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Cord blood technology: a new paradigm

P. Hollands

Consultant Clinical Scientist, Cambridge, UK

Corresponding Author: Peter Hollands, PhD (Cantab), FRSB; e-mail: peterh63@hotmail.com

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Abstract

Objective: **This opinion paper examines the current status of cord blood stem cell technology using volume reduction and describes a novel cord blood collection, processing and storage system. This technology could represent a new paradigm in using cord blood in regenerative medicine in the future. The decline in the use of cord blood to treat blood disorders, largely driven by haploidentical transplantation, may in the future be replaced by using cord blood in regenerative medicine procedures. The Evo-3medica Sterile Closed Cryo-System (ESCCS) provides a closed cord blood collection, processing and storage system which will considerably reduce the cost of cord blood collection, processing and storage. The ESCCS enables easy collection, processing and storage of whole cord blood, with all of its constituent cells available for future regenerative medicine procedures. This will enable current cord blood banks to easily and cost-effectively switch their focus to regenerative medicine procedures. This is instead of focusing on the ever-decreasing use of cord blood in haemopoietic stem cell transplantation for blood disorders.**

Introduction

Cord blood was first used as a unique source of haemopoietic stem cells (HSCs) for transplantation in 1988. It was used to treat a young patient who was suffering from Fanconi anaemia[1]. This patient was treated using whole cord blood (without volume reduction). The cord blood came from his sibling and the patient is alive and well today. There is currently an unrelated donor registry of cryopreserved cord blood which has approximately 645,646 cord blood units available globally for transplantion[2], with 300,000 cord blood units available in the USA alone[3]. There have even been suggestions that allogeneic cord blood transplantation may become a first line treatment for paediatric leukaemia and related blood disorders[4]. Cord blood may also be transplanted with a less than perfect Human Leucocyte Antigen (HLA) match making it more flexible than bone marrow as a transplant product[5,6]. Cord blood transplantation in adults is not so attractive since cord blood contains a limited number of HSCs and transplantation to adults either requires stem cell expansion[7] or the co-transplantation of multiple matched cord blood units (typically 2-3) to reach the required dose of HSCs[8].

Cord Blood Collection, Processing and Clinical Use

Cord blood collection is currently carried out either *in utero* or *ex utero* with a preference towards *ex utero* in most countries. This is because *ex utero* collection seems to provide lower levels of bacterial contamination and less incidence of low volume and clotting[9]. Cord blood is usually collected by a specifically trained phlebotomist into a 250 mL bag containing citrate-phosphate dextrose solution (CPD)[10] as an anticoagulant; the collected cord blood is stable for at least 48 hours before processing and storage[11].

On arrival at the processing laboratory almost

all cord blood undergoes "volume reduction" to obtain the buffy coat, containing CD34+ HSCs, and a total volume of 25 mL is cryopreserved. This volume reduction is achieved by the use of automated systems such as SEPAX by Cytiva (Little Chalfont, Buckinghamshire, UK) or AXP by ThermoGenesis (Rancho Cordova, CA, USA)[12]. The rationale for storing volume-reduced cord blood is to reduce the required storage space in the cord blood bank. A 25 mL frozen cord blood unit takes up much less space in the storage tank than a 150 mL frozen cord blood unit[13]. The first cord blood transplant, and many subsequent transplants, used whole blood (without volume reduction) for transplantation with no adverse events[14,15].

The volume reduction processing of cord blood must take place in an EU GMP Grade B (ISO Class 5) clean room and open procedures such as the addition of the cryoprotectant (10% v/v Dimethyl sulfoxide, DMSO) are carried out in a EU GMP Grade A Class II flow hood (ISO Class 5)[16]. Cord blood is usually stored in liquid nitrogen at -196°C (sometimes it is stored in the vapour phase at -180°C) and thawed rapidly in a 37°C water-bath in the transplant center when needed for transplant. These facilities in cord blood banks are expensive to create, run and staff but they are a regulatory requirement in most countries[17]. The cost of this advanced technology is handed on to the patient in a private cord blood bank or the charity or hospital in a public cord blood bank.

Cord blood has been transplanted over 35,000 times worldwide to treat a wide range of blood disorders which are often referred to as '80 different diseases' (they are in fact all in one group i.e., blood disorders)[18]. This use of cord blood to treat blood disorders has been on the decline in recent years because the option of haploidentical bone marrow transplantation has proved a great success[19,20]. If the demand for cord blood stem cells to transplant for blood diseases continues to decline, and as regenerative medicine procedures become more common, then preparations must begin to enable cord blood to be used in regenerative medicine.

Cerebral Palsy and Autism

The most prominent 'alternative' current use for cord blood is the use of autologous volume reduced cord blood buffy coat in the treatment of cerebral palsy (CP)[21] and autism spectrum disorder[22]. The results for CP have shown some signs of benefit but much more work is needed[21]. The results for autism spectrum disorder are currently less encouraging[22]. It is also uncertain as to which type of cell, if any, is contributing to the possible benefits which have been seen. Cord blood contains HSCs[23], mesenchymal stem cells (MSCs)[24], endothelial progenitor cells (EPCs)[25,26], regulatory T cells (Treg)[27], myeloid-derived suppressor cells (MDSCs)[28], unrestricted somatic stem cells (USSCs)[29] and pluripotent very small embryonic-like (VSEL)[30] stem cells. Is it not known which of these cells, if any, are contributing to the possible benefit in CP. Only those stem cells which can cross the blood-brain barrier can provide a cellular-based benefit within the central nervous system. One possible explanatory hypothesis is that stem cells from the cord blood, when administered intravenously, may become trapped in capillary beds (e.g., in the lungs)[31] and from there the trapped cells release growth factors[32] and/or exosomes[33] which can cross the blood-brain barrier.

A type of stem cells present in cord blood, which are currently ignored by many workers, is represented by the pluripotent VSEL stem cells[34,35]. It is possible that pluripotent VSEL stem cells in cord blood can cross the blood brain barrier and carry out repair within the central nervous system. Therefore, these cells could well be the active component in cord blood buffy coat resulting in the benefits seen so far in the treatment of CP.

Public and Private Cord Blood Banks

There are two basic models for the collection, storage and use of cord blood. The public sector has altruistic cord blood donors providing cord blood, which is processed, stored and tissue typed for use by anyone in need[36]. The HLA databases of these cord blood banks are available to be searched by transplant physicians around the world thus optimising the cord blood availability. The public cord blood banks generate income from the fee charged to release a cord blood unit for transplant. The private cord blood bank sector collects, processes and stores cord blood for use by individual families only[37]. Private cord blood banks generate their income from fees for initial collection, processing and storage, annual storage fees and fees for release when a cord blood unit is needed for transplantation in the family. Both public and private cord blood banks currently use a standard 250 mL blood bag containing CPD anticoagulant to collect cord

blood and use volume reduction to 25 mL of buffy coat prior to cryopreservation and storage.

The Use of Cord Blood in Regenerative Medicine

Cord blood is a potential source of stem cells to use in regenerative medicine procedures especially in the areas of cardiovascular, ophthalmic, neurological and endocrine diseases[38]. The range of stem cell types found in cord blood provides this wide field of potential applications and the presence of MSCs makes the cord blood a potentially important source of these cells which are already showing considerable potential in musculoskeletal diseases[39].

In addition to the cellular components of cord blood, the plasma of cord blood has been shown to have important properties which may be the basis of, or play a supporting role in, future regenerative medicine therapies[40]. Emerging technologies such as *ex vivo* gold treatment of blood using GOLDIC® (Gmund am Tegernsee, Germany) may also enhance the regenerative potential of cord blood plasma[41,42]. GOLDIC® technology seems to be capable of increasing levels of gelsolin in the blood which has potential diagnostic and therapeutic applications[43].

Evo3medica Sterile Closed Cryo-System (ESCCS)

The current declining use of cord blood as a source of HSCs for the treatment of blood disorders and the increasing potential future use in regenerative medicine procedures raises questions about the current 'gold-standard' of cord blood volume reduction[12]. The present system reduces the cord blood volume to 25 mL, and plasma and cells are as a result discarded as medical waste. The concern is that if cord blood becomes an important source of stem cells for regenerative medicine in the future, then volume reduction may have removed some of the critical cells needed. It therefore seems prudent to store whole cord blood for future regenerative medicine procedures to avoid the loss of critical cord blood components and cells. In addition, the volume reduction process is expensive because it requires high levels of technology, clean room facilities and highly trained staff. Storage of whole cord blood would reduce this cost, making cord blood technology more affordable both for patients and institutions across the globe.

The Evo3medica Sterile Closed Cryo-System (ESCCS; Leipzig, Sachsen, Germany) is a new closed cord blood collection, processing and storage system for whole cord blood, which does not require either a clean room facility or expensive processing equipment (Figure 1). It also does not require highly trained processing staff. The cord blood is collected in the same way as when a single 250 mL blood bag is used and sent to the laboratory for processing. When using the ESCCS, the DMSO is added through a sterile DMSO filter directly to

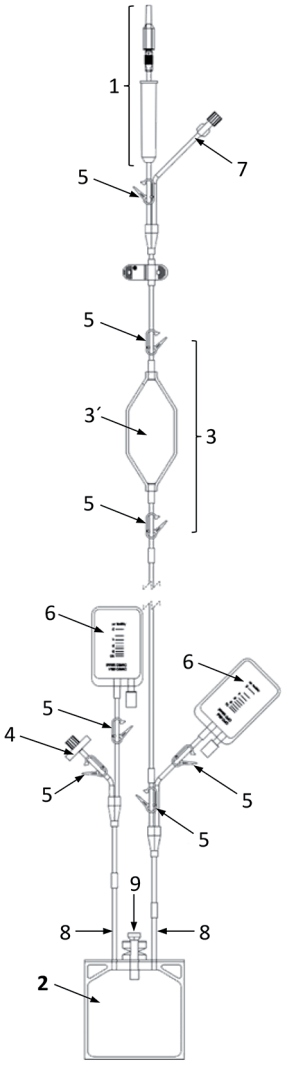

Figure 1. The Evo3medica Sterile Closed Cryo-System (ESCCS). 1. Collection needle; 2. Combined mixing and freezing bag; 3. Intermediate citrate-phosphate dextrose solution (CPD) reservoir; 4. Dimethyl sulfoxide (DMSO) Inlet/sterile filter; 5. Tube clips; 6. Satellite bags; 7. Injection port; 8. Main interconnector; 9. Storage Bag Opening Port (to use after thawing).

the whole blood. There are two satellite bags in the ESCCS system. The first bag is used to take the blood sample for infectious disease serology and flow cytometry. The second bag is used to take a small blood sample plus DMSO (analogous to the small compartment of a 25 mL pall bag) which can be stored with the main sample and used for testing at the time of transplant. All of these procedures can be carried out in a processing room without air filtration because the system is totally closed. The processing of cord blood is much quicker and cheaper using this technology and it creates less medical waste than volume reduction. The collection, freezing and storage of cord blood using the ESCCS all take place in a single Ethylene-Vinyl Acetate (EVA) bag. The ESCCS also has a novel CPD anticoagulant handling system which coats the tubing and bag before blood collection, thus minimising the risk of clotting during collection and transport.

The use of the ESCCS reduces the cost and time taken to process the cord blood and it retains all of the blood cells present, so that they can be used in future regenerative medicine procedures. This is a new paradigm which may change and improve the processing and storage of cord blood for future regenerative medicine procedures. It will reduce the cost of processing and provides a critical undepleted cord blood unit for future use.

It is theoretically possible to use technology such as the Terumo sterile tube welder to connect all of the separate processing components to the collection bag in the laboratory. This is less attractive than a pre-manufactured processing system such as the ESCCS because the tube welds may possibly introduce contamination, it requires more steps in the processing laboratory, it takes more time to carry out processing and the cost is increased because each component has to be sourced, purchased and validated separately. At the time of writing there were no publications on this system for routine cord blood processing.

There is a similar system to the ESCCS developed by Vita34 and called the Decentralised System (DeSy), which is protected by a patent[44]. This follows a traditional multi-bag system

in contrast to the advanced single-bag system used by the ESCCS. The DeSy technology, however, does not seem to play a substantial role in the overall strategy of Vita34.

In contrast, Evo3medica follows the world-wide vision that every family should be able to participate in medical progress, in general, and in the huge opportunities of regenerative medicine, in particular. Therefore, Evo3medica aims at a significant market penetration behind a global network of local laboratories which provide direct access and reasonable pricing to the customers. The ESCCS is patented in Europe (EP2889047A1)[45].

Conclusions

The cord blood industry has come to an inflexion point. The clinical use of cord blood to treat blood disorders is on the decline and the future lies in regenerative medicine. The present volume reduction of cord blood is expensive and may be resulting in the loss of essential cells and plasma which could be very important in future regenerative medicine procedures. The ESCCS provides closed whole cord blood processing and storage (without the need for a clean room) thus removing the need for expensive volume reduction. In addition, the ESCCS retains every cell contained in cord blood to optimise future use in regenerative medicine procedures. Storage facilities may have to increase capacity to accommodate larger stored volumes, but the future benefits of this whole cord blood storage using the ESCCS will far outweigh this additional storage requirement.

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ORCID:

Peter Hollands: https://orcid.org/0000-0003-4116-1954

Conflict of Interest:

Peter Hollands declares that he has no conflict of interest to disclose.

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