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Review

ERYTHROCYTE DISTRIBUTION BY SURFACE CHARGE: BIOPHYSICAL CHARACTERISTICS OF HETEROGENEITY, DIAGNOSTIC SIGNIFICANCE AND APPLICATION IN TRANSFUSIONOLOGY AND BIOBANKING

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Long-term storage of erythrocytes, a vital component of modern transfusion medicine, is accompanied by structural, metabolic, and biophysical alterations that reduce their functional activity. Differentiating erythrocytes by surface charge represents a promising approach for assessing viability, predicting post-transfusion behavior, and optimizing storage and biobanking strategies. However, the practical application of zeta potential analysis in clinical transfusion medicine remains insufficiently explored.

To summarize current knowledge on erythrocyte heterogeneity by surface charge, their biophysical properties, diagnostic value, and application prospects in transfusion medicine and biobanking.

A systematic literature search was conducted using PubMed, Scopus, Web of Science, Cochrane Library, Google Scholar, and Clinical Key, focusing on studies addressing erythrocyte biophysics, surface charge parameters, population heterogeneity, zeta potential measurement methods, and clinical applications.

Erythrocyte heterogeneity by surface charge is a fundamental population property shaped by physiological aging, oxidative stress, and membrane–cytoskeleton modifications. Reduced zeta potential is associated with enhanced aggregation, impaired deformability, and decreased microvascular passage. Analysis of zeta potential enables identification of subpopulations with varying levels of damage and prediction of their functional performance after transfusion. In transfusion medicine, this approach may improve transfusion efficiency and safety, while in biobanking it offers opportunities for better selection of cells for long-term storage. Surface charge differentiation also holds promise for predicting erythrocyte shelf life and advancing personalized transfusion strategies.

Integration of zeta potential analysis into laboratory practice could enhance the quality of blood components and support the development of personalized transfusion medicine.

Keywords: erythrocytes; zeta potential; surface charge; biophysics; transfusion; biobanking.

Introduction

Transfusion of packed red blood cells (pRBC) is one of the most important components of modern transfusion therapy and often saves the lives of patients in critical conditions. Every year, millions of recipients worldwide require blood transfusion for acute blood loss, chronic anemia, surgical interventions and other clinical situations that require the availability of effectively stored blood components. Ensuring the availability of pRBC is a complex task that involves multi-stage logistics, including donor blood collection, processing, biopreservation and transportation. According to current international recommendations, pRBC can be stored at 1–6°C under refrigerated conditions for up to 35–42 days [1, 2]. However, recent studies [2] indicate that erythrocytes stored for a long time undergo significant changes at the molecular, biochemical, morphological and functional levels compared to freshly collected cells. In particular, during storage, erythrocytes gradually accumulate such structural and metabolic disruptions as: oxidative membrane damage, degradation of cytoskeletal proteins, changes in rheological properties, decreased cell deformability, and impaired gas exchange [3, 4]. In addition, morphological changes in cells are observed - the formation of echinocytes, spherocytes, and microspherocytes, caused by impaired protein-lipid interactions in the plasma membranes of erythrocytes [5, 6]. Such modifications, combined under the term "storage damage," are actively studied in modern literature, but their clinical significance and impact on the outcome of transfusion therapy still remain a subject of debate in the scientific community [7, 1].

Of particular interest are the results of studies indicating that older erythrocytes, especially those stored for more than 35 days [8], have an increased susceptibility to intravascular and extravascular he-

molysis. This is due to their sequestration in the spleen and active erythrophagocytosis after transfusion [5]. Hemolysis leads to the release of free heme and iron into the plasma, which has a potentially toxic effect on the recipient's body. Free heme and iron can induce oxidative stress, stimulate the production of pro-inflammatory cytokines and affect the function of tissue macrophages [9, 10]. In addition, they create favorable conditions for the growth of siderophilic bacteria, which increases the risk of bacterial complications in the post-transfusion period [1, 11–13]. Thus, the accumulation of toxic erythrocyte degradation products and activation of inflammatory cascades may have negative clinical consequences, especially in vulnerable patient groups, such as patients with severe infectious pathology, sepsis, or immunosuppression.

It has been established that changes in pRBC during storage can occur under the influence of factors such as the addition of special preservative solutions, irradiation and other processing methods [14]. Non-genetic factors, environmental influences on donors, as well as a set of factors, the so-called “exposome”, influence the formation of the molecular phenotype of stored erythrocytes [15, 19], their resistance to hemolysis and clinical efficacy after transfusion. Taking into account the influencing factors should improve the practice of storing blood components.

Heterogeneity of the erythrocyte population is a fundamental biological phenomenon, reflecting a complex set of morphological, biochemical, biophysical and functional differences between individual cells within the same organism. Erythrocytes circulating in human peripheral blood demonstrate significant variability in such parameters as cell age, degree of dehydration or hydration, hemoglobin (Hb) concentration, level of metabolic activity, deformability, membrane charge, cytoskeletal stability. This diversity is formed throughout the life cycle of the erythrocyte - from the moment of its release from the bone marrow to its final elimination through the mechanisms of erythrophagocytosis in the spleen or liver. Physiological aging of cells is accompanied by the accumulation of biochemical damage, changes in the composition of membrane lipids, oxidation of cytoskeletal proteins, activation of the proteolysis system and impaired ion exchange, which leads to a gradual loss of functional properties of erythrocytes. These processes are additionally influenced by microenvironmental factors, including changes in plasma composition, the presence of pro-inflammatory mediators, hypoxia, as well as individual characteristics of the organism, in particular genetic, gender, age and concomitant diseases [16].

Understanding the nature of the heterogeneity of the red blood cell population is key to developing and improving methods for storing blood components. In biobanking practice, where special attention is paid to long-term storage of cellular products, studying the characteristics of different red blood cell subpopulations allows optimizing cryopreservation conditions, reducing the proportion of damaged cells in the concentrate, and developing more precise criteria for selecting high-quality donor products [17]. In particular, assessing the morphological and metabolic profile of red blood cells can become the basis for creating standardized markers of blood quality, which will improve the safety and effectiveness of transfusion therapy.

The **aim of the work** is to summarize current data on the distribution of erythrocytes by surface charge, their biophysical properties, diagnostic value, and possibilities of application in transfusion medicine and biobanking.

Materials and Methods

A search for scientific publications was carried out in international databases PubMed (https://pubmed.ncbi.nlm.nih.gov/), Clinical Key Elsevier (https://www.clinicalkey.com/), Cochrane Library (https://www.cochranelibrary.com/), eBook Business Collection (https://www.ebsco.com/) and Google Scholar (https://scholar.google.com/). The paper analyzed modern studies that highlight the biophysical properties of erythrocytes, the heterogeneity of their populations, methods for determining the surface charge of cells, the diagnostic value of these parameters, as well as their practical application in transfusion medicine, biobanking, and quality assurance of blood components.

At the first stage, a literature search was performed using the following keywords: "red blood cells", "erythrocyte heterogeneity", "cell surface charge", "zeta potential", "erythrocyte biophysics", "cryopreservation", "storage lesion", "transfusion safety", "erythrocyte quality control", "biobanking of blood products", "oxidative stress", "erythrocyte deformability", "post-transfusion recovery".

In the second stage, articles were selected for relevance based on the analysis of titles and abstracts. Publications that did not correspond to the research topic or had insufficient methodological quality were excluded. In the third stage, the full texts of the selected articles were studied to assess their compliance with the inclusion criteria and scientific reliability of the data.

The criteria for inclusion of publications for content analysis were as follows:

1. Coverage of the biophysical characteristics of erythrocytes, in particular surface charge parameters, population heterogeneity, morphological and functional changes during storage, as well as their clinical significance in transfusion medicine and biobanking.

2. Compliance of studies with the principles of evidence-based medicine.

3. Availability of full-text access to the publication.

The quality of pRBC is critically important for the safety and effectiveness of transfusion therapy. Current studies confirm that the heterogeneity of the erythrocyte population, in particular the distribution by surface charge, significantly affects their biophysical properties, storage capacity and functional recovery after transfusion. Analysis of the zeta potential of cells opens up new opportunities for assessing the quality of blood components, predicting their effectiveness and improving biobanking practices. The study of these aspects is an important step for the development of personalized approaches in transfusion medicine and cryopreservation of cellular blood products.

1. Biophysical charactristics of erythrocytes; the role of the surface charge

Erythrocytes are the most numerous cell population in the human body, constituting approximately 25% of the 30×10^{12} of all cells [1, 20, 21]. A mature erythrocyte contains about 250–270 million Hb molecules per cell, which provides the ability to transport approximately 1 billion oxygen molecules per erythrocyte [22]. Typical values of key biophysical characterisitics of human red blood cell are given in the Table 1.

The functional activity of erythrocytes, in particular the ability of Hb to bind and release oxygen, is regulated by a complex of metabolic and biochemical mechanisms. One of the main mechanisms is allosteric control through the binding of small molecular weight effectors, such as 2,3-diphosphoglycerate (DPG) and adenosine triphosphate (ATP), which reduce the affinity of Hb for oxygen and promote its release in tissues [23]. These modulatory molecules function as sensitive indicators of the metabolic state of the cell, responding to changes in energy levels, pH, partial pressure of oxygen, and other factors that reflect the body's oxygenation needs.

Nowadays multidisciplinary research based on the integration of omics technologies – including proteomics, metabolomics and lipidomics – with microfluidic approaches and kinetic measurements at the level of individual cells has significantly expanded the understanding of the regulation of Hb functional activity. The use of microfluidic devices for high-precision analysis of oxygen transport kinetics at the level of single erythrocytes allows to detect subpopulation differences in cell properties that were inaccessible to classical analysis methods. Recently, it has been established that in addition to well-known regulators such as DPG and ATP, indirect metabolic modulators play an important role in modulating the ability of Hb to bind and release oxygen, among which adenosine, sphingosine-1-phosphate (S1P), as well as a number of other bioactive lipids are particularly distinguished [14, 24, 25]. Their role in the regulation of oxygenation has been confirmed in studies on high-altitude hypoxia models, where a relationship between the levels of these molecules and the body's adaptive responses to reduced partial pressure of oxygen was demonstrated [26–28]. Moreover, changes in the erythrocyte metabolome, including the accumulation of adenosine, S1P and other metabolites, have been found to correlate with cellular aging processes, their ability to survive storage and the effectiveness of post-transfusion recovery [19, 29].

The complex organization of the cytoskeleton and membrane apparatus of erythrocytes is the result of an evolutionarily developed regulatory system that ensures the long-term preservation of the cell's mechanical integrity, flexibility and viability during circulation. Disruption of these mechanisms, including structural abnormalities of cytoskeletal proteins, dysfunction of ion channels or changes in the lipid composition of the membrane, can lead to the development of a number of pathological conditions, such as hereditary membrane pathologies (e.g., spherocytosis, elliptocytosis), increased fragility of cells during blood storage or increased susceptibility to hemolysis due to the influence of external stress factors.

The zeta potential (ζ-potential or ZP) is one of the key electrochemical characteristics that determine the surface properties of cellular structures, including

Table 1: Average values of key biophysical parameters of human erythrocytes

Parameter	Average value	Commentary
Cell diameter	≈ 7.5–8.5 mm	Typical value for mature human erythrocyte
Cell thickness	≈ 1.7–2.5 mm	Native shape of erythrocyte is a biconcave disc, the cell thickness is smaller in the central part, and larger on the periphery
Cell surface area	≈ 120–160 mm^2	If cell is modelled as a disc
Cell volume (MCV)	≈ 80–100 fl	Mean corpuscular volume
Zeta-potential	from –10 to –20 mV	Depending on the method and surrounding medium condition
Membrane tension	≈ 6–9 mN/m	Depends on the cytoskeletal proteins and membrane composition
lifespan within the human body	≈ 100–120 day	Physiological term of life of a red blood cell in the circulation

erythrocytes. It is formed due to the presence of an electric charge of molecules on the cell surface and their interaction with environmental ions [30, 31].

The value of ZP depends on the physicochemical parameters of the medium, such as pH, ionic strength, temperature, and the presence of certain ions or molecules that can interact with the cell membrane. A decrease in the absolute value of ZP can lead to increased intercellular interactions, aggregation, and the formation of microvascular obstructions, while a high negative charge promotes suspension stability and prevents cell aggregation [30, 31].

2. Methods for determining the surface charge of erythrocytes

The electrical properties of cells are a multifaceted phenomenon that manifests itself in various forms and is of fundamental importance for the physiological and pathological activity of the cell. The most studied parameter is the cell membrane potential (Vm), which is formed as a result of the asymmetric distribution of ions between the intracellular and extracellular environments. However, in addition to Vm, there are other important electrical characteristics of cells, including the electrical conductivity of the membrane, the membrane capacitance, the conductivity of the cytoplasm, as well as the charge localized on the cell surface, which forms an electric double layer and determines the extracellular electric potential, known as the zeta potential (ζ-potential).

The relationship between Vm and the ζ-potential has important biological consequences. In particular, changes in the electrical potential at the shear boundary can affect the ability of cells to adhere to proteins, including immunoglobulins and other inflammatory mediators, as well as intercellular interactions in the tissue microenvironment. In addition, modification of the electrostatic profile of the cell can regulate the transport of ions across the membrane, which is important for maintaining homeostasis and adapting cells to changes in the external environment [32].

The assessment of the zeta potential (ζ-potential) of erythrocytes is an important tool for studying their biophysical properties, population heterogeneity and interactions with the environment. For this purpose, various methods are used, based on the principles of electrophoresis, optical capture, laser Doppler microscopy, light scattering, impedancemetry and microfluidic technologies.

Electrophoresis and microelectrophoresis are classical methods for determining the ζ-potential. Their principle is based on the observation of the speed of movement of charged cells in a liquid medium under the action of an electric field. The electrophoretic mobility of the cell, measured by microscopic or automated tracking, is used to calculate the ζ-potential according to the Helmholtz-Smoluchowski or Henry equations. These methods are highly sensitive, affordable, and allow the study of both entire cell populations and individual cells in prepared suspensions. However, electrophoresis has limitations, including sensitivity to experimental conditions such as pH, ionic strength, temperature, and the influence of aggregates or contaminants in the sample. In addition, the method is difficult to apply to cells in physiological environments, where cell movement can be affected by additional factors, including plasma proteins [43].

Optical tweezers and laser Doppler microscopy are modern methods that allow the analysis of single cells with high precision. Optical tweezers use focused laser radiation to hold and manipulate individual cells in three-dimensional space, which allows studying their electrophysical properties without contact with other objects. Laser Doppler microscopy, in turn, allows determining the speed of cell movement based on the frequency shift of light reflected from particles moving in the field. These methods have high spatial and temporal resolution, allow studying even small changes in surface charge and obtaining data on individual cells or micropopulations. At the same time, they require specialized equipment, high operator skills and have limitations that make their use for screening studies difficult [34].

Light scattering, impedancemetry and microfluidic platforms open up new possibilities for the analysis of the ζ-potential. Light scattering (dynamic or static) allows us to assess the electrokinetic properties of cell suspensions based on changes in the intensity or angle of light scattering passing through the sample. Impedancemetry is based on measuring changes in the electrical impedance of a cell suspension at different frequencies of an electric current, which allows us to obtain information about the conductivity and capacitance of the cell membrane, as well as indirectly about the surface charge. Microfluidic platforms, thanks to the integration of microscopic channels and electrodes, allow us to perform high-precision measurements in small volumes, study cells under conditions close to physiological, and perform parallel analysis of a large number of samples. These methods are characterized by high sensitivity, but require standardization of protocols and adaptation to the specifics of cell models [35].

The challenges of standardization and comparability of results remain a pressing problem for all methods of studying the ζ-potential. The value of the ζ-potential is extremely sensitive to experimental conditions – buffer type, temperature, pH, ionic strength, cell concentration, experimental time and even the geometry of the cuvette or microfluidic channel. The lack of uniform protocols for measurements makes it difficult to compare results between different laboratories and reduces data reproducibility. To overcome these limitations, it is necessary to develop standardized methods, validate new platforms and create generally

accepted control samples [31].

3. Heterogeneity of the erythrocyte population by surface charge

Red blood cell aging is a complex multifactorial process that manifests itself as an accumulation of structural, metabolic and functional changes that gradually reduce cell viability. This process occurs both in conditions of physiological aging of cells in the body, and during the development of pathological conditions or storage of red blood cell concentrates for transfusion therapy.

Normally, the life cycle of red blood cells in human circulation is about 100–120 days, during which cells are exposed to mechanical stress, oxidative stress and changes in the microenvironment. Red blood cell aging is accompanied by a gradual loss of water, which leads to their dehydration and an increase in density. The concentration of 2,3-bisphosphoglyceric acid, an important regulator of Hb affinity for oxygen, decreases, as does the level of ATP, which affects the energy status of the cell and the stability of the membrane cytoskeleton. In addition, there is a loss of intracellular Hb, proteins, and the formation of vesicles – microscopic membrane fragments that are unlaced during microvesiculation, which leads to a decrease in the cell surface area and changes in its morphology [36].

One of the important consequences of aging is **the decrease in the total negative charge of the cell membrane**, which is manifested in a decrease in the zeta potential. This, in turn, weakens the electrostatic repulsion between cells, contributing to their aggregation, increasing blood viscosity and the risk of microvascular obstruction. The decrease in surface charge also affects the interaction of erythrocytes with endothelial cells, the immune system and plasma components, which is important for the development of immunological reactions and transfusion complications.

Particular attention is drawn to the changes that occur during the storage of erythrocytes in the form of preserved blood. According to modern research, in stored erythrocytes, the degradation of metabolic reserves progresses, the pH of the environment decreases, pro-inflammatory cytokines, oxidative stress products and other biologically active compounds accumulate [1–7]. These changes may be of clinical significance, as they reduce cell viability after transfusion and increase the risk of complications in recipients, especially in patients with severe infectious or immune pathologies [36].

Oxidative stress in erythrocytes arises from an imbalance between the formation of reactive oxygen species (ROS) and the efficiency of antioxidant systems, and the Fenton and Haber-Weiss reactions, which provide the generation of highly reactive radicals, play a key role in this process. In the Fenton reaction, the iron ion (Fe^{2+}), which is part of the heme or is present in free form, catalyzes the cleavage of hydrogen peroxide (H_2O_2) with the formation of the hydroxyl radical (·OH), one of the most aggressive oxidants that damages membrane lipids, cytoskeletal proteins and residual DNA fragments, contributing to hemolysis. The Haber-Weiss reaction involves the superoxide-dependent formation of ·OH, which occurs with the participation of the superoxide anion radical ($O_2\cdot^-$), which is formed during the autooxidation of hemoglobin (Hb → MetHb), and H_2O_2; In the first phase, superoxide reduces Fe^{3+} to Fe^{2+}, and then the Fenton reaction is triggered. The formed radicals ·OH and $O_2\cdot^-$ initiate a cascade of damage: peroxidation of membrane lipids with a decrease in elasticity and the formation of malondialdehyde (MDA), oxidation of proteins with aggregation of spectrin and actin, which leads to a loss of cell deformability. Additionally, depletion of antioxidant systems (glutathione, catalase and superoxide dismutase activity) increases oxidative damage and shortens the lifespan of erythrocytes.

A decrease in the electrophoretic mobility (EPM) of erythrocytes, for example, under the enzymatic action of neuraminidase, indicates the loss of surface N-acetylneuraminic acids (NANA) by cells, important components of the glycocalyx that carry a negative charge [37]. A decrease in the content of NANA directly leads to a decrease in the surface charge density (σ) and, consequently, to a decrease in the ζ-potential. These changes are an indicator of a violation of the electrostatic stability of the cell and potentially affect its functional activity, in particular the ability to intercellular interactions, adhesion and deformation.

The electrical charge of the cell, formed by the carboxyl groups of NANA and other ionized membrane groups, is one of the key physicochemical regulators of intercellular interactions. It has been established that the ζ-potential, measured under conditions as close to physiological as possible without aggressive treatment of cells, is an informative tool for studying membrane changes and monitoring the cellular state in real time [36].

Given the growing interest in the problems of erythrocyte aging *in vivo*, in storage systems in plastic containers, as well as in the context of cell damage during preservation [38–40], it is necessary to find out whether cell aging really leads to changes in the glycocalyx, a decrease in surface charge density and ζ-potential. The study of these issues is of key importance for understanding the mechanisms of erythrocyte aging, optimizing methods for storing blood components, and developing approaches to reduce the risk of transfusion complications.

Storage of erythrocyte mass is accompanied by a number of molecular and structural changes that affect the properties of cells, in particular their surface charge. During storage, membrane structures gradually degrade – the integrity of the phospholipid bilayer is lost, the content of sialic acids in the glycocalyx

decreases, and oxidative damage to cytoskeletal proteins, such as spectrin, ankyrin, and band 3 protein, increases. These changes lead to a decrease in the surface charge density, which is expressed in a decrease in the zeta potential (ζ). A decrease in the ζ-potential is associated with increased aggregation ability of cells, a decrease in their deformability, and the ability to pass through narrow capillaries, which is critically important for effective oxygen transport after transfusion. Furthermore, the accumulation of vesicles and the formation of microparticles in the supernatant of stored blood is also accompanied by the loss of negative charge, which may contribute to the activation of the recipient's immune system and increase the risk of transfusion reactions [41–44].

Pathological conditions accompanied by systemic or local changes in the microenvironment significantly affect the electrostatic properties of erythrocytes. In cases of inflammation, the level of pro-inflammatory cytokines (IL-6, TNF-α, IL-1β), oxidative agents and enzymes that can modify membrane proteins and lipids increases, leading to the loss of sialic acids and a decrease in the ζ-potential. This contributes to hyperaggregation of erythrocytes, increased interaction with the endothelium and an increased risk of microvascular obstruction. In anemias, in particular hemolytic, there is an increased load on the erythrocyte pool, which leads to the appearance of a larger number of young cells (reticulocytes) with a higher ζ-potential and lower aggregation ability. While aging cells demonstrate a decrease in charge and an increased tendency to hemolysis. In metabolic disorders such as diabetes or metabolic syndrome, elevated levels of glucose and its oxidation products (AGEs) alter the glycocalyx, reduce membrane charge, and disrupt blood rheology. In oncopathology, complex changes are observed: both a general decrease in ζ-potential due to chronic inflammation and oxidative stress, and an increased aggregation capacity of cells, which may contribute to the formation of microthrombi in the bloodstream of cancer patients [17, 18].

Therapeutic interventions can significantly alter the electrostatic profile of erythrocytes. pRBC may introduce a population of cells with altered ζ-potential into the bloodstream, especially when blood with a long shelf life is used. This leads to the potential mixing of cells with different electrostatic characteristics, which may affect aggregation, microcirculation, and the risk of post-transfusion complications.

Splenectomy, by removing the main organ for sequestering old and damaged erythrocytes, alters the dynamics of circulating cells with low ζ-potential, which may remain in the bloodstream longer than normal. This increases the risk of accumulation of degenerative cells and worsens the rheological properties of the blood.

Drug therapy, especially drugs that affect the redox balance (antioxidants, glucocorticoids, cytostatics) or agents that modify metabolism (e.g. enzyme inhibitors, anti-inflammatory drugs), can also affect ζ-potential by altering membrane composition, the degree of oxidative damage, and the expression of membrane proteins [53].

Thus, changes in the surface charge of erythrocytes are a dynamic marker of their functional state, sensitive to external and internal factors. Understanding these processes is critical for optimizing transfusion therapy, monitoring the condition of patients with chronic diseases, and improving treatment outcomes in clinical practice. In the Table 2 we summarise different factors affecting surface charge heterogeneity in erythrocytes.

Table 2: Sourses of the red blood cell surfase charge geterogenity

Factors	Putative mechanism / Example
Membrane composition	Variations in the content of sialic acids of glycoproteins (glycophorin A, B) Changes in the composition of phospholipids (phosphatidylserine, phosphatidylethanolamine) Oxidative modification of membrane proteins
Cell Metabolic Status	ATP depletion and changes in the ATP/ADP ratio Decreased activity of ion pumps (Na^+/K^+-ATPase, Ca^{2+}-ATPase) Disruption of antioxidant systems (glutathione, catalase, superoxide dismutase)
RBC Age	Accumulation of lipid peroxidation products Decreased sialylation of membrane proteins Formation of microvesicles and membrane fragmentation
External Factors	Action of pro-inflammatory cytokines (TNF-α, IL-1β) Hypoxia, acidosis Effect of calcium and metal ions (Fe^{2+}, Cu^{2+}) Pharmacological agents (nonsteroidal anti-inflammatory drugs, cytostatics)
Pathologocal Conditions	Anemias of various genesis (iron deficiency, sickle cell) Diabetes mellitus (glycosylation of membrane proteins) Infectious and inflammatory diseases Chronic renal failure

4. The significance of erythrocyte differentiation by surface charge for transfusion medicine

Young erythrocytes lose their unique structural characteristics as early as 21–25 days of storage, and a pronounced shift towards spherical morphology is observed after 28 days of hypothermic storage [45–48]. These morphological changes reflect the complex process of erythrocyte aging, which is accompanied by loss of shape, decreased membrane deformability, and modification of cytoskeletal proteins, in particular spectrin and actin. Under normal conditions of physiological aging in the human body, erythrocytes are removed from the circulation mainly by phagocytosis by splenic macrophages, which allows maintaining homeostasis of the cell population. However, in an in vitro system that simulates the conditions of storage of donor blood, this mechanism for the removal of senescent cells is absent, which leads to the accumulation of damaged erythrocytes and a progressive decrease in their functional capacity [46–48].

Interestingly, even up to day 28 of storage, old erythrocytes exhibit a lower membrane integrity (MI) compared to controls, indicating a significant weakening of the barrier properties of the cell membrane due to the cumulative effect of external and internal stress factors. A decrease in MI is associated with an increased risk of hemolysis, the release of free Hb and microparticles, which may have clinically significant consequences, in particular, an increased risk of transfusion-associated complications, including immunomodulation and pro-inflammatory reactions. At the same time, the presence of a high proportion of discoid erythrocytes in the population of young cells has a potentially protective value for their survival after transfusion. This is because it is the discoid morphology that provides the optimal surface area to volume ratio, which is critical for maintaining effective cell deformability and their ability to pass through the narrow capillaries of the microcirculatory bed, preventing retention in the spleen and premature clearance [45, 49].

Profiling of young and old erythrocytes is of great importance in modern transfusion medicine, as it allows the identification of markers reflecting the degree of functional viability of cells [52]. Accumulating data indicate that aging of erythrocytes is accompanied by a pronounced accumulation of oxidative stress products, such as glutathione disulfide, and depletion of antioxidant potential, which is manifested by a decrease in the concentration of metabolites, in particular S-adenosylmethionine [50, 51, 53, 54]. These metabolic changes indicate a progressive depletion of antioxidant defense mechanisms and increased vulnerability of cells to oxidative damage. One of the important observations of modern research is the identification of differences in the metabolic profile of subpopulations of young and old erythrocytes. In particular, young erythrocytes are characterized by an increased content of acylcarnitines, which reflects active processes of membrane lipid remodeling, as well as a high level of metabolites of the pentose phosphate pathway, which provides the generation of nicotinamide adenine dinucleotide phosphate (NADPH), necessary for maintaining cellular redox balance and protection against oxidative stress [45, 55]. At the same time, old erythrocytes demonstrate the presence of markers of oxidative damage, in particular glutathione disulfide, and a significantly reduced ability to maintain antioxidant protection, which is confirmed by a decrease in S-adenosylmethionine levels. These data indicate that old cells are in a state of metabolic fatigue, are characterized by a reduced ability to restore antioxidant status and, accordingly, are less viable during long-term storage. Interestingly, widely used methods for detecting intracellular reactive oxygen species (ROS) revealed the development of oxidative stress in old erythrocytes only on the 28th day of storage. This indicates a delayed nature of ROS accumulation in old cells, while in young erythrocytes increased levels of ROS are detected already at early stages after division. This phenomenon can be explained by the high metabolic activity of young cells and their greater involvement in the processes of metabolic compensation and protection from damage, which may further affect their functional capacity after transfusion.

Prediction of the duration of storage of erythrocytes and their functional activity is one of the key problems of modern transfusion medicine. The effectiveness of transfusion therapy directly depends on the ability of erythrocytes to perform the main physiological function of oxygen transport, as well as on their stability, preservation of morphological integrity and metabolic activity under conditions of hypothermic storage. Assessment of erythrocyte viability is based on a complex of morphological, biochemical and functional characteristics, among which the ability of cells to maintain deformability, counteract oxidative stress and avoid clearance in the reticuloendothelial system after transfusion is of particular importance. However, as modern studies show, the duration of storage of erythrocytes under standard conditions (4±2°C) does not always correlate with their functional capacity, since even cells stored for the standard period may demonstrate significant heterogeneity in viability, which creates risks for patients [56, 34]. Therefore, it is urgent to search for new markers that will allow more accurately predicting the functional potential of erythrocytes during and after storage, as well as optimizing the practice of their use in clinical transfusion medicine.

One of the promising areas of research is the use of erythrocyte differentiation by the ζ-potential indicator. Determination of the zeta-potential can serve as an indicator for assessing the "age" of the erythrocyte

population, as well as predicting their functional activity after transfusion. This approach allows for targeted selection of erythrocyte mass for transfusion in patients with an increased risk of complications, such as patients with chronic anemia, oncohematological diseases or critical conditions, where it is important to ensure the most efficient oxygen transport without excessive load on the reticuloendothelial system. It is important to emphasize that the zeta potential measurement technique is relatively simple to perform, noninvasive, and can potentially be adapted for routine laboratory quality control of red blood cell mass. Integrating this parameter into standard protocols for assessing the quality of blood components may improve the safety and effectiveness of transfusion therapy.

Thus, the prospects for using erythrocyte distribution by zeta potential open up new opportunities for optimizing blood transfusion practice. This approach allows not only to deepen the understanding of the biophysical properties of erythrocytes during their aging, but also creates a basis for the development of individualized strategies for transfusion therapy focused on the specific clinical needs of patients. In the context of modern research, in particular with the involvement of highly sensitive flow cytofluorimetric and nanoparticle analysis methods, the study of zeta potential can become an important tool for a deeper characterization of erythrocyte subpopulations, the detection of early markers of cellular aging and the development of new criteria for assessing the quality of blood for transfusions. Further improvement of this approach requires extensive clinical studies and standardization of methods to ensure their integration into the routine practice of transfusion medicine.

5. Red blood cell biobanking: new possibilities thanks to surface charge analysis

Modern transfusion medicine requires new approaches to extend the shelf life of erythrocytes while maintaining their functional activity. One of the promising areas is the use of differentiation of cell populations by biophysical and biochemical characteristics to optimize cryopreservation and lyophilization methods. Differentiation of erythrocytes by zeta potential, morphological features, sialic acid content, oxidative stress markers and metabolic profile allows us to identify cells capable of maintaining functional activity under extreme stress conditions, in particular during freezing.

Cryopreservation, as a method of long-term storage of erythrocytes at ultra-low temperatures, is of strategic importance for the creation of blood reserves in military medicine, emergency situations and transplantation practice. However, the freezing process carries the risk of cell membrane damage due to ice crystal formation, disruption of osmotic balance, and activation of the oxidative stress cascade.

Solutions for storing red blood cells have evolved significantly over the past decades to optimize the preservation of cell viability and functionality during hypothermic storage [57]. Over the years, the limitations of hypothermic storage of red blood cells have been gradually overcome by the development of new additive solutions that have replaced the use of saline or glucose-containing solutions [58, 59].

However, it should be recognized that even improving the composition of additive solutions with the addition of antioxidants cannot completely solve all the problems associated with hypothermic storage of erythrocytes. Among them, there are issues related to chronological aging of cells, accumulation of microparticles, changes in morphology and functional properties, as well as individual characteristics of recipients, which can affect the effectiveness and safety of transfusion therapy. Thus, further research should be aimed at a comprehensive approach that combines improving the composition of additive solutions, optimizing storage conditions and implementing methods for erythrocyte differentiation to predict their viability and develop personalized transfusion strategies.

Identification of freeze-resistant subpopulations of erythrocytes based on biophysical parameters, in particular zeta potential, allows the development of individualized cryopreservation protocols aimed at preserving maximum cell viability after thawing. Similarly, lyophilization – the process of drying erythrocytes in a vacuum at low temperatures – opens up new possibilities for long-term storage of cells without the use of liquid nitrogen, but also requires optimization of the composition of protective media and selection of cells capable of maintaining morphological integrity and metabolic activity after rehydration.

A key aspect is the ability to predict the shelf life of erythrocytes depending on their biophysical and metabolic status. The use of gradient centrifugation, flow cytometry, nanoparticle analysis and zeta potential measurement allows the identification of subpopulations of cells with an increased risk of loss of functional activity or hemolysis at the early stages of storage. This creates the basis for the formation of individual quality passports of erythrocyte mass, which will contain information on the predicted period of safe use, the level of antioxidant protection, membrane deformability and resistance to stress factors. This approach allows to increase the efficiency of using donor blood, minimize the risk of developing post-transfusion complications, and prevent the introduction of cells that have already lost their functional capacity into patients.

The use of erythrocyte distribution data is of particular importance for the development of personalized transfusion strategies that take into account the individual clinical needs of patients. For example, for

oncohematological patients, people with cardiovascular diseases, newborns or patients after organ transplantation, it is important to ensure maximum efficiency of oxygen transport without increasing the risk of oxidative stress or inflammatory response. In such cases, the use of carefully selected, high-quality erythrocyte fractions with proven stability and metabolic activity can significantly improve clinical outcomes. In addition, a promising direction is the implementation of artificial intelligence and machine learning algorithms to automate the process of assessing the quality of erythrocytes based on a comprehensive analysis of distribution parameters. This will allow the creation of predictive models that can predict cell viability during storage in real time and form individualized strategies for transfusion therapy. This approach will facilitate the transition from universal use of red blood cell mass to personalized transfusion medicine, focused on the safety and effectiveness of therapy for each individual patient.

Therefore, erythrocyte distribution as a tool for optimizing storage, cryopreservation, lyophilization, and development of personalized transfusion strategies is a promising direction that combines the achievements of cell biology, biophysics, chemistry, and medical informatics. This approach has significant potential to improve the safety and effectiveness of transfusion therapy, but requires further multicenter studies, standardization of methods, and implementation in clinical practice.

Conclusions

Distribution of erythrocytes by surface charge is an important biophysical tool for assessing their viability, preservation of morphological integrity and functional activity during storage. A decrease in zeta potential is associated with cell aging, increased aggregation, loss of deformability and the risk of hemolysis, which justifies the use of this parameter as a promising marker of pRBC quality for transfusion therapy. Inclusion of zeta potential assessment in routine blood component control can increase the safety and effectiveness of transfusions.

A deep understanding of the heterogeneity of erythrocytes by biophysical parameters, in particular zeta potential, creates a scientific basis for the development of personalized transfusion strategies. Selection of erythrocyte subpopulations with predicted stability and optimal functional potential allows minimizing the risks of post-transfusion complications, especially in patients at high risk of complications. This approach contributes to the transition from standardized use of red blood cell mass to personalized transfusion medicine focused on the needs of each patient.

Integration of erythrocyte zeta potential analysis into biobanking practice and optimization of cryopreservation and lyophilization processes opens up new opportunities for long-term storage of blood components without loss of their functional activity. The use of biophysical markers for the selection of stable cell subpopulations allows the formation of high-quality blood reserves for emergency and routine medical care. This approach increases the efficiency of donor blood use and the safety of transfusion therapy in clinical practice.

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РОЗПОДІЛ ЕРИТРОЦИТІВ ЗА ПОВЕРХНЕВИМ ЗАРЯДОМ: БІОФІЗИЧНІ ХАРАКТРИСТИКИ ГЕТЕРОГЕННОСТІ, ДІАГНОСТИЧНЕ ЗНАЧЕННЯ ТА ЗАСТОСУВАННЯ У ТРАНСФУЗІОЛОГІЇ ТА БІОБАНКІНГУ

Тривале зберігання еритроцитів, яке є основою сучасної трансфузійної медицини, супроводжується накопиченням структурних, метаболічних та біофізичних змін, що знижують функціональну активність клітин. Диференціація еритроцитів за поверхневим зарядом є перспективним напрямом, що дозволяє оцінювати життєздатність клітин, прогнозувати їх поведінку після трансфузії та оптимізувати стратегії зберігання й біобанкінгу. Попри зростання інтересу, практичне застосування аналізу дзета-потенціалу в клінічній трансфузійній медицині та біобанкінгу все ще залишається недостатньо розвиненим.

Узагальнити сучасні дані щодо розподілу еритроцитів за поверхневим зарядом, їхніх біофізичних властивостей, діагностичної цінності та можливостей застосування в трансфузійній медицині й біобанкінгу.

Проведено систематичний пошук у базах даних PubMed, Scopus, Web of Science, Cochrane Library, Google Scholar, Clinical Key та інших рецензованих джерелах. Включено публікації, що висвітлюють біофізичні характеристики еритроцитів, параметри поверхневого заряду, гетерогенність популяції, методи визначення дзета-потенціалу та їх практичне застосування.

Гетерогенність еритроцитів за поверхневим зарядом є фундаментальною характеристикою популяції, яка формується внаслідок фізіологічного старіння, окисного стресу, змін у складі мембран і цитоскелету. Зниження дзета-потенціалу асоціюється з підвищеним ризиком агрегації, зменшенням деформівності та здатності клітин проходити через вузькі мікросудини. Аналіз дзета-потенціалу дає змогу ідентифікувати субпопуляції еритроцитів з різним ступенем пошкодження та прогнозувати їх функціональні можливості після трансфузії. Використання цього параметра у трансфузійній медицині підвищує ефективність і безпеку трансфузій, а у біобанкінгу – оптимізує відбір клітин для тривалого зберігання. Диференціація за поверхневим зарядом

також має перспективи у прогнозуванні терміну зберігання еритроцитарної маси та розвитку персоналізованих трансфузійних стратегій.
Диференціація еритроцитів за поверхневим зарядом є перспективним підходом для оптимізації зберігання компонентів крові, прогнозування їх функціональної активності та забезпечення безпечної трансфузійної терапії. Інтеграція аналізу дзета-потенціалу в рутинну лабораторну практику має потенціал для підвищення якості компонентів крові, а подальші дослідження сприятимуть розвитку персоналізованої трансфузійної медицини.
Ключові слова: еритроцити, дзета-потенціал, поверхневий заряд, біофізичні властивості, старіння клітин, трансфузіологія, біобанкінг