Optimising methods of red cell sedimentation from cord blood to maximise nucleated cell recovery prior to cryopreservation

M. MADKAIKAR, M. GUPTA, K. GHOSH, S. SWAMINATHAN, L. SONAWANE and D. MOHANTY
Institute of Immunohaematology (ICMR), K.E.M. Hospital, Parel, Mumbai, India

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ABSTRACT

Human cord blood is now an established source of stem cells for haematopoietic reconstitution. Red blood cell (RBC) depletion is required to reduce the cord blood unit volume for commercial banking. Red cell sedimentation using hydroxy ethyl starch (HES) is a standard procedure in most cord blood banks. However, while standardising the procedure for cord blood banking, a significant loss of nucleated cells (NC) may be encountered during standard HES sedimentation protocols. This study compares four procedures for cord blood processing to obtain optimal yield of nucleated cells. Gelatin, dextran, 6% HES and 6% HES with an equal volume of phosphate-buffered saline (PBS) were compared for RBC depletion and NC recovery. Dilution of the cord blood unit with an equal volume of PBS prior to sedimentation with HES resulted in maximum NC recovery (99% [99.5±1.3%). Although standard procedures using 6% HES are well established in Western countries, they may not be applicable in India, as a variety of factors that can affect RBC sedimentation (e.g., iron deficiency, hypoalbuminaemia, thalassaemia trait, etc.) may reduce RBC sedimentation and thus reduce NC recovery. While diluting cord blood with an equal volume of PBS is a simple method to improve the NC recovery, it does involve an additional processing step.

KEY WORDS: Erythrocytes.
Fetal blood.
Cord blood stem cell transplantation.
Cryopreservation.
Stem cells.

Introduction

The transplantation of umbilical cord blood (UCB) is recognised increasingly as an alternative for allogeneic transplantation with curative intent.[1,2] To build up large-scale banks of unrelated UCB transplants for broader clinical use, techniques have been established to minimise the volume of the collected samples in order to reduce the need for storage space in liquid nitrogen.[3] Red blood cell (RBC) depletion not only reduces the amount of cord blood to be stored, but also reduces the risks associated with transfusing haemolysed RBCs and also large amounts of dimethyl sulphoxide (DMSO).

Clinical data have shown that a low infused cell dose is detrimental to a patient's outcome after transplantation.[1,2] Thus, investigators have focused on the problem to reduce the volume of UCB transplants without a resultant major loss of haematopoietic progenitor cells (HPCs), and have introduced techniques such as RBC depletion[4–7] and hydroxyethyl starch (HES) sedimentation.[8,9]

Currently available methods for cord blood processing include white blood cell (WBC) collection by buffy coat removal, density gradient separation and differential centrifugation, and RBC lysis with ammonium chloride and RBC sedimentation using different viscous media.[7]

The use of HES sedimentation for RBC depletion is one of the most commonly used techniques in cord blood banks worldwide. However, while standardising the procedure with HES, the authors experienced variable and significant NC loss. Hence, this study evaluates possible reasons for this and compares the results of four differential sedimentation procedures for RBC depletion and NC recovery, based on different separation media. These include the commercially available macromolecular compounds hydroxyethyl starch and high molecular weight dextran, and the low-cost gelatin.

Correspondence to: Dr. Kanjaksha Ghosh
Institute of Immunohaematology (ICMR), 13th Foor, New Multistoreyed Bldg, K.E.M. Hospital, Parel, Mumbai – 400012, India
Email: kanjakshaghosh@hotmail.com

Materials and methods

After obtaining informed written consent, 65 units of cord blood were collected from infants of more than 35 weeks' gestation at the time of delivery. All mothers were screened during pregnancy for human immunodeficiency virus (HIV) 1+2, hepatitis B and C serologically, and tested for syphilis using the VDRL method.

After delivery of the infant, the umbilical cord was clamped and cut. The umbilical vein was punctured while the placenta was still *in utero*, using a 16 G needle from a triple blood bag set containing 25 mL citrate phosphate dextrose with adenine (CPDA), and blood was allowed to drain into the bag by gravity.

Processing was carried out within 24 h of collection. The blood obtained was distributed in equal volumes and

Table 1. Results of RBC sedimentation using different techniques.

	3% Dextran	3% Gelatin	6% HES	6% HES+PBS*
WBC recovery (%)	94.5±4.2	90.5±3.6	70±10.1	99.5±1.3
RBC depletion (%)	98.7±10.3	99.2±8.2	80±9.6	98.8±3.2
Viability (%)	98±2.0	96±3.2	97±4.2	98±1.6

*$P<0.001$ against all other reagents for WBC recovery and RBC depletion;

$P>0.05$ against viability.

Sixty-five samples tested; results presented as mean± SD.

processed using four different methods of RBC sedimentation, as describe below.

Dextran procedure
High molecular weight dextran (3%, Pharmacia) was added to an equal volume of cord blood. This mixture was allowed to stand for 1 h at room temperature to allow RBC sedimentation, and then centrifuged at 2000 rpm for 10 min. The supernatant was removed and the cells were washed with PBS. The WBC preparation was resuspended to a final volume of 1 mL.

Gelatin procedure
Cold water fish skin gelatin (3%, Sigma) was mixed with an equal volume of cord blood and placed at room temperature for 1 h to allow RBC sedimentation. The WBC-rich plasma supernatant was then centrifuged at 2000 rpm for 10 min. The WBC pellet was washed with PBS, and the final collection was resuspended in a 1 mL volume.

HES procedure
The HES procedure was performed according to Rubinstein *et al.*, with slight modification. Briefly, 6% HES (Stem Cell Technology) was mixed with cord blood at a ratio of 1:5 and RBC sedimentation was carried out by centrifugation at 1500 rpm for 5 min. Then, the WBC-rich plasma supernatant was centrifuged at 2000 rpm for 10 min. The final WBC preparation was resuspended in 1 ml volume.

HES and PBS procedure
Cord blood was diluted with an equal volume of PBS and mixed with 6% HES at a ratio of 1:5. The method then followed the HES procedure above.

Results

Percentage WBC recovery, RBC depletion and viability were found to be better with 6% HES mixed with blood diluted with PBS, and using the 3% dextran method, than with the standard 6% HES method. The results are shown in Table 1.

Discussion

The standard protocol for RBC depletion used worldwide includes use of 6% HES. However, results obtained with 6% HES can be poor, with less than 70% NC recovery. Thus, the authors assessed different methods to improve the mononuclear cell yield. High molecular weight dextran, gelatin and HES with dilution of the UCB with an equal volume of PBS produced good results (Table 1). As 6% HES plus PBS gave the best results, this method has been adopted for the cryopreservation of UCB units.

Although standard procedures using 6% HES are well established in Western countries, this technique may not be applicable in India, as a variety of diseases can affect RBC sedimentation and thus reduce subsequent NC recovery. A recent study using dextran for sedimentation of cord blood showed NC recovery of only 86.1%,[10] which is lower than the recovery obtained in the present study.

Several semi-automated filtration-based techniques have also been evaluated for routine cord blood banking. One assessed Baxter top and bottom fractionation with the Optipress II system against HES sedimentation,[11] while another evaluated multiple parameters in cord blood collection using the Optipress II filtration systems developed by Asahi Kasei Medical (Japan) and Terumo against a traditional HES-based collection system.[12] In neither of the studies was cord blood diluted with an equal volume of PBS.

Traditional HES-based separation technology gave a superior NC yield in tests against filtration technologies, but the yield obtained with Optipress II was similar. Optipress and filtration were found to be fast and user-friendly technologies.

In summary, minor modification to the traditional HES-based technology by diluting the cord blood sample with PBS improves NC yield and red cell depletion significantly, without compromising cell viability or increasing the cost of the procedure substantially. However, this technique does involve an additional centrifugation step and introduces the possibility of contamination during the addition of PBS. □

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