



To Research the Effects of Storage Time on Autotransfusion based on Erythrocyte Oxygen-Carrying Capacity and Oxidative Damage Characteristics

Cell Transplantation
Volume 30: 1–10
© The Author(s) 2021
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/09636897211005683
journals.sagepub.com/home/ctj


Zhen-Zhou Li^{1,2}, Dong-Lin Jia³, Huan Wang¹, Xiao-Fang Zhou¹, Yong Cheng¹, Li-Shuang Duan¹, Lei Yin¹, Han-Wei Wei¹, Wei Guo⁴, and Jian-Rong Guo^{1,2} 

Abstract

Autotransfusion refers to a blood transfusion method in which the blood or blood components of the patient are collected under certain conditions, returned to himself when the patient needs surgery or emergency after a series of storing and processing. Although autotransfusion can avoid blood-borne diseases and adverse reactions related to allogeneic blood transfusion, a series of structural and functional changes of erythrocytes will occur during extension of storage time, thus affecting the efficacy of clinical blood transfusion. Our research was aimed to explore the change of erythrocyte oxygen-carrying capacity in different storage time, such as effective oxygen uptake (Q), P50, 2,3-DPG, Na⁺-K⁺-ATPase, to detect membrane potential, the change of Ca²⁺, and reactive oxygen species (ROS) change of erythrocytes. At the same time, Western blot was used to detect the expression of Mitofusin 1 (Mfn1) and Mitofusin 2 (Mfn2) proteins on the cytomembrane, from the perspective of oxidative stress to explore the function change of erythrocytes after different storage time. This study is expected to provide experimental data for further clarifying the functional status of erythrocytes with different preservation time in patients with autotransfusion, achieving accurate infusion of erythrocytes and improving the therapeutic effect of autologous blood transfusion, which has important clinical application value.

Keywords

autotransfusion, erythrocyte, oxygen-carrying capacity, storage time, oxidative stress, cytomembrane

Introduction

Blood transfusion is a common treatment in clinical practice, and it is also the most important means to save patients' lives in case of massive blood loss¹. However, blood transfusion has potentially dangerous, especially allogeneic blood transfusion, which mainly includes transfusion-related infectious diseases and transfusion-related adverse reactions^{2–4}. Autotransfusion refers to a blood transfusion method in which the blood or blood components of the patient are collected under certain conditions, stored and processed, and then returned to the patient when the patient needs surgery or emergency. Compared with allogeneic blood transfusion, autologous blood transfusion has the advantages of avoiding allogeneic rejection and transfusion-transmitted diseases, avoiding hemolysis, fever and allergic reaction caused by the immune reaction of antibody antigen produced by allogeneic blood transfusion, which greatly increases the safety of blood

¹ Department of Anesthesiology, Shanghai Gongli Hospital, the Second Military Medical University, Shanghai, P. R. China

² Ningxia Medical University, Gongli Hospital of Shanghai Pudong New Area Training Base, Shanghai, P.R. China

³ Department of Pain Medicine, Peking University Third Hospital, Beijing, China

⁴ Department of Intensive Care Unit, Anhui Provincial Lujiang County People's Hospital, Hefei, P.R. China

Submitted: February 22, 2021. Revised: February 22, 2021. Accepted: March 5, 2021.

Corresponding Authors:

Jian-Rong Guo, Department of Anesthesiology, Gongli Hospital, the Second Military Medical University, No. 219, Miaopu Road, Pudong New Area, Shanghai 200135, P. R. China.
Email: jr_guo@yeah.net

Wei Guo, Department of Intensive Care Unit, Anhui Provincial Lujiang County People's Hospital, Hefei 231500, P.R. China.
Email: rjdlp2@163.com



transfusion and alleviates the contradiction of blood source tension. However, autologous transfusion has its limitations, mainly because it needs to be stored at low temperature after collection *in vitro*. Some studies have shown that the structural and functional damage of erythrocytes in the process of blood preservation, namely the storage damage, which is the main problem affecting the safety of blood transfusion. At present, the function of 0–28 days erythrocytes in stock is still regarded as equal in clinical practice. This phenomenon ignores the effect of storage time on erythrocyte function and the effect of storage time on blood transfusion treatment^{5,6}.

Erythrocyte transfusion is the most important part of blood transfusion, and it is also the blood component with the largest dosage. The main function of erythrocytes is to transport oxygen and carbon dioxide to tissues and organs of the body, which plays a crucial role in maintaining normal physiological functions⁷. The main purpose of transfusion of erythrocytes is to ensure the normal supply of oxygen to the organs and tissues of patients by using their oxygen carrying function, so as to relieve symptoms and save lives. Therefore, it is very important to evaluate the function of red blood cell transfusion. Erythrocytes have a life span of 120 days *in vivo*, which only 21–42 days *in vitro*. Erythrocytes are stored at low temperature *in vitro*. Even in this low-temperature environment, the metabolism of erythrocytes does not stop⁸. Due to the occurrence of erythrocyte storage injury, the number and function of erythrocytes with different storage days are continuously decreasing. The oxygen-carrying capability of erythrocytes is the key to its therapeutic effect. Therefore, it is of great significance to define the change rules of erythrocyte function during the process of the collection of erythrocytes for clinical blood transfusion. It has also been a hot and difficult point in clinical research to establish a more complete evaluation system for erythrocyte function, guide accurate blood transfusion and minimize the adverse reactions related to blood transfusion.

Our research was aimed to explore the change of erythrocyte oxygen-carrying capacity in different storage time. Six cases of blood donors were collected as investigated subjects and this study is expected to provide experimental data for further clarifying the functional status of erythrocytes with different preservation time in patients with autotransfusion, achieving accurate infusion of erythrocytes and improving the therapeutic effect of autologous blood transfusion, which has important clinical application value.

Materials and Methods

Six cases of blood donors who met the conditions for voluntary blood donation in China. 1 unit of red blood cells (200 mL/U) was collected from each blood donor to prepare suspended erythrocytes. Defoaming agent, DMEM, FBS, Trypsin, PBS were purchased from Thermo Fisher. All the other chemicals were purchased from commercial supplier or Innochem. Oxygen, carbon dioxide and nitrogen bottles were purchased from Beijing Beipu Feilong Gas Company.

37 °C constant temperature water bath, low-speed automatic balancing centrifuge B120 purchased from American Hyclone Corporation. Add Sampler (Eppendorf, 200 µl, 1000 µl), 2,3-DPG detection kit, Na⁺-K⁺-ATP detection kit were purchased from Beyotime Biotechnology Research Institute. Fluorescence emission spectra and full wavelength absorption spectra were performed on Tecan Spark™ 10 M Multimode Microplate Reader. OD values were recorded in a 10 mm path quartz cell on a Metash UV-5100B spectrometer.

Determination of Effective Oxygen-Carrying Capacity (Q) and P50

10 mL blood was prepared suspended erythrocytes come from the 6 donors with a concentration of $3.5 \times 10^{12}/L$ final volume (3 mL). Defoaming agent (20 µL) was added into all tested samples. Simulation of arterial oxygen partial pressure, the test condition was set to: O₂ = 16 mL/min, CO₂ = 3 mL/min, N₂ = 120 mL/min, flow rate: 100 mL/min, 37 °C to sample inflatable 9 min. Finally, 1 mL of the sample was extracted for blood gas analysis⁹. The charging conditions were adjusted as O₂ = 6 mL/min, CO₂ = 3 mL/min, N₂ = 160 mL/min, and the flow rate was 100 mL/min. Balance for 10 minutes, 37 °C to sample inflatable 6 min. Finally, 1 mL of the sample was also extracted for sexual blood gas analysis. According to the calculation formula of effective oxygen-carrying capacity of erythrocytes, $Q = 20 \times (S1-S2)$, the unit was mL. When the oxygen partial pressure rose to 100 mmHg (pulmonary arterial partial oxygen pressure) and stabilized, the oxygen saturation of Hb in the solution was measured and recorded as S1. When the oxygen partial pressure of mixed gas was 40 mmHg (oxygen partial pressure of pulmonary venous blood), the oxygen saturation of Hb was measured and recorded as S2. P50 value was obtained from the blood gas analysis results when the oxygen partial pressure reached 100 mm Hg.

Determination of 2,3-DPG and Na⁺-K⁺-ATPase

The whole blood was collected from 6 healthy donors, with each blood sample being 400 mL. The blood was treated by conventional anticoagulant and centrifuged for 10 min at 4 °C with the speed of 3300 rpm/min, then removed the plasma, erythrocyte preserve fluid (CPDA-1) was added into the suspended erythrocytes. Suspended erythrocytes will come from the same patient were divided into five portions, these six samples respectively in the preservation 1, 7, 14, 21, 28 day were detected according to the 2,3-DPG and Na⁺-K⁺-ATPase kit instructions¹⁰.

Observation of Erythrocyte Morphology

Erythrocytes in different storage times were collected and the morphology of erythrocyte was characterized on H7600

TEM (Hitachi, Japan) at 0, 7, 14, 21, 28 days after the storage of erythrocytes.

Membrane Potential Assessment of Erythrocytes

Suspended erythrocytes were resuspended in 0.5 mL JC-1 working liquid after centrifuging under 400 r/min for 5 min, upside down several times, then put into incubator at 37 °C for 20 min with 5% CO₂. After 20 min, the cells were washed with pre-ice bath JC-1 buffer. Finally, added the normal medium and observed under the fluorescence microscope. The cells superposition of red fluorescence and green fluorescence could be recorded. The previous treatment was as follows, after encapsulating the probe 20 min, the JC-1 buffer was used to wash off the unloaded fluorescent probe. Finally, the fluorescence intensity was measured by Flow Cytometer¹¹.

Determination of Ca²⁺ in Cytochrome

Erythrocytes were collected after centrifuging at 1000 r/min for 5 min and washed twice with PBS, and then resuspended in PBS with a concentration of 1×10^{10} cells/mL¹². The final concentration of 5 µmol/L Fluo-3/AM Ester and 0.0625% Pluronic F-127 were added into erythrocytes suspension. The mixture was cultured at 37 °C in 5% CO₂ incubator for 45 min, and then washed with PBS for twice to remove the excess stain. The superposition of red fluorescence and green fluorescence could be recorded. The previous treatment was as follows, after encapsulating the probe 20 min, the JC-1 buffer was used to wash off the unloaded fluorescent probe. Finally, the fluorescence intensity was measured by Flow Cytometer.

Determination of Reactive Oxygen Species (ROS) in Red Blood Cells

Erythrocytes suspension 200 µL were centrifuged at 2 000 r/min for 5 min to discard supernatant¹³. Erythrocytes were gently washed with two times volume of normal saline, and centrifuged at 2 000 r/min for 5 min to discard supernatant. The erythrocytes were diluted 10 times by adding normal saline. 100 µL diluted cell suspension was centrifuged at 2 000 r/min for 5 min to discard supernatant. Diluted DCFH-DA (1:1 000 physiological saline) 200 µL was added for incubation at 37 °C for 30 min. The mixture was centrifuged at 2000 r/min for 5 min to discard the supernatant. Erythrocytes were washed three times with 200 µL normal saline. The cells were lysed with equal volume RIPA cell lysate liquid. The products of cell lysis were added to the 96-well black detection plate, and the fluorescence value was determined by a fluorescence enzyme standard (excitation wavelength 488 nm, emission wavelength 525 nm). 20 µL cell lysis product was extracted and the hemoglobin content was determined according to the kit instructions. The

ROS content in erythrocytes was expressed by the ratio of fluorescence value to hemoglobin value.

Western Blot Assay

Western blot was used to detect the expressions of Mitofusin 1 (Mfn1) and Mitofusin 2 (Mfn2) proteins on the cyto-membrane surface. The cells were added into cell lysis buffer, homogenized in an ice bath with a glass homogenizer, and the supernatant was collected by centrifugation, collected protein samples from cells were extracted with RIPA buffer, and a suitable amount of concentrated sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) protein buffer solution was added, boiled for 5 min to make the protein denature and cooled down to room temperature. Protein samples were separated by 12% SDS-PAGE and transferred to NC membranes at 200 mA for 90 min. After blocking the membranes at room temperature for 2 h in 5% low-fat milk powder in Tris-buffered saline-Tween 20, the membranes were probed with primary antibodies and incubated at 4°C overnight. All of the primary antibodies were diluted 1:100. After washing, the membranes were incubated with the appropriate horseradish peroxidase-labeled secondary antibodies (diluted 1:8000) for 1 h at room temperature. Bound antibodies were visualized with enhanced chemiluminescence reagents. After a final wash with Tris-buffered saline-Tween 20, multiple images were obtained and the protein bands were quantified by using a Bio-Rad Chemi EQ densitometer and Bio-Rad Quantity One software.

Statistical Analysis

All experiments were replicated independently at least three times. The data were analyzed using one-way analysis of variance (ANOVA) and are presented as the mean \pm standard deviation (SD). Statistical significance was defined as $P < 0.05$.

Results

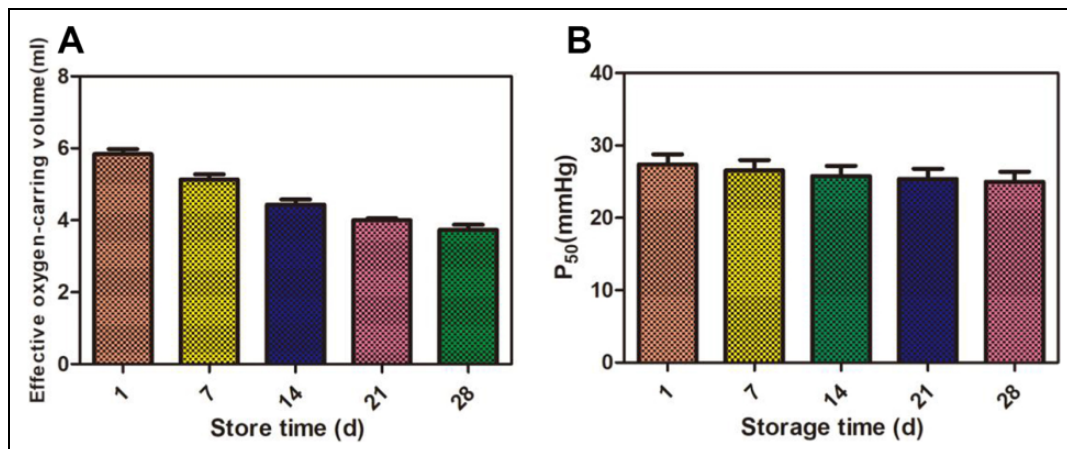
Determination of Effective Oxygen-Carrying Capacity (Q) and P50

The Q value of oxygen-carrying capacity of erythrocytes in the storage period decreased with the number of days. Effective oxygen-carrying capacity decreased 12.0% after 7 days of storage, 24.0% after 14 days of storage, 31.0% after 21 days of storage, and 43.1% after 28 days of storage.

The oxygen affinity value (P50) of suspended erythrocytes showed a gradually decreasing trend in retention period, P50 significantly decreased in the first 2 weeks, and then the change was not obvious. 3.0% decreased after 7 days of storage; 3.4% decreased after 14 days of storage, the phenomenon was not obvious in the following weeks. The detailed results were shown in Table 1 and Fig. 1. The

Table 1. Changes of Erythrocyte Oxygen Carrying Capacity at Different Storage Time.

(Day)	1	7	14	21	28
Q (mL)	5.83 ± 0.21	5.13 ± 0.21	4.43 ± 0.20	4.00 ± 0.08	3.30 ± 0.08
P50 (mmHg)	27.4 ± 2.0	26.7 ± 2.0	25.8 ± 2.0	25.4 ± 2.0	25.0 ± 2.0

**Figure 1.** The changes of carrying oxygen capacity in erythrocytes at different store time. (A) Effective oxygen-carrying volume of erythrocytes suspension; (B) P50 (mmHg) in erythrocytes suspension.**Table 2.** Changes of Erythrocyte Oxygen Carrying Capacity at Different Storage Time.

(Day)	1	7	14	21	28
2,3-DPG (μmol/mL)	1.67 ± 0.21	1.58 ± 0.20	1.40 ± 0.20	1.25 ± 0.27	0.8 ± 0.35
Na ⁺ -K ⁺ -ATPase (μmol Pi.gHb ⁻¹ hour ⁻¹)	7.23 ± 2.1	3.90 ± 1.6	2.60 ± 0.8	2.21 ± 0.7	2.01 ± 0.5

change of P50 reflected that the oxygen affinity of erythrocytes increased with the increase of preservation time, which was not conducive for hemoglobin to the release oxygen.

2, 3-DPG, Na⁺-K⁺-ATP Were Detected and Analyzed Respectively

2,3-DPG decreases gradually with the increase of storage days, and decreased slowly after 21 days, the content of 2,3-DPG rapidly decreased of 25.1%. By the end of storage time, the content of 2,3-DPG was only 52.1% of 1 day erythrocytes. The content of Na⁺-K⁺-ATPase decreased gradually with the increase of the number of days in stock. The content of 2,3-DPG rapidly decreased of 46.1 before 7 days, and it come to be slowed down to 27.8%. At the end of storage time, the content of Na⁺-K⁺-ATPase decreased of 27.8% compared with 1 day. The detailed results were shown in Table 2 and Fig. 2.

The Morphology Change of Erythrocyte

The morphology of erythrocyte was observed under an electron microscope after erythrocytes storing for 1, 7, 14, 21, and 28 days, respectively. In the first 2 weeks, the erythrocytes

were observed to be elliptical in shape, uniform and shiny, which indicated that the cells were well preserved. However, morphological map of the cells after 21 day showed that the cells were significantly sparse than those before 21 days, and even more sparse and irregular when they were stored until the 28th day. The detailed results were shown in Fig. 3.

Membrane Potential Assessment of Erythrocytes

Cells were collected and stained with JC-1, then detected by flow cytometry. The cells with membrane potential in each group were shown in Fig. 4. 1 day was considered as an experimental control. Compared with the control group, the red fluorescence of JC-1 in cytomembrane of erythrocytes was significantly decreased after 7 days of storage, and the fluorescence intensity was not significantly changed after 14 days and 21 days, while significantly decreased after 28 days of storage.

Changes of Ca²⁺ in Mitochondria of Erythrocytes

The changes of Ca²⁺ in erythrocytes were analyzed by the Ca²⁺ detection kit. The results were shown in the Fig. 5. The changes of Ca²⁺ in the first 7 days were not obvious, the

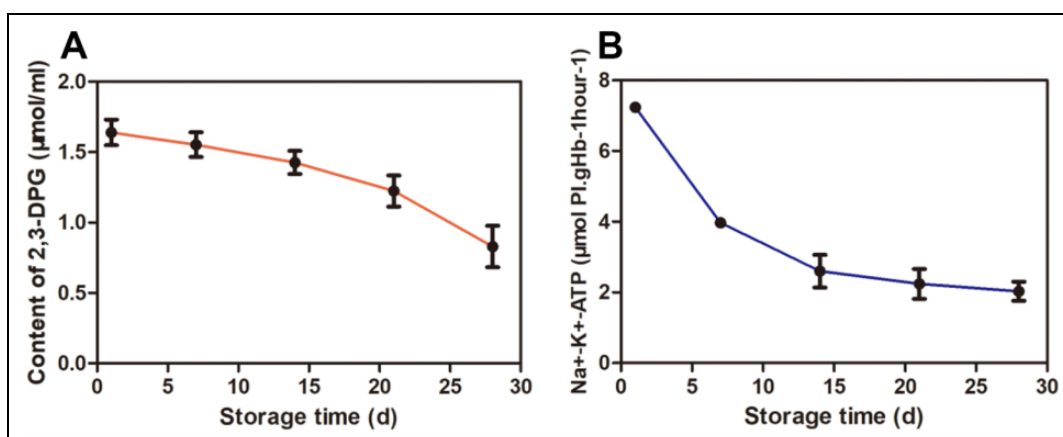


Figure 2. The changes of 2,3-DPG, $\text{Na}^+\text{-K}^+\text{-ATPase}$ in red blood cells in different storage time. (A): The content of 2, 3-DPG ($\mu\text{mol}/\text{mL}$); (B): The content of $\text{Na}^+\text{-K}^+\text{-ATP}$ ($\mu\text{mol Pi}\cdot\text{gHb}^{-1}\cdot\text{hour}^{-1}/\text{mL}$).

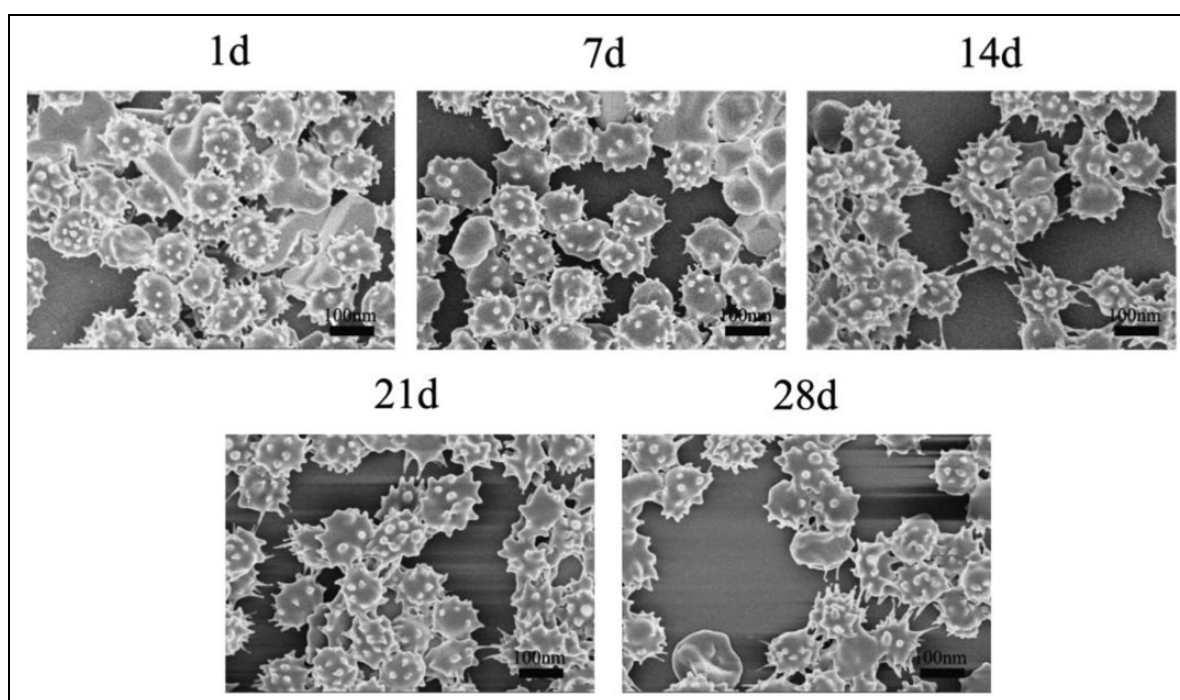


Figure 3. The change of erythrocyte morphological image in different storage time, from the 1 day to 28 day.

fluorescence intensity of Ca^{2+} was significantly increased in the 14th day. The changes of Ca^{2+} in erythrocytes were statistically significant at the 21st and 28th days, compared with the 1st, 7th and 14th days, $P < 0.05$.

Determination of ROS in Mitochondria of Erythrocytes

DCFH-DA fluorescent probe kit was used to detect the content of active oxygen (ROS) in erythrocytes. We have found that there is no or only a small amount of DCF green fluorescent before the storage of 14 days. Which indicated that less reactive oxygen species were produced. While the green fluorescence of DCF were significantly elevated after 14 days.

The relative fluorescence intensity in 14 day was 4.5 times compared with the first day ($P < 0.05$), the relative fluorescence intensity at 21 days was 7.6 times higher than that on the first day, showing a significant difference ($P < 0.05$), while at 28 days that increased 31.3 times compared with that on the first day, at day 1, showing a significant difference ($P < 0.01$). The detailed results were shown in Fig. 6.

Western Blot Assay

Western Blot was used to detect and analyzed the expression levels of Mitofusin 1 (Mfn1) and Mitofusin 2 (Mfn2) proteins on the cytomembrane. Results analysis showed that the

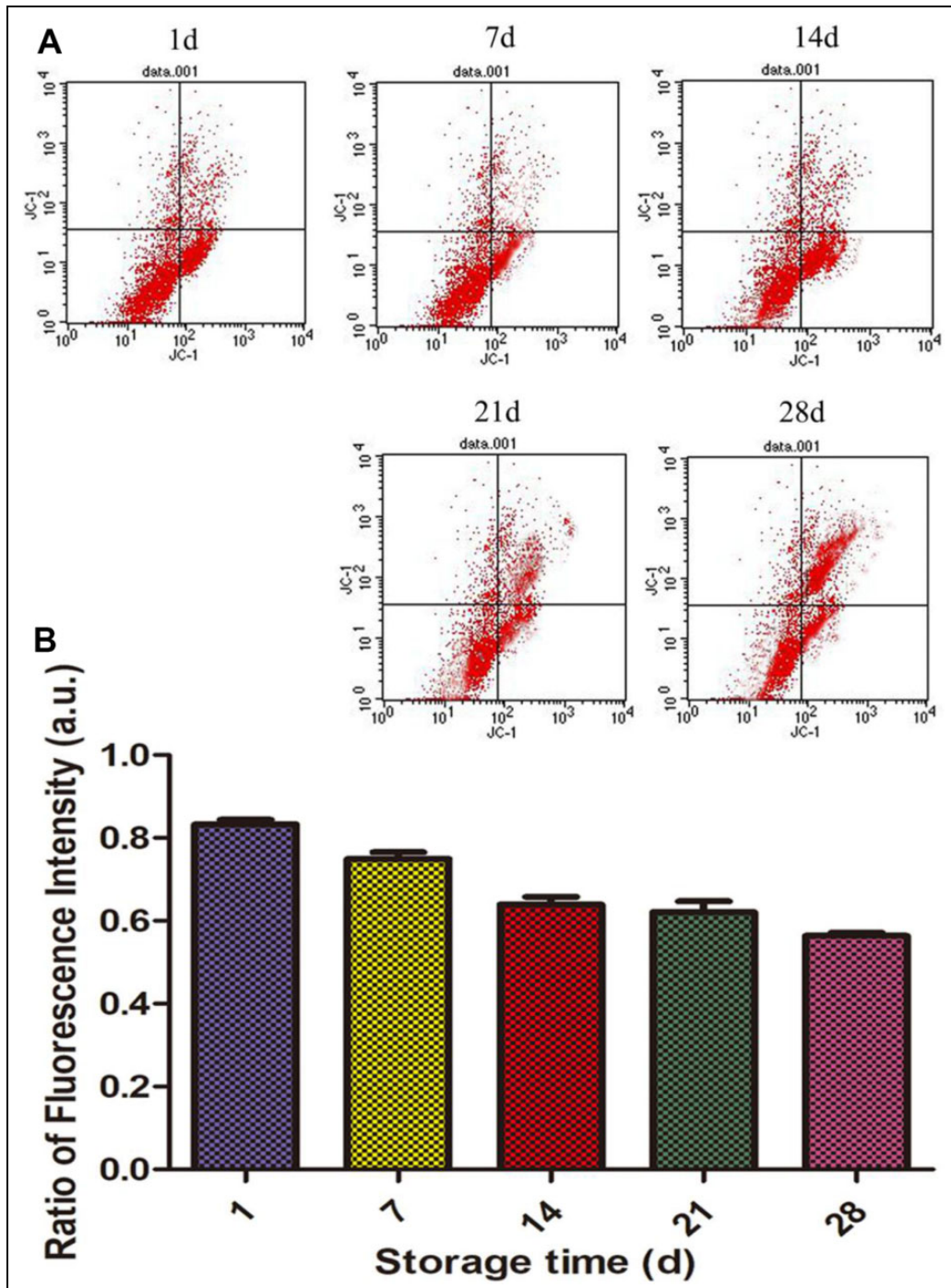


Figure 4. Membrane potential changes of cytomembrane of erythrocytes. (A): membrane potential changes were recorded by fluorescence microscopy; (B): the ratio of low membrane potential cells was recorded by flow cytometry.

expression levels of Mfn1 /Mfn2 were decreased compared with control group of erythrocytes storage of 7 days. There was a significant difference to be shown in Fig. 7.

Discussion

Effective oxygen-carrying capacity is the most intuitive indicator to evaluate the oxygen carrying capacity of

erythrocytes. It refers to the number of oxygen milliliters that 100 mL of blood can actually be transported to the tissues after a blood circulation under normal physiological conditions and standard air pressure. The actual amount of oxygen transported by erythrocytes to various tissues is related to the partial oxygen pressure in arterial blood. Therefore, the effective oxygen carrying capacity of 100 mL blood of a person under normal physiological

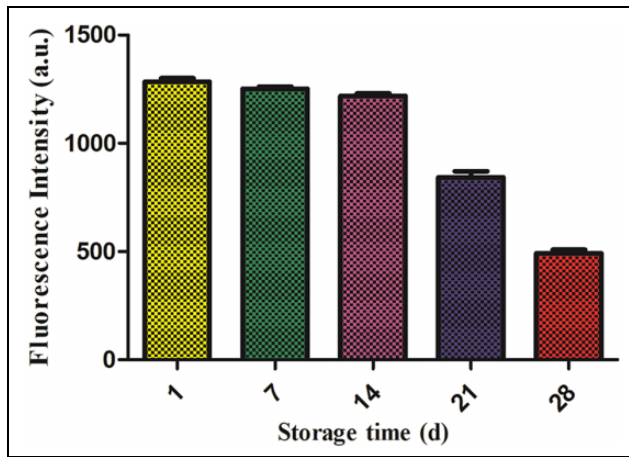


Figure 5. Fluorescence intensity of Ca^{2+} in mitochondria of red blood cells in different storage time.

conditions is about 4-5 mL in fact¹⁴. According to the experimental results, the effective oxygen-carrying capacity of the erythrocytes in the first 14 days was within the normal range, but gradually decreased from 14 to 21 days. The detection result of Q at 28 days was 3.3 mL. With the increase of erythrocyte inventory time, the effective oxygen-carrying capacity decreased gradually, which was consistent with domestic and foreign literatures¹⁵. Therefore, it is necessary to pay more attention to the storage time during autotransfusion and timely remind patients of the poor quality of stored blood.

Currently, the main oxygen dissociation curve is used to detect the oxygen carrying function of erythrocytes, and its corresponding parameter is P50, which can be used to reflect the affinity between erythrocytes and oxygen¹⁶. P50 refers to partial saturated oxygen pressure in arterial blood. It is the partial oxygen pressure at 50% oxygen saturation of hemoglobin, which reflects the oxygen affinity of hemoglobin. The smaller the P50 value is, the greater the oxygen affinity is, and the stronger cell's ability to bind oxygen is, which is not conducive to release oxygen. On the contrary, the smaller the oxygen affinity is, the weaker erythrocytes' ability to bind oxygen is, and the easier releasing of oxygen is¹⁷. We have found that P50 decreased gradually with the extension of preservation time from the results, which indicated that the ability of red blood cells to release oxygen was decreased. Moreover, P50 is affected by blood pH, temperature, partial pressure of CO_2 , 2,3-DPG concentration^{18,19}. Although the determination of P50 can largely reflect the oxygen carrying capacity of erythrocytes, the process of oxygen carrying function in erythrocytes is complex dynamic, and it is not comprehensive to evaluate the function of red blood cells only from the perspective of oxygen affinity.

The important role of 2,3-diphosphoglycerate (2,3-DPG) in erythrocytes is to regulate the oxygen-carrying function

of hemoglobin. 2,3-DPG binds to deoxyhemoglobin, thereby reducing the affinity of hemoglobin to O_2 and promoting the dissociation of O_2 with hemoglobin. Especially when blood passes through tissues, the presence of 2,3-DPG in red blood cells can significantly increase the release of O_2 for tissue needs. From the above results, it can be seen that with the increase of erythrocyte storage times, the concentration of 2,3-DPG gradually decreases, indicating that O_2 is difficult to release²⁰.

Erythrocytes adjusts the volume of cells mainly by regulating the contents of Na^+ and K^+ in the cells. These cation pumps are dependent on $\text{Na}^+\text{-K}^+\text{-ATPase}$ on the membrane, which plays an important role in maintaining normal morphology of cells, and promoting the hydrolysis of ATP for all cells to survive²¹. However, the activity of $\text{Na}^+\text{-K}^+\text{-ATPase}$ decreased, which reduced the hydrolysis of ATP and affected the normal metabolism of erythrocytes. It is because the decrease of $\text{Na}^+\text{-K}^+\text{-ATPase}$ causing the release of K^+ in the cells and the imbalance between the inside and outside ions in erythrocytes, thus affecting the oxygen carrying capacity of the red blood cells.

The production of reactive oxygen species (ROS) increased caused its antioxidant capacity decreased. ROS accumulated in the body which resulted in the opening of membrane permeability, enhanced intracellular oxidative stress and apoptosis^{22,23}. Membrane and DNA are easily oxidized under the effect of ROS, which may lead to membrane rupture, gene mutation and fragment loss, and eventually lead to cell function pathological changes or aging of the body²⁴.

The decreased expression of Mfn1/Mfn2 indicates that the cytomembrane fusion in erythrocytes is impaired and the division is strengthened, thus inducing the apoptosis of erythrocytes. The above experimental data showed that the decrease of Mfn1/Mfn2 resulted in the imbalance of erythrocytes, which affected the morphology and function of erythrocytes, reduced ATP generation, decreased membrane potential, and increased apoptosis rate, thus affecting the dysfunction of erythrocytes²⁵.

In a word, this study proposed by detecting some indicators of erythrocytes carry oxygen. To explore the apoptosis mechanism process from the changes of membrane potential and reactive oxygen species of erythrocytes in different storage days, and give a comprehensive evaluation of erythrocytes carry-oxygen function. Cellular mechanisms in detail as shown in Fig. 8. To observe the effect evaluation of erythrocyte infusion in clinical blood transfusion patients with different storage days, so as to provide experimental basis for the establishment of erythrocyte functional dose, standardize and rationalize the application of erythrocytes, and provide theoretical basis for improving the precise infusion of clinical erythrocytes.

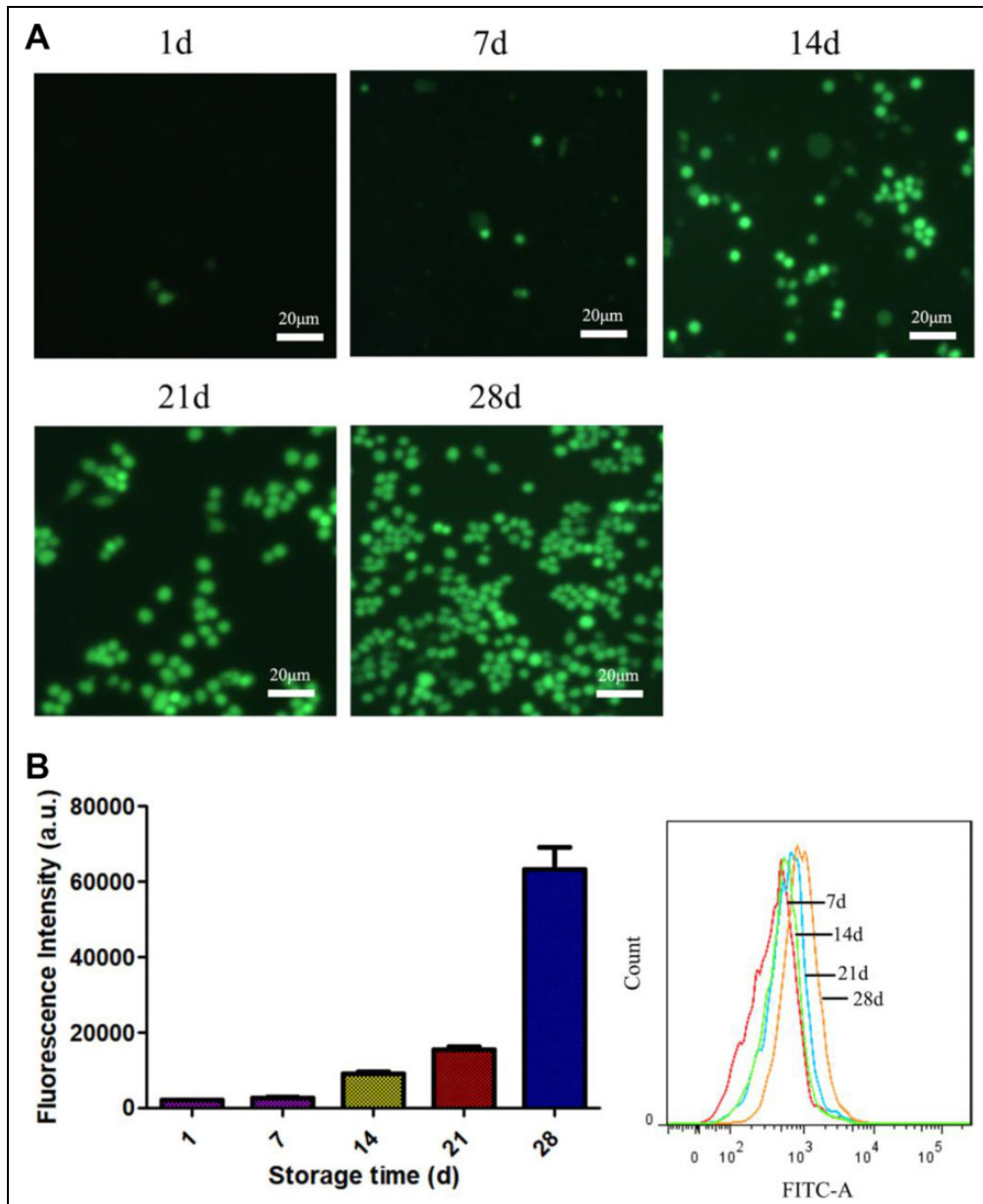


Figure 6. The level of ROS in blood cells detected with DCFH-DA probe. (A) Fluorescence imaging; (B): Fluorescence intensity of ROS in blood cells in different storage time. ($n = 3$).

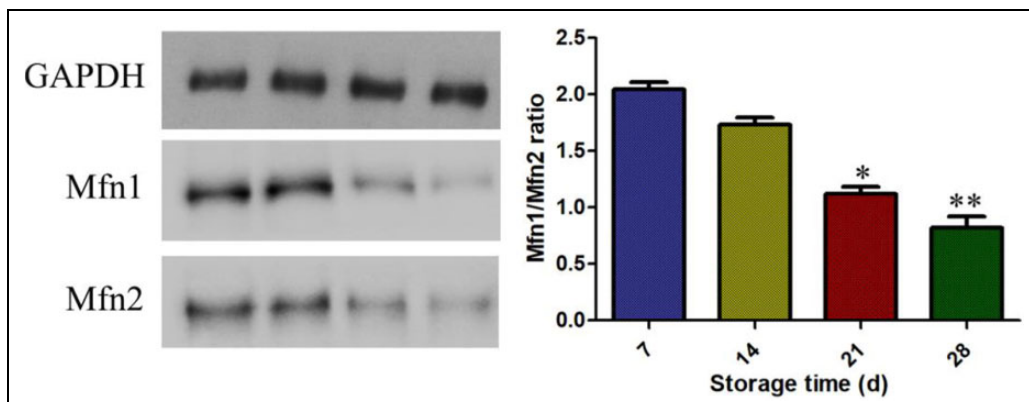


Figure 7. Western blot analysis of expression levels of Mitofusin 1 (Mfn1) and Mitofusin 2 (Mfn2) on the cytomembrane. ** $P < 0.01$ compared with the storage of 7 days.

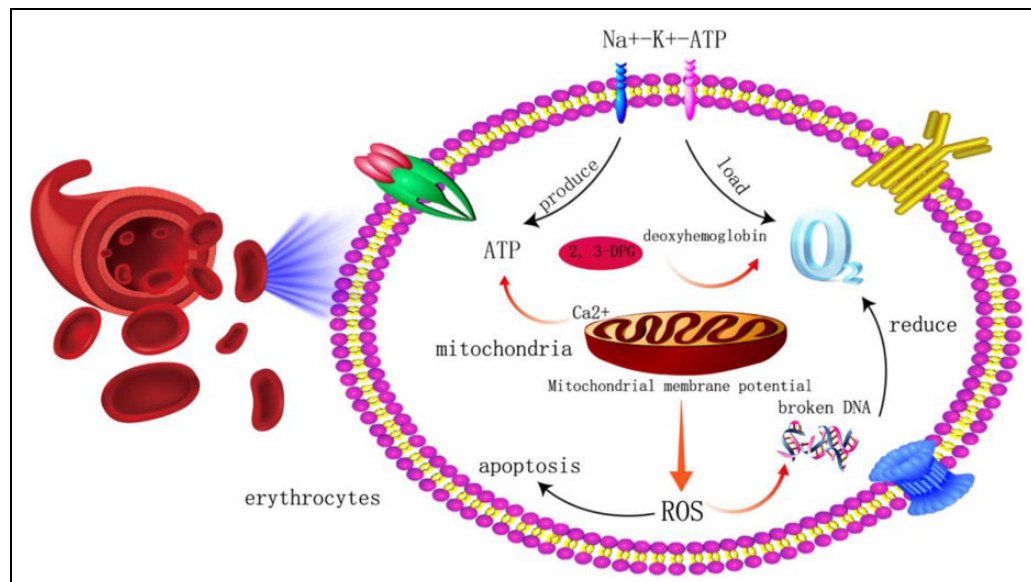


Figure 8. Schematic diagram of erythrocyte oxygen transport and apoptosis.

Conclusion

The change of erythrocyte oxygen-carrying capacity in different storage time, such as effective oxygen uptake (Q), P50, 2,3-DPG, $\text{Na}^+\text{-K}^+\text{-ATPase}$ will affect membrane potential, the change of Ca^{2+} , and reactive oxygen species (ROS) change of erythrocytes. The different storage time will improve generation of oxidative stress to produce ROS, and further damage the function of erythrocytes.

Authors Note

Zhen-Zhou Li and Dong-Lin Jia co-first author.

Zhen-Zhou Li and Dong-Lin Jia are authors contributed equally to this work.

Acknowledgement

We would like to acknowledge the reviewers for their helpful comments on this paper.

Authors Contribution

Each author has made an important scientific contribution to the study and has assisted with the drafting or revising of the manuscript.

Consent to Publish

All of the authors have Consented to publish this research.

Ethics, Consent and Permissions

Ethical approval was given by Gongli hospital, The Second Military Medical University.

Ethical Approval

Ethical approval was given by Shanghai Gongli Hospital, the Second Military Medical University, Shanghai, China.

Statement of Human and Animal Rights

All procedures in this study were conducted in accordance with Shanghai Gongli Hospital, the Second Military Medical University, Shanghai, China.

Statement of Informed Consent

Written informed consent was obtained from the patients for their anonymized information to be published in this article.


Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work is supported by Key Disciplines Group Construction Project of Pudong Health Bureau of Shanghai (No. PWZxq2017-10), and National Natural Science Foundation of China (No. 81870147).

ORCID iD

Jian-Rong Guo  <https://orcid.org/0000-0001-9692-6614>

References

1. Lu J, Liu YG, Qian YB, et al. The effect of different transfusion proportion on the prognosis of patients with trauma transfusion. *Chinese Journal of Trauma*. 2017;33(5):453–458.
2. Wang QL. Research progress on erythrocyte storage injury. *Chinese Journal of Blood Transfusion*. 2012;25(6):515–519.
3. WJ Ma, MH Bian, MJ Zheng, et al. Study on apoptosis of suspended erythrocytes induced by in vitro storage. *J Anhui Med Univers*. 2015;(2):189–193.
4. Holmberg JA. Blood you can trust: global trends in transfusion safety. *MLO Med Lab Obs*. 2015;47(10):20–22.

5. D Orlov, K Karkouti. The pathophysiology and consequences of red blood cell storage. *Anaesthesia*. 2015;70(1):29–37.
6. Barshtein G, Gural A, Manny N, Zelig O, Yedgar S, Arbell D. Storage-induced damage to red blood cell mechanical properties can be only partially reversed by rejuvenation. *Transfus Med Hemother*. 2014;41(3):197–204.
7. Lückner A, Weber B, Jenny P. A dynamic model of oxygen transport from capillaries to tissue with moving red blood cells. *Am J Physiol Heart Circ Physiol*. 2015;308(3): H206–H216.
8. Tuo WW, Wang D, Liang WJ, Huang YX. How cell number and cellular properties of blood-banked red blood cells of different cell ages decline during storage. *Plos one*. 2014;9(8):e105692.
9. Bei-zhan YAN, Hui-min MA, KONG Cun-quan LIANG Yu, Wei-yan ZHU, Shu-ting JIANG. Research on the oxygen-carrying capacity and survival changes of red blood cell in different preservation time. *Chinese J Health Lab Technol*. 2017;12:15.
10. Yi X, Liu M, Wang J, Luo Q, Zhuo H, Yan S, Wang D, Han Y. Effect of phase-change material blood containers on the quality of red blood cells during transportation in environmentally-challenging conditions. *PLoS One*. 2020;15(1):e0227862.
11. Yang J, Cheng Y, Du L, Gong W, Shi S, Sun J, Chen B. Analyzing the effect of the incidence angle on chlorophyll fluorescence intensity based on laser-induced fluorescence lidar. *Opt Express*. 2019;27(9):12541–12550.
12. H Qing, LJ Yang, SQ Zhang, et al. Effects of sinoaortic denervation on mitochondrial Ca^{2+} and membrane potential of nerve cell in rats. *Chinese J Int Med Cardio-/Cerebrovascular Dis*. 2016; 14(17):1999–2001.
13. SL Wang, W Han, LJ He, et al. Correlation analysis between ATP, 2,3-DPG concentration in suspended red blood cells without leukocyte and Oxidative stress during storage. *J Hebei Med Univers*. 2017; 38(2):192–195.
14. Hamasaki N, Yamamoto M. Red blood cell function and blood storage. *Vox Sang*. 2000;79(4):191–197.
15. YJ Li, X Wang, K Lan. Effects of blood preservation on erythrocyte oxygen-carrying function and energy metabolism. *Chinese journal of blood transfusion*. 2009; 22(6): 439–443.
16. Zhong R, Liu H, Wang H, Li X, He Z, Gangla M, Zhang J, Han D, Liu J. Adaption to high altitude: an evaluation of the storage quality of suspended red blood cells prepared from the whole blood of Tibetan plateau migrants. *PLoS One*. 2015;10(12): e144201.
17. Mairbaurl H. Red blood cells in sports: effects of exercise and training on oxygen supply by red blood cells. *Front Physiol*. 2013;4:332–336.
18. M Luo, YY Huang, BC Su, et al. Study on the dynamic change of erythrocyte oxygen carrying function with incubation time. *Spectroscopy and spectral analysis*. 2015; 5(12):3350–3355.
19. BZ Duan, RJ Zhang, JH Jiao. Effects of blood preservation on erythrocyte oxygen-carrying function and energy metabolism. *Int J Lab Med*. 2015;36(4):556–557.
20. A Heaton T, Keegan S, Holme. In vivo regeneration of red cell 2,3-diphosphoglycerate following transfusion of DPG-depleted AS-1, AS-3 and CPDA-1 red cells. *Br J Haematol*. 1989;71(1):131–136.
21. Jinghan L, Qun L, Wei H, Haibao W, Qingmei W, Xijin L. Experimental research of blood preservation under non-4 °C conditions. *Med J Chin PLA*. 1998;23(2):103–104.
22. Ferdous Z, Beegam S, Tariq S, Ali BH, Nemmar A. The in vitro effect of polyvinylpyrrolidone and citrate coated silver nanoparticles on erythrocytic oxidative damage and eryptosis. *Cell Physiol Biochem*. 2018;49(4):1577–1588.
23. Nemmar A, Beegam S, Yuvaraju P, Yasin J, Shahin A, Ali BH. Interaction of amorphous silica nanoparticles with erythrocytes in vitro: role of oxidative stress. *Cell Physiol Biochem*. 2014; 34(2):255–265.
24. Gerhard GS, Benko FA, Allen RG, Tresini M, Kalbach A, Cristofalo VJ, Gocke CD. Mitochondrial DNA mutation analysis in human skin fibroblasts from fetal, young, and old donors. *Mech Ageing Dev*. 2002;123(2/3):155–166.
25. Hall AR, Burke N, Dongworth RK, Kalkhoran SB, Dyson A, Vicencio JM, Dorn GW, Yellon DM, Hausenloy DJ. Hearts deficient in both Mfn1 and Mfn2 are protected against acute myocardial infarction. *Cell Death Dis*. 2016; 26(7):e2238.