *The Journal of Physiology* 552.2 (2003): 335-344.

2. Hayyan M., *et al*. "Superoxide ion: generation and chemical implications". *Chemical Reviews* 116.5 (2016): 3029-3085.

3. Devasagayam TPA., *et al*. "Free radicals and antioxidants in human health: current status and future prospects". *The Journal of the Association of Physicians of India* 52 (2004): 794-804.

4. Waszczak C., *et al*. "Reactive oxygen species in plant signaling". *Annual Review of Plant Biology* 69 (2018): 209-236.

5. Jacobasch G., *et al*. "Metabolism of the hexose monophosphate shunt in glucose-6-phosphate dehydrogenase deficiency and closely interrelated reactions". *Haematologia (Budap)* 15.4 (1982): 401-407.

6. Guitton J., *et al*. "Hexose monophosphate shunt activities in human erythrocytes during oxidative damage induced by hydrogen peroxide". *Archive Toxicology* 77.7 (2003): 410-417.

7. Pastore A., *et al*. "Determination of blood total, reduced, and oxidized glutathione in pediatric subjects". *Clinical Chemistry* 47.8 (2001): 1467-1469.

8. Pompella A., *et al*. "The changing faces of glutathione, a cellular protagonist". *Biochemical Pharmacology* 66.8 (2003): 1499-1503.

9. Novello F and McLean P. "The pentose phosphate pathway of glucose metabolism. Measurement of the non-oxidative reactions of the cycle". *Biochemical Journal* 107.6 (1968): 775-791.

10. Lu Shelly C. "Glutathione synthesis". *Biochimica et Biophysica Acta (BBA) - General Subjects* 1830.5 (2013): 3143-3153.

11. Halprin K M and Ohkawara A. "The measurement of glutathione in human epidermis using glutathione reductase". *The Journal of Investigative Dermatology* 48.2 (1967): 149-152.

12. Murakami K., *et al*. "Impairment of glutathione metabolism in erythrocytes from patients with diabetes mellitus". *Metabolism Clinical and Experimental* 38.8 (1989): 753-758.

13. Scholz R W., *et al*. "Mechanism of interaction of vitamin E and glutathione in the protection against membrane lipid peroxidation". *Annals of the New York Academy of Sciences* 570.1 (1989): 514-517.

14. Mills B J., *et al*. "Glutathione disulfide variability in normal human blood". *Analytical Biochemistry* 222.1 (1994): 95-101.

15. Richie Jr J P., *et al*. "Long-term stability of blood glutathione and cysteine in humans". *Clinical Chemistry* 42.7 (2021): 1100-1105.

16. Schafer FQ and Buettner GR. "Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple". *Free Radical Biology and Medicine* 30.11 (2001): 1191-1212.

17. van 't Erve T J., *et al*. "The concentration of glutathione in human erythrocytes is a heritable trait". *Free Radical Biology and Medicine* 65 (2013): 742-749.

18. Belousov A N. "The use of magnetite nanoparticles in applied medicine". *Materials Science Forum* 694 (2011): 205-208.

19. Belousov AN. "Myth and reality application of magnetite nanoparticles as selective contrasting means of the malignant tumors in MRI investigation". *Biomedical Engineering Research* 2.3 (2013): 147-152.

20. Belousov A N. "The role of magnetite nanoparticles (ICNB) in discovery new factor which influence on permeability of erythrocytes and eryptosis". *Open Access Library Journal* 1 (2014): e1055.

21. Belousov AN., *et al*. "Application of the standardized form magnetite nanoparticles (ICNB) in creature simple and practical method of additive modernization of preservation solutions for red blood cells". *Global Journal of Anesthesia and Pain Medicine* 1.1 (2018).

22. Belousov A., *et al*. "Innovative method of nanotechnology to increase the storage time of RBCs due by stabilizing the molecular structure of proteins and lipids of erythrocyte membranes". *Biomedical Journal of Scientific and Technical Research* 13.4 (2019): 10079-10087.

23. Belousov A., *et al*. "Study of effects a new resuspending solution which was nanotechnologically upgraded on lipoperoxidation, catalase activity and red blood cell peroxidation resistance in donor blood components". *Archives in Biomedical Engineering and Biotechnology* 6.2 (2021): 1-6.

24. Mranova I S. "Determination of 2,3DPG and ATP in red blood cells". *Laboratory Business* 7 (1975): 652-654.

25. Eschenko ND., *et al*. "Methods of biochemical studies". Sankt-Petersburg National University, Leningrad (1982).

26. Moin VM. "A simple and specific method for determining glutathione peroxidase activity in erythrocytes". *Lab Delo* 12 (1986): 724-727.

27. Severin S E. "Practical work on biochemistry: Textbook". Allowance. Severin, S. E., Solovyov, G. A., Eds., MGU, Moscow (1989).

28. Benesch R and Benesch R E. "The effect of organic phosphates from the human erythrocyte on the allosteric properties of hemoglobin". *Biochemical and Biophysical Research Communications* 26.2 (1967): 162-167.

29. Brewer G J. "2,3-DPG and erythrocyte oxygen affinity". *Annual Review of Medicine* 25 (1974): 29-38.

30. Melkonian E A and Schury MP. "Biochemistry, anaerobic glycolysis" (2022).

31. McMahon T J., *et al*. "Generation and export of red blood cell ATP in health and disease". *Frontiers in Physiology, Sec. Red Blood Cell Physiology* 5.12 (2021): 754638.

32. Francis R O., *et al*. "Donor glucose-6-phosphate dehydrogenase deficiency decreases blood quality for transfusion". *Journal Clinical Investigation* 130.5 (2020): 2270-2285.

33. Yoshida T., *et al*. "Red blood cell storage lesion: causes and potential clinical consequences". *Blood Transfusion* 17.1 (2019): 27-52.

34. Timothy J McMahon., *et al*. "Generation and Export of Red Blood Cell ATP in Health and Disease". *Frontiers in Physiology, Sec. Red Blood Cell Physiology* 5.12 (2021): 754638.

35. Pietkiewicz J., *et al*. "Influence of water polarization caused by phonon resonance on catalytic activity of enolase". *Journal of the American Chemical Society* 6 (2021): 4255-4261.

36. Agarwal V., *et al*. "Hemolytic response of iron oxide magnetic nanoparticles at the interface and in bulk: extraction of blood cells by magnetic nanoparticles". *ACS Applied Materials and Interfaces* 14.5 (2022): 6428-6441.