Research Article

Assessment of physical damage of cryopreserved RBCs during thawing by impedance spectroscopy

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Abstract

Background: Preservation of red blood cells (RBCs) is an important task to ensure a long-term, readily available, safe blood supply for transfusion during emergency. Effective preservation procedures are required at various steps for the long-term storage of an RBC product including testing, inventory, quality control, and product distribution. Biopreservation is the process of maintaining the integrity and functionality of cells outside the native environment for long storage times. Hypothermic storage, cryopreservation, and lyophilization are the various methods of biopreservation.

Objective: To monitor the physical integrity of erythrocytes after cryopreservation with the help of impedance spectroscopy.

Materials and Methods: Cryoprotectants were added to protect the erythrocytes from cryoinjuries. In addition, the erythrocytes viability was monitored through hemolysis test. Afterward, the exact numbers of live or lysed cells were observed by the effect of thawing rate on the cells through impedance spectroscopy, as we know that there is a relationship between impedance, frequency, and temperature.

Result: As the frequency increases, the impedance decreases, which indicates that the RBCs are lysed. The impedance starts decreasing at –200 °C and drastically decreased at –140 °C.

Conclusion: This study indicates the real viability of the RBCs through impedance measurement by the impedance spectroscopy.

KEY WORDS: Cryopreservation, lyophilization, cryoprotectants, impedance spectroscopy, cryoinjuries

Introduction

This study deals with the monitoring of cryopreserved erythrocytes by impedance spectroscopy. The life span of erythrocytes does not last longer than 21 days at normal conditions.[1] Owing to the short shelf life of erythrocytes, the transfusion problems in case of emergency conditions such as cardiovascular surgery are prominent. The objective of cryopreservation is to cease all the biochemical and metabolic activities of the erythrocytes.[2] Biopreservation is the process of maintaining the physical integrity and functionality of cells in ex vivo to increase the viability for longer period.[3] The cryopreservation of red blood cells (RBCs) also fulfills the demand for the rare blood groupers and during the wars and natural disasters.[4] Cryopreservation may alter membrane's phase and permeability.[5] As RBCs are delicate in nature, their integrity may alter during cryopreservation because of (a) ice crystal formation by nucleation and (b) alteration of intra- or extracellular solute concentration.[6] So, to avoid the chilling injuries caused during cryopreservation at subzero temperature, cells and tissues are cryopreserved along with the cryoprotectants [cryoprotective agents (CPA)].[7] These agents work by inhibiting the nucleation of ice crystals, thereby ceasing the freezing injuries.[8,9] Various cryoprotectants being used are glycerol, dimethyl sulfoxide (DMSO), sugars, egg yolk, bovine serum albumin, and so on.[10]

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The viability of RBCs after cryopreservation can be monitored through impedance spectroscopy at various temperatures.[11–13] Electrical impedance is used to characterize the cellular alteration quantitatively.[14] This functions on the basis of conductance and monitors the RBCs' plasma membranes, cell volumes, and the intra- and extracellular conductivity parameters.[15] Bioimpedance works with some passive electrical properties of tissue, the ability to resist electric current flow.[16] Tissue may be concerned as a volume conductor or a dielectric.[17] In the frequency range <100 kHz, most tissues are predominantly electrolytic conductors.[18] However, at low frequencies, close to ~10 Hz, tissues show some dielectric characteristics and act as capacitors, which is totally facilitated by tissue structure (i.e., inner and outer cellular compartments and the cellular membrane).[19] This frequency-depending electrical properties of cells or RBCs are the basic key parameters to measure impedance of the cell. This study shows the relationship between impedance, frequency, and temperature. As the frequency increases, the impedance decreases, which indicates that the RBCs are disintegrated.[20] The experiments were carried out at different thawing rates, because during thawing at low warming rates, recrystallization can occur: the ice crystals that entered the cell during freezing process will have time to grow and eventually reach a lethal size that can damage the cell.

Materials and Methods

Chemicals and Reagents

All chemicals used during experiments were analytical grade. The distilled water was used during preservation; solutions were purified by Millipore water purification system. DMSO and filter papers were purchased from Qualigen, Mumbai, India. The cryovial and Eppendorf tubes were obtained from Tarson, Pvt., Ltd., Kolkata, India. Trisodium citrate was obtained from Loba Chemie, Pvt., Ltd., Mumbai, India.

For long-term preservation, we use various types of CPAs in different concentrations to know their protective effect during the storage of RBCs. After plunging into liquid nitrogen, the samples are thawed and then checked for their viability in terms of percentage hemolysis and plasma potassium concentration.

Blood Sample Collection

The various human blood samples were collected in the citrate phosphate dextrose bag from healthy, adult volunteers from the blood bank of CWS Hospital, Jagda, Rourkela, Orissa, India, and used for preservation of isolated RBCs in different cryoprotective solutions. For the impedance spectroscopy analysis, the blood was drawn with the help of 5-mL syringe and further processed for the isolation of RBCs.

Isolation of RBCs

The erythrocytes were separated from the whole blood by centrifugation (329*g*, 14 min, at 4 °C).[15,16] The cells were then washed three times in isotonic CPD-A solution by centrifugation at 515*g* for 10 min.

Formulation of Buffer and Protective Solutions

CPD and CPD-A were used as protective solutions and Adsol, Nutricel, and Optisol as additive solutions. The buffer that showed lower hemolysis during preservation was further used for the formulation of different CPAs for control rate preservation of RBCs in liquid nitrogen.

Control Rate Freezing of RBCs

The controlled rate freezing minimizes the effect of low temperature and osmotic shock during preservation of biological samples at low temperature. To investigate the effect of thawing rate on the viability of RBCs, experiments were performed with different temperature. The thawing rate was decreased from –1 °C/min up to –60 °C. Then, the temperature was increased at a rate 5 °C from –60 to –20 °C. Finally, the temperature was increased at a rate of 1 °C up to +20 °C.

Real-Time RBCs Viability Measuring Through Impedance Spectroscopy

The frequency-dependent electrical behaviors of cells mainly help us in measuring their instantaneous viability, as all cells contain cytoplasm and the cytoplasm contains various types of ions. An intact cells or viable cells behave similar to a capacitor. So, the instantaneous viability of RBCs was measured by considering the very basic principal that, as hemolysis increases, the conductivity of solutions also increases.

Formulation of Solutions

1. Distilled water
2. Normal saline (NS)
3. DMSO
4. NS + RBCs
5. NS + lysed RBCs

The solutions were kept in vessel one by one for impedance measurement.

Experimental Setup for Measuring Impedance Spectroscopy

For the experiment, a typical impedance spectroscopy, also called as electrochemical impedance spectroscopy, was used, which measures the dielectric properties of a medium as a function of frequency. It was based on the interaction of an external field with the electric dipole moment of the sample, often expressed by permittivity, and an experimental method for characterizing the electrochemical systems. The technique measures the impedance of a system over a range of frequencies, and, therefore, the frequency response of the system, including the energy storage and dissipation properties, was revealed. Impedance is the contradictory flow of alternating current (AC) in a computer system. A passive

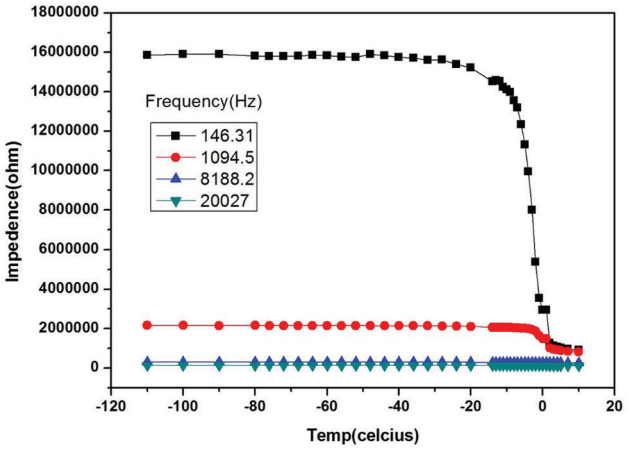

Figure 1: Impedance of RBC in distilled water.

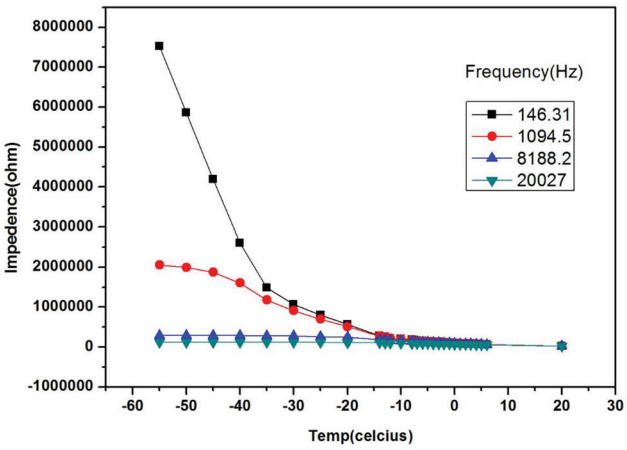

Figure 3: Impedance of RBC in DMSO.

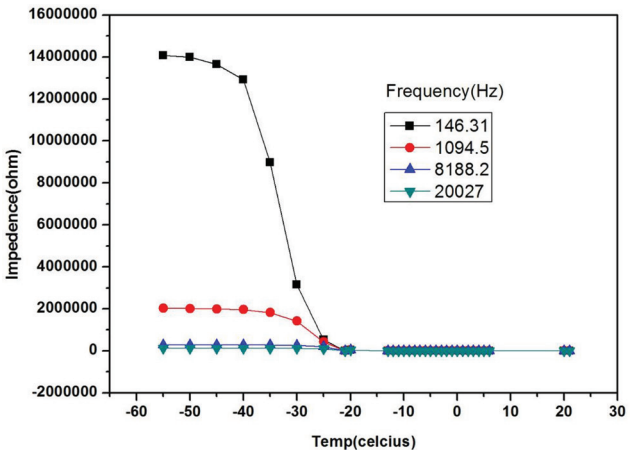

Figure 2: Impedance of lysed RBC in normal saline.

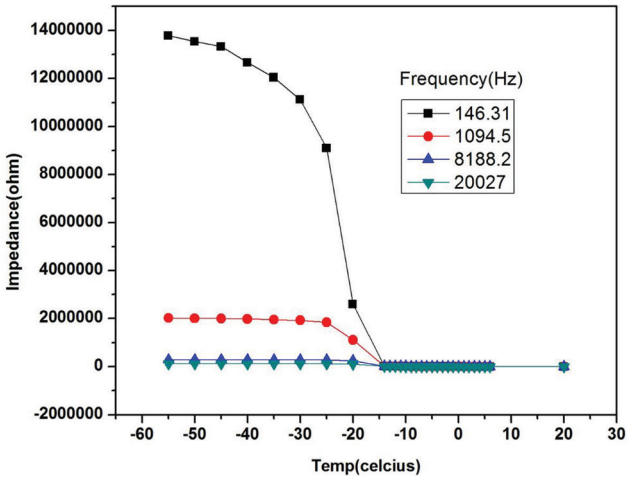

Figure 4: Impedance of RBC in normal saline.

complex electrical system consists of both energy dissipater (resistor) and energy storage (capacitor) elements. If the system is purely resistive, then the opposition to AC or direct current (DC) is simply resistance.

The impedance spectroscopy was connected to the electrochemical chamber that had two electrodes for the measurement of RBCs viability. The experimental electrochemical chamber contained: normal saline, 0.9% NaCl; 99% DMSO; and distilled water. The number of RBCs were 10×10^5 cells.

Result

After the separation of RBCs from the collected blood, the cryopreservation of RBCs was carried out. Then, the cryopreserved RBCs were real monitored using the impedance spectroscopy, which provided the information regarding the lysis of RBCs at a particular temperature. By this method, the optimum temperature for cryopreservation of RBCs was known in a particular medium.

Figure 1 shows that there is a relationship between impedance, frequency, and temperature. As the frequency increases, the impedance decreases, which indicates that the RBCs are lysed. The impedance started to decrease at –20 °C and, then, drastically decreased up to –14 °C at the frequency 146.31 Hz. Except this frequency, all the other frequencies showed constant impedance.

At the frequency 8,188.2 Hz and 20,027 Hz, the impedance remained constant as the temperature increased. At the frequency 1,094.5 Hz, the impedance slowly decreased at –20 °C, but at frequency 146.31 Hz, the impedance drastically reduced at –20 °C up to –14 °C, as shown in Figure 2.

In Figure 3, the frequency is not at peak; it shows that at –35 °C, the impedance at the frequencies 146.31 Hz and 1094 Hz declined, showing the cryoprotection effect by DMSO.

Figure 4 shows that, at the frequency 1,094.5 Hz and at the temperature –25 °C, the impedance reduced; but, for the frequency 146.31 Hz, the impedance declined at a slow rate.

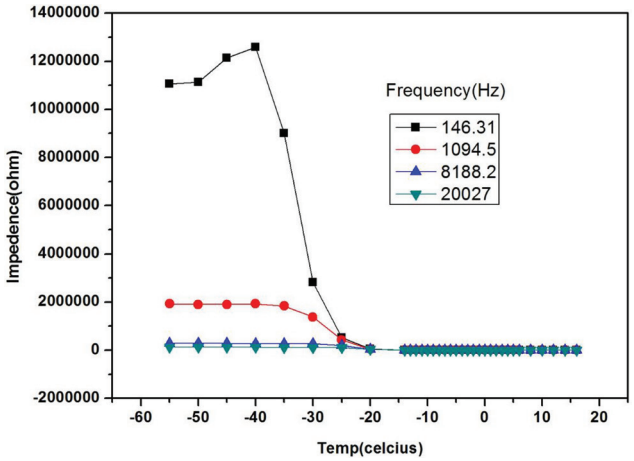

Figure 5: Impedance of lysed RBC in normal saline.

Figure 5 shows that, at the frequency 1,094.5 Hz, the graph shows declination at –35 °C; at the frequency 146.31 Hz, the graph is constant at the temperature –55 to –50 °C It, then, slowly increases from –50 to –40 °C, after which sharply decreases at –40 °C. However, the rest two frequencies show a constant graph.

Discussion

The preservation of blood components for a long time is the foremost important task for emergency purposes. Hence, from the results, it has been proved that electrochemical impedance spectroscopy is a reliable method to measure the hemolysis of RBCs after thawing. The CPAs used during the experiment minimized the disintegration of RBCs. In this research, DMSO has been proved to be highly capable of cryoprotecting the RBCs.

Conclusion

This study indicates the analysis of real viability of the RBCs through impedance measurement using the impedance spectroscopy.

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