

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Cryopreservation of Blood

Miloš Bohoněk

*Department of Hematology,
Biochemistry and Blood Transfusion,
Central Military Hospital Prague, Praha,
Czech Republic*

1. Introduction

Various methods of cryopreservation of blood, especially erythrocytes, are generally known and have been used for a long time. Storage of blood in the frozen state presented one of the alternative ways of storing blood components; this possibility was intensively explored in the 1950's and 60's, when the shelf life of non-frozen red blood cells did not exceed 21 days at those times. This time limitation significantly reduced flexibility of usage of RBC products and contributed to their dramatically high and wasteful expiration reaching up to 30%. The short shelf life of the RBCs resulted in the transfusion services not being able to meet demands of quickly evolving surgical disciplines, particularly cardiovascular surgery and radical surgical oncology. In military and emergency healthcare, utilization of these three-week products as a way of creating blood supplies was even more complicated, almost unthinkable. The storage of frozen red blood cells therefore presented a great prospect.

Following the implementation of modern resuspension solutions with addition of polysaccharides, phosphates and adenine into everyday practice in the 1970's and 80's, the general emphasis on the long-term storage of erythrocytes was withdrawn into background. In many fields, the now-normal 42-day-long life virtually eliminated the need to perform further research since no more than 5 percent of the stored RBC products had to be destroyed due to expiration.

Nevertheless, some areas with a need for long-term storage of red blood cells still remained – example being the military transfusion, emergency transfusion service, storage of rare blood cells or special autotransfusion programs. In these cases, substantially higher costs of the red blood cells production are accepted (when compared to the common storage in liquid state). Blood substitution and blood supply is a permanent strategic and logistic problem of the military medical services across the world arising from the blood, which is a biological drug, has a limited shelf life and needs the special transport and conditions of use. The same problem must solve the national health-care authorities in programs of the national blood crisis policy, where to get a huge amount of blood supply any time at any place in the case of disaster, terrorist attack and war. The therapeutic problems in immunohematology cases can solve by stock of rare blood, storage of autologous blood for patients with rare erythrocyte antigens and storage of autologous blood for patients with red blood cell alloantibodies with no chance to use common blood. All mentioned demands

highly correspond with stock of frozen blood. New global security risks exalt this problem to all-society relevancy.

2. Methods of cryopreservation of living tissues and cells

Throughout the years, tackling the matter of cryopreservation of cells and tissues has been deriving from findings about the protection of cells against the impacts of frost, especially from the knowledge about production of ice crystals that cause the subsequent destruction of cellular structures and membranes [1916]. As early as 1866, French naturalist Félix-Archimède Pouchet first described that frozen erythrocytes are destroyed after thawing [16]. This effect had long been attributed solely to the mechanical damage that cells suffer from ice crystals generated in the course of freezing.

As we know now thanks to physical chemistry, however, during the changing process of aqueous solutions into solid state, it is water that changes its state of matter first. Water crystals are created from pure water, while the space in between them is filled with concentrated electrolyte. This leads to cellular dehydration and to the pH change. Based on this knowledge, James Lovelock came up with a hypothesis in 1953, suggesting that the damage effects of frost to cells first induce the cells dehydration and pH changes. Those mechanisms destroy cell membrane before mechanical injury caused by ice crystals. This evolved into a generally accepted theory [6,10,11,19].

Protection of cells from freezing is achieved by adding so-called cryoprotective substances. Since these cryoprotectants usually cause a significant increase in osmolality, it is nevertheless necessary to have all the procedures monitored, and to have osmotic changes under control, in order to avoid an irreversible damage to cellular structures and membranes caused by them [6,11,17].

With regard to the types of their effects, cryoprotectants are divided into two groups:

2.1 Intracellular (penetrative) cryoprotectants

Due to their relatively simple chemical structure, these substances penetrate the cellular membrane and do not present any toxic danger for the cell when in low concentration. Glycerol, dimethyl sulfoxide (DMSO) and certain types of glycol (ethanol, propylenglycol, methanol, etc.) belong among them. In terms of utilization, they are mostly applied when the long-term preservation of frozen tissues is needed (e.g., sperm banks, erythrocyte cryobanks, banks for stem cells and umbilical cells).

There has not been fully clarified the mechanism of the effect of penetrative cryoprotectants yet. Initially, the damage of cells was associated with the effect of ice crystals only. Cryoprotective substances, nevertheless, besides limiting the creation or frozen crystals, also modify these crystals' shape and size, and by changing their ionic ratio intracellularly as well as extracellularly, they also eliminate the damage caused by osmotic shock which otherwise occurs during freezing. During the freezing process, penetrative cryoprotectants increase output of intracellular water, maintaining the osmotic balance in a partially frozen extracellular solution in this way. It results in not only reducing the cells' volume but also in the reduction of the osmotic load. With regard to subsequent survival of cells after their

thawing, a significant effect is the inhibition of APT caused by some of the cryoprotectants such as glycerol and DMSO [15,23].

Glycerol: Cryoprotective effect of glycerol, that is also called low-molecular nonelectrolyte, was first described on sperms. Its cryoprotective effect lies in penetrating the cellular membrane into the cell's nucleus and creating a hyperosmotic environment. It used to be utilized for cryopreservation of large numbers of mammals' cells, as well as for embryos and unfertilized eggs. Now it is mostly used for erythrocyte cryopreservation. Glycerol is mostly used in concentration of 1.0 - 2.0 M, 20 - 40% (55% max.) respectively.

Dimethyl sulfoxide (DMSO): Just like glycerol, DMSO is a nonelectrolyte with low molecular mass. With regard to its cryoprotective quality, it has a similar effect to glycerol. As opposed to glycerol, however, it is more likely to become toxic - already at a concentration of 1.0 M.

Propylene glycol (PROH): Its cryoprotective effects are caused by its entirely amorphous state that it reaches in an aqueous solution. PROH excels in stability in temperatures below the freezing point. Thereby it limits crystallization during the freezing and de-freezing process. PROH is usually used in combination with other substances, aiming to limit the possibility of a specific toxic and osmotic damage to tissues and cells.

2.2 Extracellular (non-penetrative) cryoprotectants

Due to their molecular mass, these substances do not penetrate cellular membrane and are mostly used for rapid and ultra-rapid freezing. There are many possible examples, of which several should be named: monosaccharides (glucose, hexose), disaccharides (sucrose, trehalose), trisaccharides (raffinose) and polymers (polyvinylpyrrolidone, polyethylene glycol) and other macromolecular substances such as dextran, modified gelatin, hydroxyethyl starch or albumin.

The mechanism of the non-penetrative cryoprotectants effect lies in their ability to stabilize cellular membrane and also in so called vitrification. When there is water (with temperature below 0°C) turning into ice, non-penetrative cryoprotectants remain outside the cells, where they secure the creation interspaces between cellular membrane and extracellular environment. Electrolytes segregated from freezing solutions are being concentrated in these interspaces. As a consequence to change in osmotic ratio, extracellular hypertonicity removes water from a slowly freezing intracellular space. Moreover, the subsequent decrease in the osmotic differences does not damage the cell membrane. One needs to be cautious with the increase in hypertonicity of cooled cells as it may results in osmotic shock if the thawing process takes too long [5,18].

3. Cryopreservation of red cells

Only recently the great limiting factor of cryopreservation of red blood cells has been at least partially solved - the life of erythrocytes after thawing was merely one day, which drastically limited the operational use of the given product. With the discovery of new technologies and resuspension solutions in the past years, the life of the blood after its thawing ("reconstitution") was extended to 1 - 3 weeks.

The current practice takes advantage of the erythrocytes cryopreservation and the intracellular cryoprotectant glycerol is used almost exclusively. Its cryoprotective effects has been known since 1949 when the sperm cells were frozen. Only a year later, in 1950, glycerol was firstly used for the freezing of erythrocytes. Thawed RBCs were first successfully transfused in 1951 by Mollison [13]. The method used low, 20% concentration of glycerol and quick freezing, discovered by A.U.Smith [17].

The routine clinical use of cryopreservation of erythrocytes was first implemented in the United States in early 1960's. Since the beginning, a method of cryopreservation was used freezing erythrocytes in high glycerol, i.e. with the addition of 40% glycerol to erythrocytic suspension. This method is now common in most departments dealing with RBC. The freezing process is slow, and it takes place at -80°C in mechanical freezers. The frozen red cells are then stored in -65°C . The method was first described by J.L.Tullis in 1958 [21,22].

Notwithstanding, the main reason for introducing this method was pragmatic - standard PVC bags were not applicable for this method and there were problems with obtaining license that would give way to the production of aluminum containers for the storage of RBC in liquid nitrogen. In other words, it was not the question of the quality of the erythrocytes that were frozen using this method. The complication of this method was caused by storage issues. Although special plastic bags for cryopreservation at -200°C did appear on the market in the next 10 years, the already suffered massive investments into mechanical freezers, as well as high cost of liquid nitrogen in the U.S., meant that the method of freezing in high glycerol became the North American standard.

An alternative method of freezing and storing red blood cells in glycerol is rapid freezing in liquid nitrogen at -196°C ($40^{\circ}\text{C} / \text{min}$) and subsequent storing in nitrogen vapors at -170°C . The method was first described by Pert et al. in 1963 [15]. In addition to lower concentrations of glycerol (5-20%) which is easier to wash off, the advantage of this method lies in its independence on the sources of electrical energy, thus reducing the risk of endangering the stored product. There are significant practical weak points of this method: they are handling of liquid nitrogen containers, transport issues and also the scheme of Dewar containers. This method had become widely used in Europe for some time, where cryopreservative procedures were being implemented into practice 10 years later than in the US, i.e. in early 1970's. At that time, plastic bags suitable for use in extremely cold temperatures were already available, liquid nitrogen was more affordable in Europe and, unlike in the US, mechanical deep-freeze boxes represented a very high investment.

There was another reason why the way of European practice headed towards this method. It was the simplicity of the deglycerolization process which can be, at low concentration, accomplished by manual methods, or by using special rinsing attachments in a centrifuge, such as the ADL cell-washing bowl (in contrast with rinsing of highly concentrated glycerol which requires more complex technique and more sophisticated technical equipment). The comparison of RBCs cryopreservation in high glycerol and low glycerol is displayed in Table 1.

Turning to the non-penetrative cryoprotectants, there is particularly the hydroxyethyl starch (HES) in the spotlight. Its usage in the field of erythrocytes cryopreservation was patented in the U.S. in 1991. Its benefits are the bio-compatible properties of macromolecular polysaccharides (including HES, which is commonly used as volume expander) at higher concentrations, that do not require any complex rinsing procedure during the process of

thawing and reconstitution, and so the defrosted product may be directly transfused. Disadvantage of HES is the freezing and storage in liquid nitrogen and relative higher haemolysis after thawing.

	glycerol 40%	glycerol 20%
Freezing temperature	-80°C	-197°C
Freezing rate	slow	fast
Freezing technique	mechanically freezer	liquid Nitrogen
Storage temperature	min. -65°C	min.-120°C
Impact of temperature changes	thawing and refreezing is possible	critical
Containers	PVC, polyolefin	polyolefin
Transportation	dry ice	Nitrogen vapor
Time of deglycerolization	60 min.	30 min.
Hematocrit	55-70%	50-70%
Leucodepletion	94-99%	95%

Table 1. The comparison of RBCs cryopreservation in 40% glycerol and 20% glycerol

Despite the fact that deep-frozen tissues and cells (i.e. erythrocytes) can be preserved almost indefinitely (on condition they are provided with an appropriate storage temperature), it was necessary to determine an administratively acceptable storage time. Only in 1987 the United States Food and Drug Administration (FDA) set the life of at -80°C frozen RBCs to be 10 years. This time limit was adopted by other countries as well. In 2010, European legislation prolonged the frozen erythrocytes’ administrative life up to 30 years, depending on the storage method (Directive 2044/33/EC). According to the *Guide to the Preparation, use and quality assurance of blood components* (No.R Recommendation (95) 15 Council of Europe), “the storage of frozen RBCs may be extended to at least ten years if the correct storage temperature can be guaranteed.” [7,26,27].

From the practical point of view, the usability of erythrocytes after reconstitution, i.e. after defrosting and deglycerolization, is a more important parameter than their storage time in a frozen state. For a long time, reconstituted erythrocytes expired after 24 hours, which greatly limited their flexibility and possibilities of their usage. This did not change even after the introduction of semi-automated deglycerolization systems in 1970’s (Haemonetics ACP-115, IBM Cell Washer, Elutramatic cell washer). There came a revolutionary breakthrough and significant prolongation of the erythrocytes life after thawing after the fully automated device (Haemonetics ACP-215) had been invented and homologated for general usage by FDA in 2011. This invention made the glycerolization as well as the deglycerolization possible in a fully closed manner, while locking it out of external environment and thus performing it out of contact with this external environment.

C. Robert Valery and his team from the Naval Research Blood Laboratory in particular proved the applicability of cryopreserved red blood cells 7 days after reconstitution when using conventional resuspension solutions (SAG-M), and 14 days after reconstitution when using

AS-3 solution (Nutricel) [4,24,25]. Subsequently, Bohonek et al. proved the applicability of cryopreserved erythrocytes 3 weeks after reconstitution, also when using AS-3 [1].



Fig. 1. The line of deglycerolization (washing) machines Haemonetics ACP-115 – first fully automated machines



Fig. 2. Haemonetics APC-215, fully automated deglycerolisation (washing) machines with „close system“



Fig. 3. Mechanical freezers for deep freezing (-65°C - -80°C)



Fig. 4. Stock of frozen blood.



Fig. 5. Frozen RBCs units

4. Cryopreservation of platelets

Platelet could be cryopreserved in different cryoprotectives: intracellular (DMSO, glycerol) as well as in extracellular (HES, dextran).

The most widely used method for the platelets cryopreservation is freezing in 5-10% DMSO at -80°C with their storage at -65°C . This method is easy and does not need any technical equipment. After thawing, the platelets are suspended in thawed plasma and there is no need to wash out the cryoprotectant.

Although the platelets stored by cryopreservation are efficient in hemostasis, they are affected by a number of functional defects during storage and preparation for transfusion. Approximately 15% of cryopreserved platelets lost surface-bound GPIb, while there was no measurable loss of GPIIb/IIIa during cryopreservation. The cryopreserved platelets also showed a significant decrease in aggregation to ristocetin, but no loss of response to the stronger agonist, thrombin. Even though these defects are of a minor clinical relevance and the cryopreserved platelets were shown to be safe and effective for treatment of abnormal bleeding, it is still necessary to reckon with these changes [2,3,14,20].

5. Conclusion

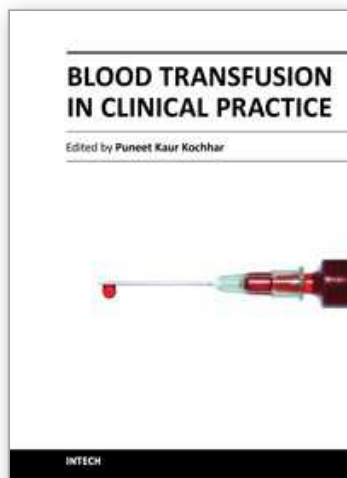
The cryopreservation of blood is a method which solves various problems in blood transfusion service. The main application is in military medicine and blood crisis policy, but also in special transfusiology fields, such as the storage of rare red blood cells and long-term storage of autologous blood. Thanks to modern procedures, which allow for prolonged shelf time after thawing and reconstitution of frozen blood, the use of frozen blood is now more flexible and less limited.

6. References

- [1] Bohonek M, Petras M, Turek I, Urbanova J, Hradek T, Chmatal P, Staroprazska V, Kostirova J, Horcickova D, Duchkova S, Svobodova J, Tejckova E., Quality evaluation of frozen apheresis red blood cell storage with 21-day postthaw storage in additive solution 3 and saline-adenine-glucose-mannitol: biochemical and chromium-51 recovery measures, *Transfusion*, 2010, 50:1007-1013
- [2] Daly PA, Schiffer CA, Aisner J, Wierni PH, Successful transfusion of platelets cryopreserved for more than 3 years, *Blood*, 1979, 54:1023-1027
- [3] Handin RL, Valeri CR, Improved Viability of Previously Frozen Platelets, *Blood*, 1972, 40:509-513
- [4] Hess JR, Hill HR, Oliver CK, Lippert LE, Greenwalt TJ., The effect of two additive solutions on the postthaw storage of RBCs., *Transfusion*. 2001 Jul;41(7):923-7.
- [5] Horn EP, Sputtek A, Standl T, Rudolf B, Kühnl P, Schulte Transfusion of autologous, hydroxyethyl starch-cryopreserved red blood cells, *Anesthesia & Analgesia*, 1997;85:739-745
- [6] Huggins CE, Frozen Blood, *Europ.surg.Res.* 1969; 1: 3-12
- [7] Lecak J, Scott K, Young C, Hannon J, Acker JP., Evaluation of red blood cells stored at -80 degrees C in excess of 10 years., *Transfusion*. 2004 Sep;44(9):1306-13.
- [8] Lelkens CCM, Koning JG, de Kort B, Floot IBG, Noorman F, Experiences with frozen blood products in the Netherlands military, *Tnsfus Apher Sci*, 2006, 34:289-98
- [9] Levin RL, Cravalho FG, Huggins CE, Effect of hydration on the water content of human erythrocytes, *Biophys.J.*, 1976;16:1411-14260
- [10] Lionetti, F. J. and Hunt, S. M., "Cryopreservation of Human Red Cells in Liquid Nitrogen with Hydroxyethyl Starch", *Cryobiology*, 1975; 12:110-118
- [11] Lovelock JE, The Protective Action of Neutral Solutes against Haemolysis by Freezing and Thawing, *Biochem.J.*, 1954; 56:265-270
- [12] Meryman HT, Hornblower M, Red Cell Recovery and leukocyte Depletion Following Washing of Frozen-Thawed Red Cells, *Transfusion*, 1973; 13(6):388-393
- [13] Mollison PL, Sloviter HA, Successful transfusion of previously frozen human red cells, *Lancet*, 1951; 261:862-864
- [14] Owens M, Werner E, Holme S, Afferbach C, Membrane glycoproteins in cryopreserved platelets, *Vox Sanguinis*, 1994, 67:28-13
- [15] Pert JH, Schork PK, Moore R, A new method of low temperature blood preservation using liquid nitrogen and glycerol sucrose additive, *Clinical Research*, 1963; 11:197
- [16] Pouchet MFA, Recherches expérimentales sur la congélation des animaux, *Journ..L'Anat. et Physiol*, 1866;.iii:1-36
- [17] Smith AT., Prevention during haemolysis during freezing and thawing of red blood cells, *Lancet*, 1950, 259: 910-911,
- [18] Sputtek A, Singbartl G., Langer R., Schleinzer W., Henrich HA, Künl P., Cryoperervation of red blood cells with the non-penetrating cyoprotectant hydroxyethylstarch, *Cryo Letters* 16, 1995; 283-288
- [19] Sputtek A, Kryokonservierung von Blutzellen, *Transfusionsmedizin*, 2nd.ed. (Ed.:C.Mueller-Eckhardt), 125-167, Springer, Berlin 1996
- [20] Taylor MA, Cryopreservation of platelets: an in-vitro comparison of four methods, *J Clin Pathol*, 1981, 34:71-75

- [21] Tullis JL, Ketchi MM, Pyle HM, Penneli GB, Gibson JG, Tinch RJ, Studies on the in vivo survival of glycerolized and frozen human red blood cells, J.of the Am.Med.Association, 1958; 168:399-404
- [22] Tullis JL, Haynes L, Pyle H, Wallach S, Pennell R, Sproul M, Khoubesserian A, Clinical use of frozen blood, Arch.Surg., 1960, 81:169
- [23] Valeri CR, Frozen Blood, New Engl.J.Med., 1966; 25:425-431
- [24] Valeri CR., Pivace LE., Cassidy GP., Rango G., The survival, funktion, and hemolysis of human RBCs stored at 4°C in additive slution (AS-1, AS-3, or AS-5) for 42 days and then bochemimically modifie, frozen, thawe, washed, and stored at 4°C in sodium chloride and glucose solution for 24 hours, Transfusion, 2000, Nov; 40(11): 1341-5.
- [25] Valeri CR, Ragno G, Pivacek LE, Srey R, Hess JR, Lippert LE, Mettillie F, Fahie R, O'Neill EM, Szymanski IO., A multicenter study of in vitro and in vivo values in human RBCs frozen with 40-percent (wt/vol) glycerol and stored after deglycerolization for 15 days at 4 degrees C in AS-3: assessment of RBC processing in the ACP 215., Transfusion. 2001 Jul;41(7):933-9.
- [26] Commission Directive 2004/33/EC of March 2004, Annex IV.
- [27] Guide to the preparation, use and guality assurance of blood components, Council of Europe, Recommendation No.R(95) 15

IntechOpen



Blood Transfusion in Clinical Practice

Edited by Dr. Puneet Kochhar

ISBN 978-953-51-0343-1

Hard cover, 272 pages

Publisher InTech

Published online 16, March, 2012

Published in print edition March, 2012

Blood Transfusion in Clinical Practice focuses on the application of blood transfusion in different clinical settings. The text has been divided into five sections. The first section includes a chapter describing the basic principles of ABO blood group system in blood transfusion. The second section discusses the use of transfusion in various clinical settings including orthopedics, obstetrics, cardiac surgery, etc. The third section covers transfusion transmitted infections, while section four describes alternative strategies to allogenic blood transfusion. The last section speculates over immunomodulatory effects of blood transfusion.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Miloš Bohoněk (2012). Cryopreservation of Blood, Blood Transfusion in Clinical Practice, Dr. Puneet Kochhar (Ed.), ISBN: 978-953-51-0343-1, InTech, Available from: <http://www.intechopen.com/books/blood-transfusion-in-clinical-practice/cryopreservation-of-blood>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen