Evaluation of red blood cells stored under various temperature profiles

Thomas Wagner[1], Maria Anna Pabst[2], Alexandra Winkler[1], Gerhard Lanzer[1] and Berthold Huppertz[2]

[1] Department of Blood Group Serology and Transfusion Medicine, and [2] Institute of Cell Biology, Histology and Embryology, Medical University of Graz, Austria

Corresponding author: thomas.wagner@medunigraz.at
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Background: For transfusion purposes red blood cells (RBCs) are traditionally stored at 4°C for maximally 42 days, while transport temperature should not exceed 10°C. During shelf life, RBCs may be exposed to different temperature profiles mostly exceeding 10°C and then need to be used or discarded. At the same time, the influence of variations in temperature is not well documented. Therefore, experiments including multiple cooling and rewarming processes were performed to assess the influence of temperature profiles on morphology, quality and functional parameters of stored RBCs.

Material and Methods: Buffy coat depleted and in-line filtered RBCs were used as standard products in all tests performed. In a first set of experiments RBCs were stored constantly at 4°C, 13°C or 22°C. In a second set of experiments RBCs were stored at 4°C, but exposed to 10°C, 13°C or 22°C for 24 hours once a week. After storage the following parameters were measured: pH, K^+, glucose, lactate, 2,3-DPG, ATP, osmotic resistance, haematocrit, free haemoglobin, free total protein, free and degraded spectrin. Ultrastructure of erythrocytes was assessed by transmission and scanning electron microscopy.

Results: RBCs stored constantly at 22°C or 13°C showed an increase in the percentage of haemolysis starting at day 21 and 35 respectively, compared to controls stored at 4°C (Figure 1A). In scanning electron microscopy (SEM) increased numbers of crenate shaped RBCs were detected in all storage temperatures. After storage at 4°C and 13°C crenate shaped RBCs seem to augment in numbers, but even after 42 days of storage nearly round RBCs could be detected in SEM. After storage at 13°C for 42 days occasionally fine fibrillar material appeared. Storage at 22°C showed that on day 42 the erythrocytes agglutinated and in SEM a lot of fibrillar material was found on and between the agglutinated cells (Figure 2B). Additionally small particles were seen in between the erythrocytes.
If RBCs were stored at 4°C and for one day once a week at peak temperatures (10°C and 22°), differently shaped RBCs were found with SEM after different storage conditions. Also under these conditions more crenate erythrocytes appeared after 42 days of storage than after 3 days (Figures 2A, C). Additionally, after 42 days between erythrocytes a few fine filaments were detected after a peak temperature of 22°C once a week (Figure 2C).

Conclusions: RBCs that have been warmed up to room temperature for maximally 24 hours on five different occasions during shelf life can subsequently be cooled down to 4°C and used as planned without quality loss. This is important data since it needs to be stringently

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evaluated to what extent and duration an RBC unit may be warmed up and subsequently cooled down without loss of quality and harm to patients.

References:
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Figure 1

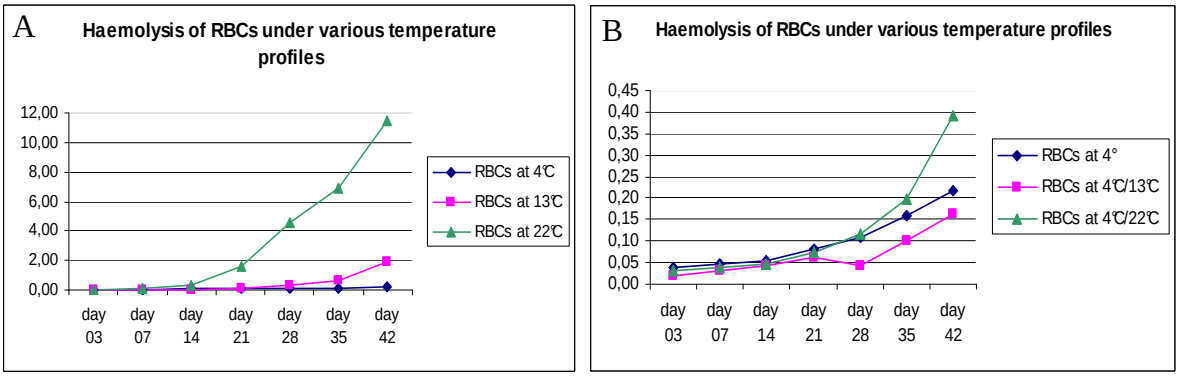

Figure 1: Haemolysis of RBCs stored for 42 days under various temperature profiles. Percentage of haemolysis after 42 days of storage should not exceed 0.8%.
(A) RBCs constantly stored at 13°C or 22°C show a significant increase in haemolysis starting at 21 or 35 days of storage respectively compared to the control group stored at 4°C.
(B) RBCs stored at 4°C but exposed to 13°C or 22°C for 24 hours once a week do not show any differences in haemolysis as compared to the control group stored at 4°C only.

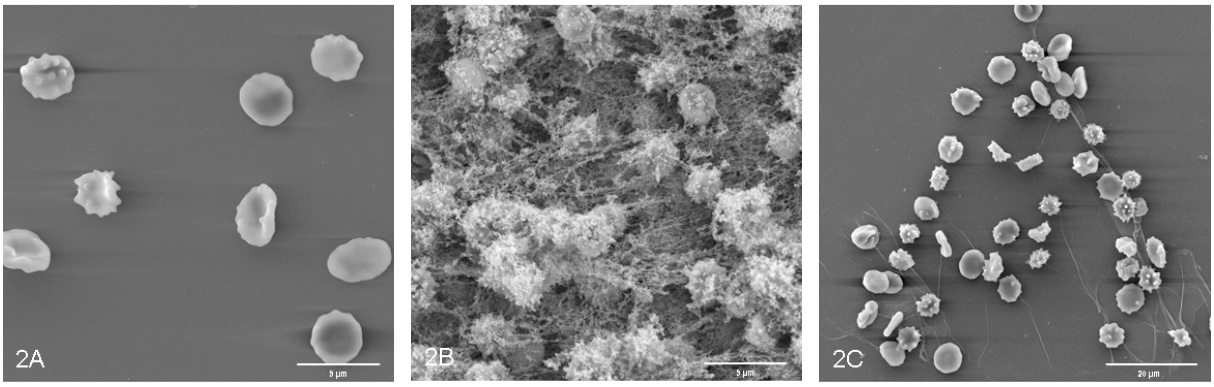

Figure 2: Scanning electron microscopy of RBCs.
(A) fresh RBCs on day 3 of storage at 4°C.
(B) RBCs constantly stored at 22°C showed agglutinated erythrocytes on day 42. In SEM a lot of fibrillar material was found on and between the agglutinated cells.
(C) RBCs stored at 4°C and exposed to 22°C for 24 hours once a week. After 42 days of storage more crenate erythrocytes appear than after 3 days. Additionally, after 42 days between erythrocytes few fine filaments were detected.

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