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Review Article

Red blood cell "freshness" dilemma: storage lesions

Yu Zhang[1,2], Yating Ling[1,2], Yu Jin[1,2], Qiang Fu[1,2,*], Chengtao He[1,2,*]

[1] *Nanjing Red Cross Blood Center, Nanjing, Jiangsu 210003, China;*
[2] *Key Laboratory of Transfusion Medicine, Nanjing Red Cross Blood Center, Nanjing, Jiangsu 210003, China.*

ABSTRACT

Storage lesions of red blood cell (RBC) refer to a series of physiological, biochemical, and morphological alterations that occur during extracellular preservation, leading to diminished functional capacity and reduced cellular lifespan. This paper reviews the primary pathological mechanisms of RBC storage lesion, including morphological alterations, energy metabolism disorders (decreases in ATP and 2,3-G levels), acidosis, ion pump dysfunction (K^+ loss and Na^+ accumulation), oxidative lesion (lipid peroxidation and structural changes of band 3 protein), and degradation of membrane phospholipids. An in-depth understanding of these mechanisms can help optimize RBC storage strategies and improve the safety and effectiveness of blood transfusions.

Keywords: red blood cell, storage lesion, morphological change, metabolism, oxidative stress, membrane structure

INTRODUCTION

Red blood cell (RBC) transfusion is a vital therapeutic intervention for the management of anemia, trauma, and perioperative blood loss in clinical practice. To ensure the safety of blood supply, collected RBCs need to be stored at 4 ℃ for a period of 35 to 42 days (depending on the type of preservation solution)[1]. However, during storage, RBCs undergo a series of structural, metabolic, and biochemical alterations collectively known as RBC storage lesion[2]. RBC storage lesion is characterized by the loss of the biconcave discoid structure (increased proportion of spherocytic cells), energy metabolism disorders (decrease in adenosine triphosphate (ATP) levels), accumulation of oxidative stress markers such as malondialdehyde (MDA) elevation, and degradation of membrane skeleton proteins (band 3 protein aggregation)[3]. These pathological changes not only result in a reduced survival rate of RBCs following transfusion but may also trigger adverse reactions, such as transfusion-related immunomodulation[4]. Given the substantial impact of storage lesion on the efficacy of blood transfusions, comprehensive investigation into the molecular mechanisms of RBC storage lesion has become a focus in the field of blood preservation. This paper presents a systematic review of significant advancements in the mechanisms of RBC storage lesion, focusing on morphological changes, metabolic regulation, and oxidative and membrane stability, thereby providing a theoretical foundation for the development of novel blood preservation technologies.

THE "METAMORPHOSIS" OF RED BLOOD CELLS: CHANGES IN MORPHOLOGY

Under normal conditions, RBCs adopt a biconcave

*Corresponding to: Chengtao He and Qiang Fu, Nanjing Red Cross Blood Center, 3 Zizhulin, Gulou District, Nanjing, Jiangsu 210003, China. E-mails: hechengtao413@126.com and fuqiangnj@hotmail.com.

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disc shape, a distinctive morphology that confers high deformability and a large surface area, thereby facilitating efficient gas exchange and smooth passage through narrow capillaries. As the storage duration increases, the structure of RBC membranes keeps changing. The shape gradually transforms from a double-concave disc-shaped structure to an echinocyte, ultimately evolving into a smooth spherical shape[5–7]. The morphological alterations of RBCs are one of the core manifestations of storage lesion, with their dynamic progression comprising two key stages.

Initial reversible morphological alteration

In the early stages of storage, the morphology of RBCs begins to undergo subtle changes, gradually transforming from the original disc shape into echinocyte. This morphological change is closely related to energy supply in RBCs, specifically linked to the reduction of ATP levels[8]. The primary source of ATP production in RBCs is anaerobic glycolysis. When ATP supply is insufficient, there is a decrease in the activity of the sodium-potassium pump (Na^+/K^+-ATPase), abnormal phosphorylation of membrane skeleton proteins, and accumulation of Ca^{2+} within the cell, followed by structural and functional alterations of the cell membrane and morphological changes of RBCs changes from their normal disc shape to a spine-spherical form[9]. The study found that the proportion of spiculated RBCs significantly increased when ATP levels declined to 36.4% of the initial value (approximately 1.5 μmol/g Hb) on the 21st day of storage, and pointed out that the level of ATP was closely related to the morphological integrity of RBCs[10]. However, if ATP levels are restored during this period, the morphology of RBCs can also be reversed, returning to approximately to their normal state. Aujla H *et al*. treated RBCs stored for 42 days with a PIPA solution containing hypoxanthine, pyruv, phosphate, and adenine for 2 hours, resulting in a significant recovery of ATP and 2,3-Diphosphoglyceric acid (2,3-DPG) levels, as well as a partial restoration of morphology from spiculated forms to a biconcave disc shape, with a significant improvement in membrane deformability[11]. This indicates that during the initial stages of storage, the morphological changes of RBCs can be regulated and restored to a certain extent.

Irreversible deformation in later stage

With the further extension of storage time, the morphological alterations of RBCs become increasingly pronounced[12]. During this period, several alterations were observed in both the lipid bilayer and membrane proteins of RBC membranes, mainly manifested as lipid rearrangement[13–14], outward flipping of phosphatidylserine (PS) from the inner to the outer leaflet of the plasma membrane[15], and abnormal expression of surface molecules such as membrane proteins. Lu C *et al*. showed that after 28 days of RBC storage, membrane lipid asymmetry was completely lost, with the proportion of externalized PS increasing from 1.5% at day 14 to 8%–10% at day 28. Moreover, extending the storage period to 42 days could further elevate externalized PS levels to 18.4%[16]. Roussel C *et al*. demonstrated that approximately 23.6% of RBCs underwent irreversible morphological alterations, transforming into "small red cells" with a reduced surface area of 15% to 19.9% after 42 days of storage, primarily due to the loss of membrane area resulting from the release of membrane particles (MPs)[17]. At this stage, the morphology of RBCs is severely deformed, and this deformation is often irreversible. Severely deformed RBCs have difficulty traversing narrow capillaries and transporting oxygen effectively. Their survival ability within the body after infusion will be significantly diminished. Severely deformed RBCs may also be identified and eliminated by the body's immune system, thus failing to perform normal physiological functions[18].

METABOLIC "SLOW LANE": METABOLISM SLOWS DOWN

Mature RBCs, which lack a nucleus and mitochondria, rely primarily on glycolysis (anaerobic metabolism) for energy, with a metabolic pathway differing from that of other cells, and their basal metabolic rate (BMR) is only one-tenth that of nucleated cells[19–20], functioning as a "power-saving mode" or "slow lane" metabolism. The rate of glycolysis decreases by 12% to 16% per week during RBC storage, providing direct evidence of an additional slowdown in the "slow lane"[21]. Although the "slow lane" metabolism of RBCs is energy-efficient, it simultaneously results in limited repair capacity and aggravates the irreversibility of storage lesion.

The depletion of "power source": ATP concentration decreased

ATP, often referred to as the "energy currency" within cells, plays a core role in the normal physiological functions of RBCs. The decline in ATP concentration during the storage of RBCs is a critical alteration that affects their morphology, metabolism, and function[22]. During the initial storage period (0–7

days), the ATP concentration within RBCs may not immediately decrease; instead, it may slightly increase. At this stage, RBCs themselves activate compensatory mechanisms by upregulating the activity of glycolytic enzymes, such as hexokinase (HK) and phosphofructokinase (PFK), and increasing the rate of glycolysis to temporarily maintain ATP levels. ATP concentrations typically reach their peak and then gradually decline as the storage duration approaches approximately 2 weeks[23]. Through the metabolomic analysis of RBCs from 13 029 donors, Nemkov T *et al*. found that PFK polymorphism was significantly associated with ATP levels at the end of storage. A compensatory increase in PFK activity at the beginning of storage could transiently increase ATP concentrations, suggesting that upregulation of PFK activity is a key factor in maintaining ATP[24]. Burger P *et al*. showed that supplementing the storage solution with 1 mM hypoxanthine and adenine prolonged the ATP peak from 4 to 21 days; this effect was primarily attributed to the synergistic effect of hypoxanthine and phosphate, which enhanced ATP synthesis the glycolysis purine salvage pathway[25].

Acid-base "imbalance crisis": acidosis

Acidosis is an important alteration observed during the storage of RBCs, and serves as a key indicator of storage-related metabolic disorders (SRAM)[26]. Lactic acid accumulation and citrate metabolism contribute to the decrease in pH during storage.

Lactic acid accumulation and acidosis

During glycolysis, glucose is progressively decomposed into pyruvic acid, which is further converted into lactic acid under anaerobic conditions[27]. With the extension of storage duration, RBCs continuously undergo glycolysis, resulting in the consistent production and gradual accumulation of lactic acid within the storage solution. Guppy M *et al*. showed that during storage, lactate accumulated at a rate of 0.3 mmol/L/day during the first week, while the pH declined from 7.4 to 6.7 by the fourth week. Although lactate generation slowed subsequently, its overall concentration reached a peak[28].

Citrate metabolism inhibition and acid-base imbalance

Anticoagulants are typically employed during the storage of RBCs to prevent blood coagulation. Citrate is one of the commonly used anticoagulants. It can effectively inhibit blood coagulation by chelating Ca^{2+} without altering the initial pH value[29]. Citrate undergoes a series of metabolic reactions within the body, ultimately being converted into bicarbonate. Although bicarbonate itself is an anine substance, its metabolism in the acidic environment of RBC storage has a complex effect on acid-base balance. The rate of citrate conversion to bicarbonate is pH-dependent, and citrate metabolism is inhibited under acidic conditions. Nemkov T *et al*. demonstrated that citrate metabolism in RBCs mainly depends on citrate lyase (CL) and malate hydrogenase (MDH) in the cytoplasm. When the pH is below 7.0, the activity of these enzymes is significantly reduced due to the protonation, which inhibits citrate cleavage and results in a decrease of 80% in the rate of citrate metabolism[30]. The extensive metabolism of citrate into bicarbonate may disrupt the acid-base balance that has already been affected by lactic acid accumulation, thereby further aggravating the degree of acid-base imbalance and promoting the occurrence and development of acidosis[31].

The influence of acidosis on red blood cells

Under acidic conditions, the proteins and lipids of the RBC membrane undergo structural and functional changes, resulting in increased membrane permeability and compromised stability. Acidosis activates phospholipase A2 (PLA2), accelerating the hydrolysis of membrane phospholipids. When the pH is below 6.8, membrane skeleton proteins (such as spectrin) undergo degradation and the lipid bilayer permeability increases[32]. Acidosis can also affect the enzyme activity within RBCs, reducing the activity of enzymes involved in key metabolic processes such as glycolysis and pentose phosphate pathway, and further influencing the energy supply and metabolic function of RBCs[33]. Research indicated that when the pH is below 7.0, the activities of HK and pyruvate kinase (PK) decrease by 30%[34]. When the pH of stored RBCs drops to 6.8, it results in reduced cellular deformability, a 40% increase in retention within capillaries, and a 25% decrease in tissue partial pressure of oxygen (pO_2), impairing their ability to traverse narrow capillaries and adversely affecting oxygen transport and tissue oxygenation[35].

Oxygen-carrying "signal" weakened: 2,3-DPG concentration decreased

2,3-DPG is a critical intermediate product in RBC metabolism and plays a key role in regulating the affinity of hemoglobin (Hb) for oxygen[36]. The concentration of 2,3-DPG changes significantly during the storage of RBCs. Kurup PA *et al*. found that in CPDA-1 preservation solution, 2,3-DPG concentration rapidly decreased in the early stage of storage (14 days), reaching approximately 6% of the initial value by 28 days[37]. This occurs because, during the storage process, the metabolic activities of

RBCs are gradually inhibited, causing decreased activity of enzymes involved in the synthesis of 2,3-DPG and thus a reduction in the synthesis of 2,3-DPG[38].

Factors within the storage environment, such as changes in pH value and decreases in ATP concentration, can further influence the metabolism and stability of 2,3-DPG, accelerating its decline[39]. In CPDA-1 preservation solution, when the pH is below 7.2, the activity of diphosphoglycerate mutase (DPGM) decreases by 60%, whereas the activity of 2,3-DPG phosphatase (DPGP) increases, resulting in a 2-fold increase in the degradation rate of 2,3-DPG[40]. When ATP levels drop below 1 μmol/g Hb, glycolytic flux declines significantly and DPGM ceases to synthesize 2,3-DPG due to the absence of substrates such as 1,3-Bisphosphoglycerate (1,3-BPG)[41].

Since 2,3-DPG is a key modulator of Hb's affinity for oxygen, a decrease in its concentration will significantly weaken the oxygen release capacity of Hb, resulting in a leftward shift of the oxygen dissociation curve (P50 value shifts to the left). A decrease in 2,3-DPG levels from the normal approximately 5 μmol/g Hb to 2 μmol/g Hb results in a reduction of the P50 value from 27 mmHg to 16.5 mmHg, and the oxygen-releasing capacity declines significantly[42]. With the recovery of 2,3-DPG concentration, the oxygen dissociation curve gradually shifts to the right, and RBCs also regain the ability to deliver oxygen to peripheral tissues. P50 returns to normal within 24 hours following the restoration of 2,3-DPG, indicating that, to some extent, RBCs possess the capacity for self-regulation and recovery[43–44]. However, for critically ill patients requiring immediate, large-volume blood transfusions, this recovery process may fail to meet the oxygen demand of their tissues in a timely manner, thereby adversely affecting treatment outcomes and prognosis[4].

Ion "steady-state" broken: cation pump function loss

Under normal physiological conditions, the cation pumps on the RBC membrane, especially the Na^+/K^+ pump, play a crucial role in ion balance within the cells[45–46]. As storage time is extended, the ATP levels in RBCs progressively decline due to the inhibition of the glycolytic pathway, which results in decreased ATP synthesis while consumption persists[47]. ATP is the energy source for the Na^+/K^+ pump to function. When the ATP concentration decreases to a certain extent, the active center of the pump cannot bind to ATP, and the function of the Na^+/K^+ pump will be inhibited, preventing the completion of ion transmembrane transport[47]. Consequently, potassium gradually diminishes within the cell while sodium continuously accumulates in the cytoplasm[48–49]. Nogueira D *et al*. indicated that during the storage of RBCs, potassium ions in the storage solution showed a gradual increase, while sodium ion concentration correspondingly decreased. A significant statistical difference was observed in the concentrations of sodium and potassium ions between day 7 and day 42 when compared to day 0[50].

An imbalance of ions directly affects the osmotic pressure equilibrium of RBCs, as the osmotic pressure maintained by the Na^+/K^+ pump accounts for 55% to 65% of the total osmotic pressure equilibrium[51]. Ionic imbalance can also substantially influence the deformability of RBCs[51]. Lang F *et al*. indicated that a high Na^+ and low K^+ environment can induce stiffening of the lipid bilayer in RBC membranes, activate calcium-dependent proteases (such as calpain) and Ca^{2+}-sensitive potassium channels (Gardos channels), and accelerate cell dehydration and morphological changes. These processes further compromise the membrane skeleton, resulting in increased membrane rigidity and decreased elasticity[52]. Due to their limited deformability when traversing narrow capillaries, they are susceptible to blockage, which affects the normal blood flow and oxygen transport.

"OXIDATIVE ATTACK" STRIKES: OXIDATIVE DAMAGE

Oxidative damage to RBCs during preservation is one of the central mechanisms of storage lesion, involving the accumulation of reactive oxygen species (ROS), failure of the antioxidant defense system, oxidation of membrane lipids and proteins, and Hb auto-oxidation. Ultimately, this leads to a decline in RBC functions[53].

Excessive generation of reactive oxygen species

During RBC storage, the activity of glycolytic enzymes decreases, leading to a reduction in ATP generation and an imbalance in the cellular redox state. This shift promotes the enhancement of the pentose phosphate pathway, producing more $NADP^+$ and subsequent excessive generation of ROS[54]. The Hb in RBCs gradually releases iron ions during preservation. Iron ions can catalyze the generation of highly oxidizing hydroxyl radicals from hydrogen peroxide through the Fenton reaction, triggering oxidative damage to biomacromolecules such as

lipids, proteins, and nucleic acids[23,55].

Failure of antioxidant defense system

During the preservation process, the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) within RBCs gradually decline, failing to promptly eliminate the generated ROS. This leads to the accumulation of ROS within the cells and its attack on biological macromolecules (such as membrane lipids and Hb), causing oxidative damage[56]. Meanwhile, the content of non-enzymatic antioxidants such as glutathione (GSH) also decreases due to increased consumption and reduced synthesis, resulting in a decline in the ability to eliminate ROS and an increased risk of oxidative damage to RBCs[57].

Membrane lipid peroxidation

RBC membranes are rich in polyunsaturated fatty acids (PUFAs) and are susceptible to ROS attack, leading to lipid peroxidation reactions. ROS attack PUFAs in the cell membrane, triggering a lipid peroxidation chain reaction that produces final products such as MDA. These products alter the fluidity and permeability of the cell membrane, affect the function of membrane proteins, and consequently cause membrane damage, thereby aggravating the oxidative stress response in the cells[55,58]. Rajendra Chaudhary's study proved that MDA is directly related to membrane damage. Their data showed that MDA levels significantly increased after 28 days of RBC storage, and the membrane mean osmotic fragility rose from 1.07% at day 0 to 16.2% at day 28[59].

Hemoglobin auto-oxidation

During RBC storage, ferrous ions (Fe^{2+}) in Hb are susceptible to oxidation into ferric ions (Fe^{3+}) and may further form ferryl ions (Fe^{4+}) in a higher oxidation state, releasing superoxide anions (O_2^-) and hydrogen peroxide (H_2O_2), and then oxygenating Hb (HbO_2) into ferric Hb (***Fig. 1***)[60]. Methemoglobin cannot carry and release oxygen normally, which reduces the oxygen transport capacity of RBCs; additionally, its formation generates ROS, aggravating oxidative damage[61]. Following the formation of methemoglobin, it undergoes further degradation to form methemochromogen, which subsequently deposits and binds to the lipids and cytoskeleton of RBC membrane, leading to the aggregation of band 3 protein, exposure of aging antigens, and recognition by natural IgG[62].

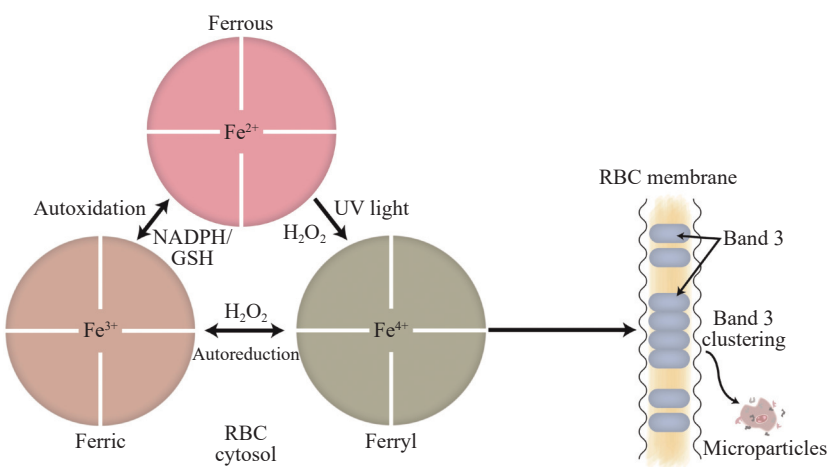

Fig. 1 **The auto-oxidation process of hemoglobin (Hb).** Heme iron (Fe^{2+}) in Hb can oxidize to nonfunctional Fe^{3+} and, under oxidative stress, highly reactive Fe^{4+}. Although red blood cells (RBCs) have reductive systems to maintain functional Hb (Fe^{2+}), oxidation still occurs, especially during storage or pathogen reduction, and potentially damages cell integrity[60]. NADPH: nicotinamide adenine dinucleotide phosphate hydrogen; GSH: glutathione.

MEMBRANE STRUCTURE "DESTRUCTION STORM"

In recent years, researchers have proposed the concept of "membrane destruction storm", emphasizing the irreversible damage to RBC membranes caused by a cascade reaction of band 3 protein aggregation, lipid peroxidation, membrane phospholipid digestion, and membrane microvesicle shedding during storage[61]. The RBC membrane is composed of a lipid bilayer (rich in phosphatidylcholine (PC), sphingomyelin, and PS) and membrane skeleton proteins (spectrin, actin, band 3 protein, *etc*.). However, during storage, disruptions in energy metabolism (depletion of ATP and 2,3-DPG) and oxidative stress (accumulation of ROS) stimulate a

"destruction storm" within the membrane structure[63].

Aggregation of band 3 protein: the "trigger" for membrane disruption

Band 3 protein is a crucial transmembrane component in RBC membrane, facilitating anion exchange (HCO_3^-/Cl^-) and anchoring the membrane skeleton[64–65]. During storage, the following changes led to its abnormal functioning.

Oxidative damage and aggregation

During storage, ROS accumulate in RBCs, directly oxidizing the cytoplasmic domain of band 3 protein, attacking its thiol groups, which leads to disulfide bond crosslinking, oligomerization, and the formation of high-molecular-weight complexes[66]. These aggregates expose "senescent cell antigens" (SCA), combine with natural IgG and complement C3b, and promote the clearance of macrophages.

Membrane skeleton dissociation

Band 3 protein is linked to spectrin *via* ankyrin to maintain membrane mechanical stability[67]. With prolonged storage, ROS-induced oxidation of cysteine 538 of band 3 protein forms diside bond cross-linking, and the phosphorylation of tyrosine residues tyrosine8 and tyrosine21 on band 3 protein decreases its binding capacity to ankyrin by 60%, thus weakening the membrane-cytoskeleton connection[68]. The interaction between band 3 protein and spectrin is weakened, leading to a destabilized membrane skeleton and reduced deformability of RBCs[69].

Lipid peroxidation: the "disintegrator" of membrane integrity

The RBC membrane is rich in PUFAs and is extremely vulnerable to ROS attack, triggering a lipid peroxation chain reaction: (1) free radical attack: Free radicals seize hydrogen atoms from PUFAs to form lipid free radicals, which then react with O_2 to generate lipid peroxide free radicals and eventually decompose into end products such as MDA and 4-hydroxynonenal (4-HNE)[56]. (2) membrane fluidity reduction: Lipid peroxidation can induce increased membrane rigidity and decreased fluidity, impairing the ability of RBCs to traverse the microcirculation. (3) phospholipase activation: Oxidative stress activates PLA2, which hydrolyzes membrane phospholipids (such as PC), releasing lyso-phospholipids and further disrupting membrane structure[70].

Membrane phospholipid digestion and loss of asymmetry

The phospholipids in normal RBC membranes are asymmetrically distributed: PC and sphinomyelin (SM) are primarily localized in the outer leaflet, while PS and phosphatidylethanolamine (PE) are predominantly found in the inner leaflet. During storage, the following changes aggravate membrane damages: (1) phospholipid flipping: Decreased ATP-dependent flippase activity fails to maintain the inward flip of PS, resulting in the externalization of PS to the surface of red cell microparticles and serving as a signal for macrophage recognition and clearance[2]. During storage, Ca^{2+}-ATPase failure causes an elevation in cytosolic Ca^{2+} levels (~10 μM), which activates the scramblase, promoting the externalization of PS, and exposed PS is recognized by macrophage surface receptors, triggering phago-cytosis[71]. (2) microvesicle release: Microvesicles are mainly formed through the localized shedding of the cell membrane, carrying Hb, band 3 protein, and PS; once formed, these microvesicles cannot be recovered, leading to irreversible damage to RBC membrane[72]. The regions of membrane asymmetry loss and the aggregation of band 3 protein are more susceptible to detachment and microvesicle formation, accelerating the reduction in cell volume and functional decline[73–74].

EXPLORING THE "WAY TO BREAK THE DEADLOCK": COPING STRATEGIES AND PROSPECTS

In response to the challenges posed by RBC storage lesion, researchers have adopted a series of effective countermeasures, aiming to minimize the damage to the greatest extent, enhance the storage quality, and improve transfusion outcomes.

Optimizing the storage additive formula

To reduce oxidative stress during RBC storage, researchers have attempted to add antioxidants such as vitamin E, GSH, and vitamin C to the preservation solution in order to enhance the antioxidant capacity of the storage environment[75–76]. GSH, an important antioxidant, can maintain the redox balance within RBCs and safeguard them against oxidative stress. When the GSH concentration exceeds 2 mmol/L, it can inhibit protein fragmentation caused by oxidative stress. The addition of a preservation solution containing GSH (5 mmol/L) reduces markers of membrane protein oxidative damage (such as protein carbonylation) by 35%, thereby preserving the normal carrying capacity of RBCs[77]. Sodium pyruvate (SP), the sodium salt of pyruvate, is an intermediate metabolite of glycolysis and has antioxidant effect,

improving storage damage through a dual mechanism of providing glycolytic substrates and scavenging H_2O_2[78]. Meyer found that preservation solutions containing SP increased the ATP levels in RBCs by 2.5 times and enhanced their functional performance[79].

Low-oxygen storage technology

Reducing the partial pressure of oxygen in the storage environment can decrease the oxidative damage of RBCs[80]. D'Alessandro A. proposed that anaerobic storage preserves the metabolic homeostasis of RBCs by inhibiting the generation of oxygen free radicals[81]. Multiple studies have demonstrated that hypoxic storage (O_2 < 1%) can double the duration of the Na^+/K^+ pump and ATP activity maintenance, delay the occurrence of ion imbalance, reduce the oxidative damage markers (such as lipid peroxidase MDA) by up to 50%, inhibit externalization of PS by 70%, and decrease the hemolysis rate by 50%[80–81].

Meng Q *et al*. showed that, compared to RBCs stored under normal conditions, removal of oxygen and replacement with helium resulted in a significant increase in ATP levels, a 30% reduction in glucose consumption, a 40% decrease in lactic acid accumulation, and after 9 weeks of storage, marked reductions in free Hb and extracellular vesicle concentrations[82]. Research conducted by Yoshida T. and Dumont LJ. showed that storage under anaerobic conditions can diminish oxidative damage, maintain the relatively stable state of RBCs, and mitigate storage damage[83–84].

Nanotechnology intervention

Nanomaterials can eliminate free radicals or repair membrane damage. Singh S. demonstrated that nanoparticles, including cerium nanoparticles (CeONPs), gold nanoparticles (AuNPs), and iron oxide nanoparticles (IONPs), have the inherent property to eliminate ROS[85]. Liu T *et al*. showed that cerium oxide nanoparticles (CeO_2-NPs) reduced ROS levels by 60% and decreased the hemolysis rate by 50%[86]. In animal experiments, Kotsuruba AV *et al*. administered 0.1 mg/kg of nanocerium (CeO_2-NPs) to rats for 14 consecutive days and found that nanocerium could reduce ROS production and stabilize the function of RBCs[87].

Cryopreservation technology

At present, cryopreservation is the most widely used method for storing cells, wherein cellular metabolic activity is effectively suspended under ultra-low temperature conditions[88]. However, the formation of ice crystals during the freezing and thawing processes can cause mechanical damage to the cell membrane, ultimately leading to the direct rupture and death of cells. Cryoprotectants can effectively protect cells from damage by ice crystals during the freezing process. Glycerol is primarily utilized for the cryopreservation of RBCs; however, excessive glycerol intake may cause symptoms such as renal failure[89]. Therefore, researchers have explored new safe cryoprotectants in recent years to ensure the quality of cells stored at low temperatures.

Zhu W *et al*. demonstrated that employing zirconium (Zr)-based metal-organic framework (MOF) NPs with well-defined surface chemistries for the cryopreservation of RBCs could achieve recovery rates of approximately 40%[90]. Yao J *et al*. investigated the use of an alginate microencapsulation method combined with 0.7 M trehalose to cryopreserve RBCs, and the RBCs cryopreserved by the above method showed no significant morphological changes following freeze-thaw and washing procedures[91]. Dou M *et al*. explored the combination of proline and trehalose as a cryoprotective scheme for freezing RBCs. The results demonstrated a reduction in hemolysis rates of RBCs after thawing, with no observed alterations in the activities of Na^+/K^+-ATPase and Ca^{2+}/Mg^{2+}-ATPase[92].

SUMMARY

RBC storage injury involves complex changes in morphology, metabolism, energy, ion balance, oxidative stress, and membrane structure, which are interconnected and mutually influential, leading to impaired RBC function and affecting transfusion efficacy and patient prognosis. A comprehensive investigation into the mechanisms of RBC storage lesion, integrated with existing research findings, enables the continuous exploration of effective intervention strategies. Through systematic improvements in preservation solution formulations, optimization of storage conditions, and advancements in gene editing and pharmaceutical development, we can potentially mitigate RBC storage damage through multiple dimensions, thereby enhancing the quality of RBC products for clinical applications.

Author contributions

Original Draft Preparation, Yu Zhang; Review & Editing, Yu Zhang; Visualization, Yating Ling; Supervision, Yu Jin; Project Administration, Qiang Fu

and Chengtao He; Funding Acquisition, Qiang Fu and Chengtao He.

Ethics statement

Not applicable.

Informed consent statement

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Declaration of competing interest

The authors declared no conflict of interests.

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