Cryobiology 78 (2017) 65–69

Contents lists available at ScienceDirect

Cryobiology

journal homepage: www.elsevier.com/locate/ycryo

Quality comparison of umbilical cord blood cryopreserved with conventional versus automated systems

Kyeong Mi Kim [a, 1], Ji Young Huh [a, 1], Jin Ju Kim [b], Myung Seo Kang [a, c, *]

[a] Department of Laboratory Medicine, CHA Bundang Medical Center, CHA University, 59, Yatap-ro, Bundang-gu, Seongnam-si, Gyeonggi-do, South Korea
[b] Department of Laboratory Medicine, Inha University College of Medicine, 27, Inhang-ro, Jung-gu, Incheon, South Korea
[c] CHA Medical Center Cord Blood Bank, 335, Pangyo-ro, Bundang-gu, Seongnam-si, Gyeonggi-do, South Korea

ARTICLE INFO

Article history:
Received 9 November 2016
Received in revised form
31 March 2017
Accepted 6 July 2017
Available online 14 July 2017

Keywords:
Umbilical cord blood
Cryopreservation
Transient warming event

ABSTRACT

Umbilical cord blood (CB) banks usually freeze and store CB for clinical transplantation using conventional controlled-rate freezer or the automated BioArchive system. The aim of this study is to compare the quality of CB cryopreserved with conventional and automated methods and to make clear the cause of the quality difference between the two methods. The experiment used 80 CB units: 40 were conventionally cryopreserved and the remainder were cryopreserved with a BioArchive. After thawing, the following measures of CB quality were compared: recovery rates of cell count, cell viability of total nucleated cells (TNCs), mononuclear cells (MNCs), and CD34+ cells, as well as colony-forming unit-granulocyte/macrophage (CFU-GM) content. Additionally, processing and storage records were reviewed to quantify the number of exposures of CB units at room temperature (transient warming event, TWE), which was analyzed in relation to CB quality. MNC and CD34+ cell viability were as follows: MNC, 78.2% ± 6.8% (conventional), 81.7% ± 7.2% (automated); CD34+ cell, 90.6% ± 6.9% (conventional), 94.7% ± 3.5% (automated). The absolute CFU-GM content per CB unit was $7.1 \times 10^5 \pm 5.9 \times 10^5$ with conventional cryopreservation and $12.3 \times 10^5 \pm 12.0 \times 10^5$ with automated cryopreservation. CBs cryopreserved with BioArchive showed significantly higher MNC and CD34+ cell viability, and CFU-GM content than those conventionally cryopreserved. The CB quality comparison depending on the amount of TWEs showed no significant quality difference between groups that were more exposed to TWEs and groups that were less exposed. CBs cryopreserved with BioArchive were of higher quality than conventionally cryopreserved CBs, and the cause of quality difference might be due to the difference of freezing conditions rather than the TWE effect.

1. Introduction

Since the first cord blood (CB) bank for clinical transplantation was established in 1992, CB is increasingly used as an alternative source of hematopoietic stem cells for transplantation in malignant and nonmalignant disorders [4,8,9]. A recent estimate indicated that more than 1,000,000 CB units are stored in over 100 quality controlled public CB banks and over 130 private banks, worldwide [2].

To maintain the hematopoietic reconstitution potential of cryopreserved CB units, meticulous handling is necessary at each step of cryopreservation [1]. In conventional CB banking procedures, processed CB units are frozen in the controlled-rate freezer and transferred to liquid nitrogen tanks for storage in most CB banks. The transfer from freezer to storage tank inevitably leads to exposure of frozen CB units to room temperature resulting in transient warming events (TWEs).

Recent advances in cryopreservation technology have developed an automated liquid nitrogen tank for CB cryopreservation, which combines the controlled-rated freezer and liquid nitrogen tank into one unit (BioArchive system, Thermogenesis, Rancho Cordova, CA, USA). Therefore, using the BioArchive system, CB units do not need to be exposed to room temperature during the freezing process. In addition, since each CB unit is kept separately, CB units in the BioArchive can be stored or taken out when it is requested for use without disturbing or being disturbed by any other unit. A few recent studies have reported high cell viability in CB units

* Corresponding author. 59 Yatap-ro, Bundang-gu, Seongnam, Gyeonggi-do 463-712, South Korea
E-mail address: olive@chamc.co.kr (M.S. Kang).
[1] KMK and JYH contributed equally to this study as first authors.

http://dx.doi.org/10.1016/j.cryobiol.2017.07.001
0011-2240/

Abbreviations

CB	cord blood
TWE	transient warming event
TNC	total nucleated cell
DMSO	dimethyl sulfoxide
TB	trypan blue
ISHAGE	International Society of Hematotherapy and Graft Engineering
7-AAD	7-aminoactinomycin-D
CFU-GM	colony-forming unit-granulocyte/macrophage
MNC	mononuclear cell

cryopreserved with BioArchive [5–7]. However, few comparisons have been made between the quality of CB units frozen conventionally versus using the automated system. In this study, we examined whether conventionally cryopreserved CB differed in quality from those cryopreserved with BioArchive, as measured by the recovery rates of total nucleated cell (TNC) and CD34+ cell, cell viability, and progenitor cell assay (Fig. 1).

2. Material and methods

2.1. CB samples

The experiment used 80 CB units donated to a public CB bank (CHA Medical Center Cord Blood Bank, Korea) between April 2013 and October 2014. These units were ineligible for clinical use due to low TNC count. Half of the CB units were randomly assigned to conventional cryopreservation and the other half to the automated system. All CB units were cryopreserved for a median of 12 months (range, 1–19 months).

This study was approved by the Institutional Review Board of the CHA Bundang Medical Center (BD2014-098).

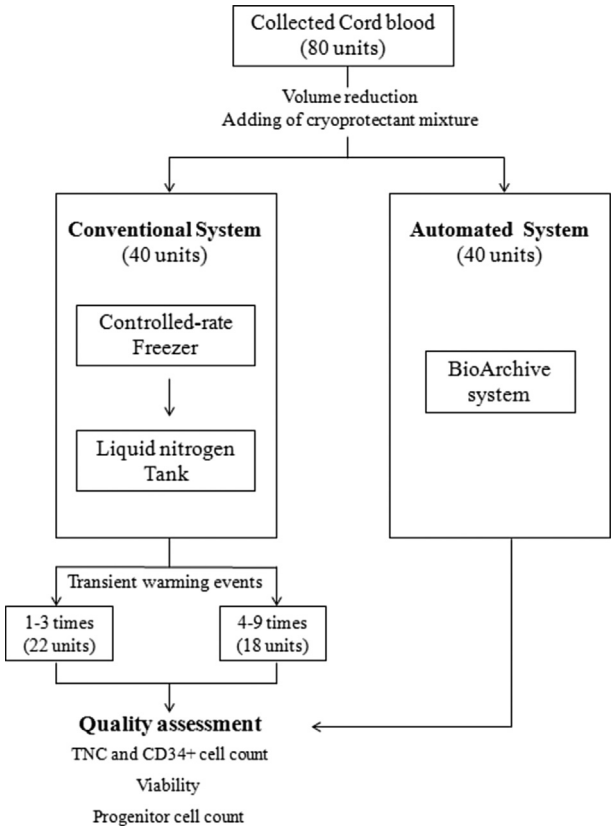

Fig. 1. Flow diagram summarizing study procedure, TNC: total nucleated cell.

2.2. Collection and processing of CB units

Each CB unit was collected after neonate delivery and umbilical cord ligation but before the expulsion of the placenta. All CB units were shipped to the CB bank at room temperature within 24 h post collection and processed within 36 h, following established CB bank standard operating procedures as previously described [3]. In brief, the buffy coat of donated CB units was separated by volume reduction and red blood cell (RBC) depletion, and a 5-mL mixture of 100% dimethyl sulfoxide (DMSO) and 5% dextran 40 was added before cryopreservation. The final volume of the processed CB units was 25 mL and achieved a final concentration of 10% DMSO. Before processing, aliquots were taken of CB units for cell count and cell viability measurements.

2.3. Freezing and storage of CB units

Under conventional cryopreservation, processed CB units were equilibrated at 4 °C for at least 15 min before being placed in the controlled-rate freezer, which was programmed as follows: step 1, cooling rate of 1 °C/min between 4 and -15 °C; step 2, cooling rate of 25 °C/min to −50 °C; step 3, warming rate of 10 °C/min to −25 °C; step 4, cooling rate of 1 °C/min to −45 °C; step 5, cooling rate of 10 °C/min to −90 °C. After freezing, the CB units were transferred to a liquid nitrogen tank and stored at below −150 °C.

Cryopreservation with the automated BioArchive system used the following protocol: pre-freeze rate, −10 °C/min; freeze start temperature, −1 °C; freeze power, 100%; freeze exit temperature, −11 °C; post-freeze rate, −2 °C/min; target temperature, −50 °C.

2.4. Thawing of CB units

The CB units were removed from the liquid nitrogen tanks and thawed rapidly in a 37 °C water bath. Immediately after the thawing process and without a washing step, two aliquots were taken from every thawed unit: one was used for measuring cell count, and cell viability, whereas the other was used for the progenitor cell assay.

2.5. Trypan blue (TB) exclusion staining

Pre-freezing and post-thawing CB samples were each mixed with an equal volume of TB dye (0.4%, Gibco, Grand Island, NY, USA). At least 100 cells were microscopically analyzed in duplicate for viability in a hemocytometer chamber. The mean percentage of living cells in the two analyses was calculated.

2.6. Cell count and viability

TNC count in pre-freezing and post-thawing CB samples was determined with an automated hematology analyzer (DxH-800, Beckman-Coulter, Miami, FL, USA).

CD34+ cells in pre-freezing and post-thawing CB samples were counted using flowcytometry (Navios, Beckman Coulter, Fullerton, CA, USA) following methods from the International Society of Hematotherapy and Graft Engineering (ISHAGE), with a commercially available CD34+ enumeration kit (STEMKIT, Beckman Coulter) in accordance with manufacturer protocol. Cell viability