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Comparison of manual and automated closed system in umbilical cord blood stem cells separation

Faezeh Tajalli[1]*, Reza Eftekhari Vakili[2]

[1]Academic Staff in Molecular Medicine Research Department, ACECR-Mashad Branch, (IRAN)

[2]Academic Staff in Azad University-Mashad Branch, Mashad, (IRAN)

E-mail : Tajalli@acecr.ac.ir

ABSTRACT

According to advanced human diseases and its interest in longevity, the sciences of stem cell biology and repair tissues and damaged organs have been used routinely to develop the cell therapies and regenerative medicine for more than three decades. Therefore, the aim of stem cells utilization as a source of appropriate differentiated cells is to replace those lost through physical, chemical or ischemic injury, or as a result of degenerative disease. For this purpose, there are two main kinds of stem cells according to their origin containing embryonic and adult stem cells. Essentially, embryonic stem cells are extracted fetal blood, amnion fluid and membrane or umbilical cord blood (UCB), however adult stem cells are commonly isolated from bone marrow, peripheral blood or skin[6,10]. UCB is a source of hematopoietic progenitors cells coupled to the immaturity of the immune system at birth that is one of the advantages of using these cells for transplantation. So the separation of these useful cells requires a standard manual technique or some important protocols and technological innovations. Nowadays, an accurate and efficient system is the automated closed system "Sepax®" that it is employed largely in the routine processing of cord blood banks worldwide.

KEYWORDS

Stem cells;
Sepax®;
Cell therapy;
Umbilical cord blood.

INTRODUCTION

Bone marrow (BM), peripheral blood progenitor cells (PBPCs), and umbilical cord blood (UCB) are three different sources of available for hematopoietic progenitor cells (HPCs)[13]. UCB stem cells that residing in waste tissues have many therapeutical advantages such as their collection without any medical or ethical problems concerning mother or newborn baby, routinely use for clinical transplantations, and the available standardized and automated methods for their collection, isolation and freezing[9]. This important biological product is applied for treatment of hematology (eg. Leukemia), heart (myocardial infarct) and vessel diseases (diabetis), orthopedics (bone fractures) and etc. UCB is an alternative source of haematopoietic stem cells for bone marrow and peripheral blood. To date, it is UCB progenitor cell transplant is an effective source of haematopoietic stem cells for bone marrow reconstitution when using suitable units. The importance of cell dose in the clinical outcome has motivated the need to develop techniques aimed at reducing cell losses,

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increasing reproducibility, decreases the risk of contamination and reduces final sample volume and subsequent to it to diminish of DMSO consumption. In this respect, many of cord blood banks (CBB) have been encouraged to develop new techniques aimed at reducing cell losses[11]. So, Hematopoietic progenitor cells (HPCs) of UCB are a suitable replacings in autologous or allogeneic settings that collection, processing, and storage, the infusion of them is an important step in the transplant[13]. So, With the development of cord blood banking, solutions have to be found to solve the storage space problem, by reducing the volume of (CBU)[8] which for mentioned purpose this article introduces the automated closed system "Sepax®" (Figure 1) and its advantages. It is now a common and accepted technology and device in umbilical cord blood (UCB) banks worldwide.

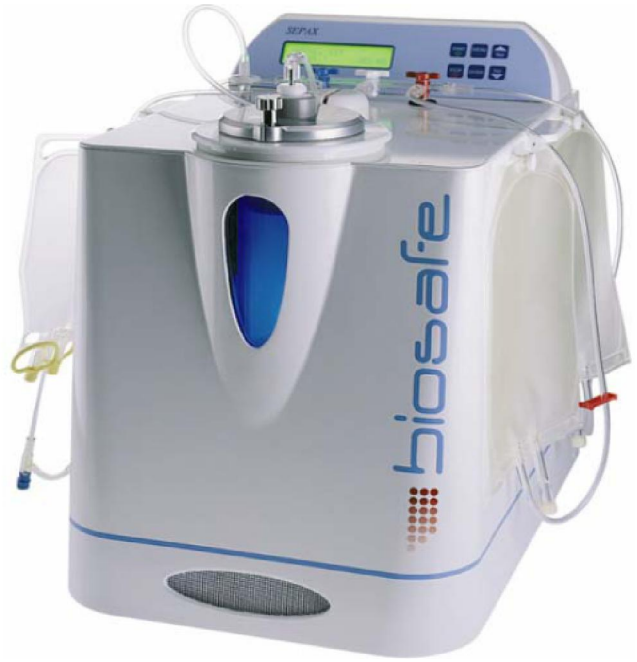

Figure 1 : Sepax® system

SEPAX® SYSTEM

Sepax, Biosafe's lead technology platform, is a versatile cell separation technology of which the unique proprietary separation chamber is core. The combination of the compact Sepax main processing unit and single-use kits allows the controlled separation of cellular products in a fully automated and closed evironment - no other equipment is required.

Sepax is equipped with protocols that allow the processing of cord blood, bone marrow and peripheral blood using different separation methods, such as volume reduction, nucleated cell component (buffy-coat) concentration and density gradient separation. Each separation protocol has an associated single-use kit consumable specifically configured to optimize the efficacy of the separation protocol.

SINGLE-USE CELL SEPARATION KIT

The Sepax single-use separation kit (Figure 2 – Figure 5) is an integral part of the Sepax system and provides a sterile, functionally-closed environment in which the cellular product is separated into its components. A common element of every single-use kit is the proprietary separation chamber that is core to the Sepax technology. The separation chamber is a rotating syringe-pump providing simultaneous separation and transfer of cellular product components to optimize cell recovery. The syringe-pump design allows the processing of variable volumes from a minimum of 35 ml up to 880 ml through multicycling of the 220 ml separation chamber. The single-use kits may be equipped with a final-product collection bag that allows the immediate addition of a cryopreservant for subsequent cryogenic storage or direct therpaeutic use, minimizing handling and the risk of microbial contamination.

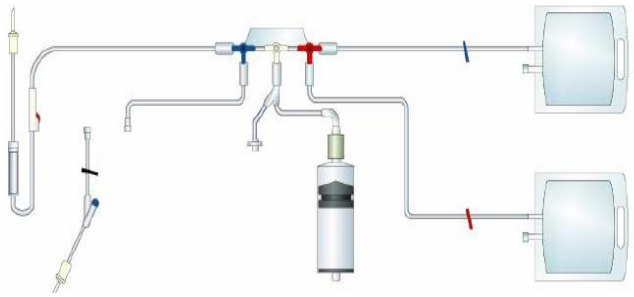

Figure 4 : CS-480 : Recommended for UCB-HES, PBSC

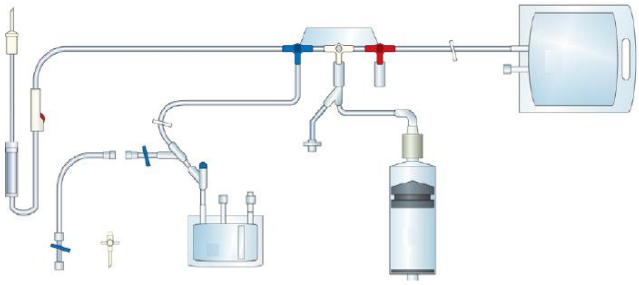

Figure 3 : CS-530: Recommended for UCB-HES

Figure 2 : CS-490: Recommended for UCB, PBSC

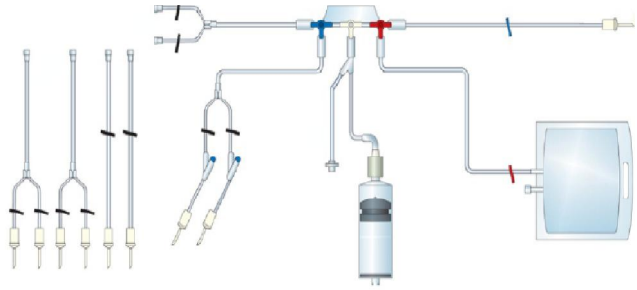

Figure 5 : CS-600: Recommended for UCB-W, PBSC-W

HES PROCEDURE

Hydro-Ethyl-Starch (HES) is a high molecular-weight polysaccharide compound isolated from corn and is closely resembling glycogen that does not freely penetrate the cell membrane[5,15]. HES in blood cell separation for granulocyte collection and in total state is used as an osmotic plasma-volume-expanding agent that protects the cell by forming a viscous, glassy shell that retards the movement of water, thereby preventing progressive dehydration as water is incorporated into the extracellular ice crystals[5,13]. Therefore, It functions as cryoprotectant, sedimenting agent and as a plasma substitute. HES macromolecules gravity sedimentation is occurred simplicity and fast in a closed-system, safe, and effective method for volume reduction and red cell removal from UCB[5].

In HES procedure that is applied in Sepax® (Biosafe SA) and in accordance with internal validation procedure, a HES solution (HES 450-0.7-6%) corresponding to 20% of the UCB input volume (UCB + CPD anticoagulant) is prepared.After filling a syringe with the prepared HES at room temperature, under LAMINAR FLOW conditions, the syringe's needle is inserted in the dedicated port of the UCB input bag, and then manually agitate the UCB input bag with one hand while injecting the HES with the other hand at a rate of approximately 2 seconds per ml : this ensures a homogeneous HES dispersion in the bag. The complete process takes approximately 45 minutes (30 minutes for sedimentation, 10 minutes for centrifugation, and 5 minutes for diversion of supernatant and cell resuspension)[15].

RESULT

On base of previous experiences, TNC and MNC recovery that are obtained after Sepax® processing are significantly higher ($P<0.0001$) whereas red blood cell is no significance in final bag[2]. Also, other results illustrate the volume reduction procedure is caused an average MNC recovery rate, cell viability, CD34+ cell recovery rate, TNC recovery and volume reduction 91±5%, ▪90%, 92±4%, 80.3±7.7% and 88±4.5%, respectively. In this researches, in vitro clonogenic potential of cells isolated with Sepax® had not differed from this of cells isolated according to the standard HES sedimentation protocol and protocol of sepax-based washing of thawed cells had resulted of 9±4% cell loss and 10±6% decrease in cell viability, when compared to thawed non-processed cells[8,9].

CONCLUSION

To date, the available documents of the previous experiences on production of stem cell from fresh cord blood show importance of the automated cell processing system Sepax® (Biosafe SA) in order to maintain GMP conformity for mononucleated cell (MNC) separation from fresh UCB, volume reduction, washing out DMSO after cell thawing, elimination of red cells, viability, CD34+ cell recovery[1]. This system is a time saving method providing clinical grade MNC isolated automatically[2].

Nowadays, it is proved that one of the best methods is using the automated closed system "Sepax®", whereas this system is a simple, safe, user-friendly and a time-saving method which provides automatic clinical-grade stem cells, the more standardized and user independent processing procedure, releases high amount of stem cells as a final product ready for cryopreservation, decreases the risk of contamination, reduces final sample volume efficiently that causes to

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solve the storage space problem and decreasing the cost of cryogenic storage in cord blood banks, providing optimal red blood cell depletion for the storage of the final cellular product[2,3,4,7,8,12,14].

Finally, it is concluded that Sepax® separator can be used for cord blood volume reduction, DMSO elimination from thawed samples[9], to maintain GMP conformity[1], higher TNC and $CD34^+$ cell recoveries[2].

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