Umbilical Cord Blood Processing Techniques and Their Comparative Advantages: A Review

Abstract

Background: Umbilical Cord Blood (UCB) has steadily gained prominence in haematopoietic stem cell transplantation (HSCT). Despite its many advantages, the principal drawback of UCB in haematopoietic stem cell transplantation (HSCT) is its limited cell dose. Initially UCB was processed and cryopreserved as whole cord blood banking leading to the problem of storing sufficiently large number of cryoprotected UCB units which requires vast amounts of costly storage space in liquid nitrogen. The sole purpose of processing is to concentrates the stem cells and reduce the volume for storage. Different UCB processing methods have been developed.

Aim: This review is aimed at bringing together the literature on the different processing methods and highlighting the underlying principles of each method, the relative efficiency and advantages of the methods.

Methodology: The work involved mainly the critical review of all available academic, professional and industry documents on cord blood processing. The relevant information was obtained from textbooks, academic journals, conference proceedings, the internet among others. The major UCB processing methods include Plasma Depletion, Density Gradient Centrifugation (DGC), Hetastarch, PrepaCyte-CB and SEPAX. A study of the potential impact of Hetastarch and PrepaCyte processing methods on transplantation outcomes revealed no significant difference between patient receiving cells from each processing regimen.

Results: A comparison of the engraftment time of PrepaCyte-CB with five other processing methods revealed a quicker engraftment time for PrepaCyte-CB processed cord blood units compared to other processing methods. PrepaCyte-CB also recovers significantly more viable stem cells than AutoXpress (AXP) and HES processing methods. Other workers demonstrated that SEPAX depletion gives a higher recovery of nucleated cells. The effect initial cord blood volume had on recovery of nucleated cells for the different processing methods were also compared. Recovery using SEPAX is reduced as the size of unit processed increases. Hetastarch, density gradient and plasma depletion separation were also affected in this way, but PrepaCyte-CB processing was not affected by the initial volume of the collected unit. The benefit of SEPAX is that it is a fully automated system which allows for the mass processing of samples, which is suitable for larger cord blood banks. For red blood cell (RBC) removal, density gradient separation is the most effective method. PrepaCyte-CB is the second most efficient method for removing RBC. The result of Total Nucleated Cells (TNC) and Mono Nucleated Cells (MNC) recovery rate of Hespan and SEPAX against AXP processing methods shows that both Hespan and SEPAX reproducibly recover greater than 95% of the cord blood stem cells in a typical collection and result in a reduced final volume for final storage.

Conclusion: The five most popular processing techniques are Plasma Depletion, Density Gradient, Hetastarch, PrepaCyte-CB and Automated Centrifugal Machine (SEPAX). Most methods involve centrifugation, sedimentation and/or filtration for reducing the red cell content, plasma volume, or both. The different UCB processing methods each has its advantages and disadvantages.

Key words: *Umbilical cord blood(UCB), Haematopoietic stem cell transplantation, UCB processing methods, Cord blood stem cells, Total nucleated cells, Mono nucleated cells, cord blood banks.*

1. Introduction

Despite its many advantages, the principal drawback for Cord Blood (CB) in haematopoietic stem cell transplantation (HSCT) is its limited cell dose[1-2]. The sole purpose of processing is to separate stem cells from the cord blood so that a sample is produced can concentrates the stem cells and that can be used safely. Initially, CB was processed and cryopreserved as whole cord blood, which Chow *et al*.[2] referred to as zero generation process. Rubinstein[3] pointed out that the first problem of cord blood banking is that banking a sufficiently large number of cryoprotected CB units requires vast amounts of costly storage space in liquid nitrogen (LN). It was for this reason that volume reduction processing of CB units was developed, with associated reduction of the 'bulk of the Red Blood Cells (RBC) and depletion of plasma. Umbilical cord blood banks use two major methods to store frozen umbilical cord blood (UCB): red cell reduction (RCR) or plasma depletion (PD). The RCR method centrifuges cord blood in hetastarch or albumin to isolate 21 ml of cord blood containing mostly white blood cells, adds 4 ml of 50% dimethyl sulfoxide (DMSO), and then freezes the resulting 25 ml of cell suspension[4]. The PD method removes plasma, saves all the cells, and freezes the cells in 10% DMSO[4].

It has been reported that the red blood cell (RBC) reduction processing method increased the number of units that could be stored in the same freezer space by as much as 10-fold, and thus provided significant economic advantage[3]. Volume-reduced process involves reducing the size of the sample by removing the harmful waste products (roughly 90% of the sample), then isolating and protecting the valuable stem cells in storage[5]. Waste products refer to red blood cells within a cord blood sample[5]. They do not cope well in storage and can rupture releasing a toxin into the sample. This can kill off valuable stem cells, meaning higher cell loss as well as being toxic for the recipient in transplant. In umbilical cord blood banking, volume and RBC reduction of the collected UC blood allows more efficient long-term storage and decreases infusion-related hemolysis and DMSO toxicity[5]. To establish an adequate panel, therefore, the haematopoietic cells of placental cord blood (PCB) units need to be concentrated into units of much smaller volume.

2. Umbilical Cord Blood Processing Techniques

Today, UCB processing laboratories use a variety of techniques for volume reduction: plasma depletion, removal of red cells, or both. Most methods involve centrifugation, sedimentation, and/or filtration for reducing the red cell content, plasma volume, or both[6]. Five major separation methods used are Plasma Depletion, Density Gradient, Hetastarch, PrepaCyte and Automated Centrifugal Machine (Sepax)[7-10]. MacoPress and AutoXpress (AXP), are machine types that all perform the volume reduction process.

2.1 Plasma Depletion

Limited cell dose hampers wider use of Cord Blood Transplantation (CBT). Plasma Depletion (PD) was developed by Chow and others in the late 1990s[4]. In the PD method, the UCB is centrifuged to separate the cells and plasma. The plasma is squeezed into a separate plasma bag, and 50% DMSO is added until the final DMSO concentration is 10% in the cord blood. The cord blood is then transferred to a freeze bag[4]. By depleting plasma but not RBC during processing, nucleated cell (NC) loss is reduced to <0.1% which increases significantly the proportion of high cell dose products by 3-fold[11]. A retrospective audited analysis was performed on 118 Plasma Depleted (PD) cord blood transplantation by[11]. These results demonstrated that plasma Plasm depleted cord blood transplantation is safe and effective, and that eliminating RBC reduction or depletion improves cell recovery during CB processing, resulting in a larger proportion of the inventory with high Nucleated cell (NC) number. Many other workers have compared the performance of PD with other processing methods[4,12-14]. Plasma depleted UCB units are more troublesome to thaw and wash, due to their larger and variable volume. However, when they are properly thawed and washed, PD units not only have more total nucleated cells (TNCs), CD34+ cells, and colony-forming units (CFU) than RCR units but also have high engraftment rates and may be more effective for treating certain conditions such as β-thalassemia[2,4].

2.2 Density Gradient Centrifugation (DGC)

Density gradient centrifugation (DGC) and the density gradient ultracentrifugation (DG-UC) method are widely used for UCB processing[15]. They work by applying a density gradient to the sample, by which the components, move to their equilibrium density (relative to the medium), separating them based on size and mass density in the case of top-down gradients, or solely based on mass density in the case of bottom-up gradients, with denser soluble constituents

collecting at the bottom. To enable the desired separation of different samples different gradient media, such as Ficoll, Percoll, Ficoll-Paque were developed. The Ficoll method was first used in 1968[16].

Almici *et al*.[17] compared separation procedures based on different density gradients in the attempt to obtain the highest reduction of red blood cells (RBC) while maintaining the highest recovery of progenitor cells. They compared three different densities of Percoll (1.069 g/ml, 1.077 g/ml, 1.084 g/ml), sedimentation over poligeline and sedimentation over poligeline followed by separation over Ficoll-Paque. Separation by sedimentation over poligeline followed by Ficoll-Paque allowed the highest reduction of RBC (hematocrit of the final cellular suspension 0.4 +/- 0.1%) while maintaining high recovery of CD34+ cells (85.3 +/- 5.6%) and total recovery for colony forming unit-granulocyte-erythrocyte-monocyte-megakaryocyte(CFU-GEMM), burst forming unit-erythroid (BFU-E) and colony forming unit-granulocyte-macrophage/monocyte (CFU-GM).

Kuljeet *et al*.[1] compared four protocols for processing CB, using different combinations of density-gradient centrifugation, hydroxyethyl starch (HES) and ammonium chloride (NH_4Cl) treatment, regarding the yields of CD45+, CD34+/CD133+ and colony-forming cells and stated that the highest yields of nucleated and progenitor stem cells were obtained with a two-step processing of cord blood. The CD133+ cells obtained by this method are expected to yield enough hematopoietic progenitors for potential allogeneic transplantation.

Sedimentation methods reduce the number of RBC to be infused. Red blood cell reduction reduces the risk of incompatible reaction. Sedimentation reduces side-effects of the DMSO cytotoxicity[18].

Taina and Jarmo,[19] demonstrated that mononuclear cells are easily isolated by density gradient centrifugation. In Ficoll-Paque density gradient centrifugation, anticoagulant-treated and diluted cord blood is layered on the Ficoll-Paque solution and centrifuged. During centrifugation, erythrocytes and granulocytes sediment to the bottom layer. Lower density lymphocytes,

together with other slowly sedimenting cells such as platelets and monocytes, are retained at the interface between the plasma and Ficoll-Paque, where they can be collected and subjected to subsequent isolation of hematopoietic stem cells or to the culture of mesenchymal stem cells[19].

2.3 Hetastarch

Hetastarch is a synthetic colloid made from natural sources of starch. The chemical name for Hetastarch is Hydroxyethyl Starch (HES)[20]. Hespan is a brand name of Hetastarch. Hetastarch (HES) processing of umbilical cord blood has been the industry standard since 1988, and thousands of transplants using HES-processed cord blood have been successful. The most common means of reducing red cell content has been the use of sedimenting agents such as hydroxyethyl starch (HES), gelatin, poligeline, and dextran[21].

Red blood cell (RBC) reduction is required to reduce the cord blood unit volume for commercial banking. Red cell sedimentation using hydroxyethyl starch (HES) is a standard procedure and it is the most common protocol in the cord blood banks[22]. The quality of UCB volume reduction is guaranteed by minimum manipulation of cord blood samples in the closed system[23]. Milad *et al.* carried out a study aimed at analyzing and comparing cell recovery and viability of UCB processed using the SEPAX automated system in the presence and absence of HES and showed that processing of UCB using the SEPAX system with the without-HES protocol due to the lower manipulation of samples could be used as an eligible protocol to reduce the volume of UCB[23]. Mutiea *et al*. demonstrated that incubation time of hydroxyethyl starch sedimentation increases cell recovery in umbilical cord blood processing by automated system[24].

2.4 PrepaCyte-CB

In 2009, a new advanced technology for processing cord blood known as PrepaCyte-CB was developed. It is a completely closed, sterile system that greatly reduces the chance for contamination during processing[25]. PrepaCyte-CB is a two-bag device that can interconnect with any freezing and storage bag of the user choice. The first bag is pre-filled with the PrepaCyte-CB separation solution, and the second bag is used to separate the blood's components during processing. The system’s interconnected, closed-bag set limits cell manipulation and helps minimize environmental contamination and identification errors[9,26].

CryoCell International was the first major cord blood bank to embrace this technology, which is reported to yields the maximum recovery of healthy stem cells and provides superior red blood cell reduction over all other methods[25]. PrepaCyte-CB has been shown to lead to earlier engraftment. For conditions where chemotherapy or other methods that affect the immune system are utilized, an earlier engraftment time means the patient will spend less time in the vital stage where they do not have an immune system capable of fighting pathogens. It can also translate to less time in the hospital and less stress and worry waiting for the patient to feel better[25]. Basford *et al*[26]. showed that PrepaCyte-CB offers high recovery of TNC, particularly after removal of granulocytes. This is because, as yet, current technology is not advanced enough to allow granulocytes to survive the freeze–thaw process[3,27].

2.5 SEPAX

Historical data on the use of SEPAX revealed an average TNC recovery of approximately 80% for CBUs with a processed volume of <220 ml. However, as volume increased to 270 ml, TNC recovery fell to <50%[28]. Sepax runs with the new program showed an increased TNC recovery for large volume units in comparison with similar historical runs, although not to the recovery seen in lower volume cords. The addition of RBC removal allowed for the desired high TNC recovery[28].

Automation of the cell concentration steps post-thaw with the use of the Sepax-2 device was introduced in routine practice about 2013. Initial performance qualification runs showed promising results and fitness of purpose for using Sepax-2 in concentrating thawed cells while removing > 95% of DMSO[2]. They reported on the reproducibility of the process of using Sepax-2 device when used to serially wash and concentrate two thawed UCB bags.

Shoulars *et al*., reported 352 UCB transplants (287 pediatric, 65 adult) performed between 2000 and 2017, with a cumulative incidence (CI) of primary graft failure (PGF) of 17.4% in pediatrics and 27.6% in adults. They showed that there was a trend toward lower PGF in pediatric Sepax-processed UCB and improved time to neutrophil engraftment for Sepax processed UCB[28].

Table 1 outlines the clinical Outcomes for Non-Sepax and Sepax processed umbilical cord blood transplants.

Table 1: Clinical Outcomes for Non-Sepax and Sepax Processed Umbilical Cord Blood Transplant (2000-2017).

Clinical Characteristics	Non-Sepax	Sepax
Number of UCB Transplant	329	23
Cumulative incidence (CI) of primary graft failure (PGF)	20.4%	4.5%
Cl of PGF: Adult UCB Transplant	27.4%	33.3%
Cl of PGF: Pediatric UCB Transplant	18.7%	0%
Time to neutrophil engraftment: Median (range) days	20 (6-141)	18 (12-35)
Time to platelet engraftment : Median (range) days	45 (18-184)	38 (9-76)
Cl of Acute GVHD	50.7%	60.8%
Cl of Chronic GVHD	28.5%	21.7%
Overall survival	48%	64%

Log rank analysis. All other *p*-values by Fisher's Exact Test. GVHD- Graft versus host disease

Source: Shoulars *et al*.,[28]

Shoulars *et al*., also reported excellent engraftment with no cases of primary graft failure in 20 consecutives pediatric UCB transplants using Sepax processing. Also, Sepax UCB processing has the advantage of prolonging product viability (>94% at 24 hours post-processing) and removal of >95% of DMSO prior to infusion[28].

3. Comparison of Umbilical Cord Blood Processing Techniques

The processing technique may influence the final concentration of the haematologic measurements and can be adjusted according to the operator's necessity. Therefore, it will be necessary to validate the process to obtain a good cellular recovery and the establishment of a quality standard for these red blood cells units[30]. Many workers have compared the effectiveness of different UCB processing techniques in achieving different purposes[9,2,31,32,25].

3.1 PrepaCyte-CB Versus Hespan Grafting Success

The Saint Louis Cord Blood Bank (SLCBB) which is one of the largest public cord blood banks in the world utilize both Hetastarch and PrepaCyte-CB UCB processed units for transplantation. Saketh *et al*.,[32] studied the potential impact of these processing methods on transplantation outcomes. One-year overall revealed no significant difference between patient receiving cells from each processing regimen. Neutrophil engraftments arising from Hespan and PrepaCyte-CB cord blood units were compared. Engraftment was defined as achievement of ≥500 absolute neutrophil count (ANC) by post-transplant day 42 (D42).

Figure 1 compares patient engraftment data for units processed with Hespan to those processed with PrepaCyte-CB. Median time to engraftment is similar, with interquartile ranges as shown. PrepaCyte-CB and Hespan had engraftment success of 98.1% and 94.5% respectively. Among the engrafted cells 9.3% died by day-42 in the Hespan processed samples while a slightly less percentage (7.5%) died in the PrepaCytes-CB samples.

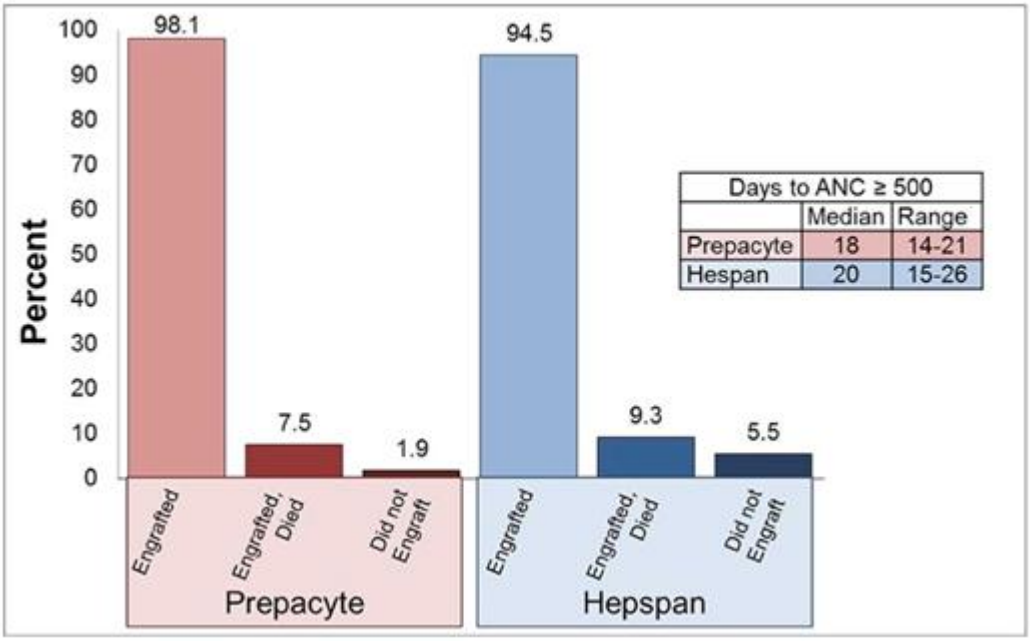

Figure 1: Day 42 Neutrophil Engraftment State: Prepacyte-CB Versus Hespan.

Source: *Saketh et al*.,[32]

3.2 Engraftment Time: PrepaCyte-CB Versus Five Other Processing Methods

CryoCell International[25] used data from five cord blood banks in the United State to compare the engraftment time of PrepaCyte-CB with five other UCB processing methods. As shown in Figure 2, the data show a quicker engraftment time for PrepaCyte-CB processed cord blood units compared to other processing methods.

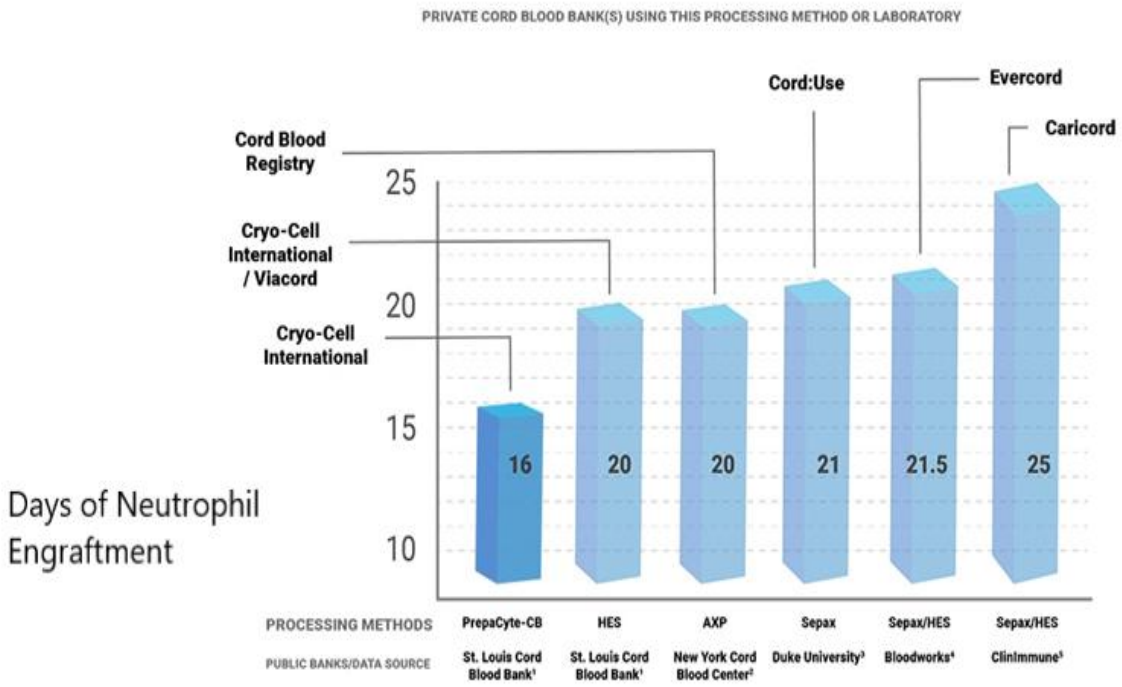

Figure 2: Comparison of Days for Neutrophil Engraftment: PrepaCyte-CB Versus Five Other Processing Methods

Source: CryoCell International[25]

3.3 Percentage Recovery of Colony-Forming Unit and Red Blood Cell from PrepaCyte-CB and Three Other Umbilical Cord Blood Processing Methods

CryoCell International,[25] also compared the percentage recovery of CFU and RBC from PrepaCyte-CB and three other UCB Processing Methods. PrepaCyte-CB recovers significantly

more viable stem cells than other processing methods. In a comparison conducted by the St. Louis Cord Blood Bank, PrepaCyte-CB recovered the highest percentage of colony–forming stem cell units (CFUs), capturing 51 percent more than the standard HES method and 70 percent more than the AutoXpress (AXP) method. PrepaCyte-CB also reduces red blood cell (RBC) as much as 99%. Fewer red blood cells post-processing means fewer toxic side elements and less chances of contamination. AutoXpress can reduce the red cell count by up to 70 percent, HES can reduce its volume by up to 82 percent and Sepax can reduce its RBCs by 84.7 percent (Table 2).

Table 2: Percentage Recovery of Colony-Forming Unit(CFU) and Red Blood Cell(RBC) from Different Umbilical Cord Blood Processing Methods

	PrepaCyte-CB	**Sepax**	**HES**	**AXP**
CFU Recovery (%)	80.2	62.7	52.9	47.0
RBC Depletion (%)	99.0	84.7	82.0	70.0

Source: CryoCell International[25]

3.4 Comparison of the Major Clinical Processing Techniques

Basford *et al*.,[9] carried out a comparison of the major clinical processing techniques for cord blood stem cells. They evaluated five separation methods: plasma depletion, density gradient, Hetastarch, a novel method known as PrepaCyte-CB and an automated centrifugal machine Sepax. The summary of the findings of Basford *et al*.,[9] shows that Sepax depletion gives a higher recovery of nucleated cells (Figure 3A), crucial for successful engraftment. After exclusion of granulocytes recovery, Sepax still remained the greatest at 78.8% compared to other methods (Figure 3B).

Basford *et al*.,[9] also examined the effect initial cord blood volume had on recovery of nucleated cells. Recovery using Sepax is reduced as the size of unit processed increases. Hetastarch, density gradient and plasma depletion separation were also affected in this way, but PrepaCyte-CB processing was not affected by the initial volume of the collected unit, and recovery of both

TNC and CD34+ progenitor cells was as efficient with smaller volumes as it was with larger units. Density gradient separation showed a reverse correlation: as UCB volume increase, so does recovery. Although interesting, it does not fairly compare to the other methods as the maximum volume processed with density gradient was 90 ml and all other methods were routinely tested with units of over 100 ml essential to promote engraftment[33]. From the results of this study Basford *et al*.,[9] suggested that Sepax, offers the best recovery of TNC, with PrepaCyte-CB and plasma depletion close behind.

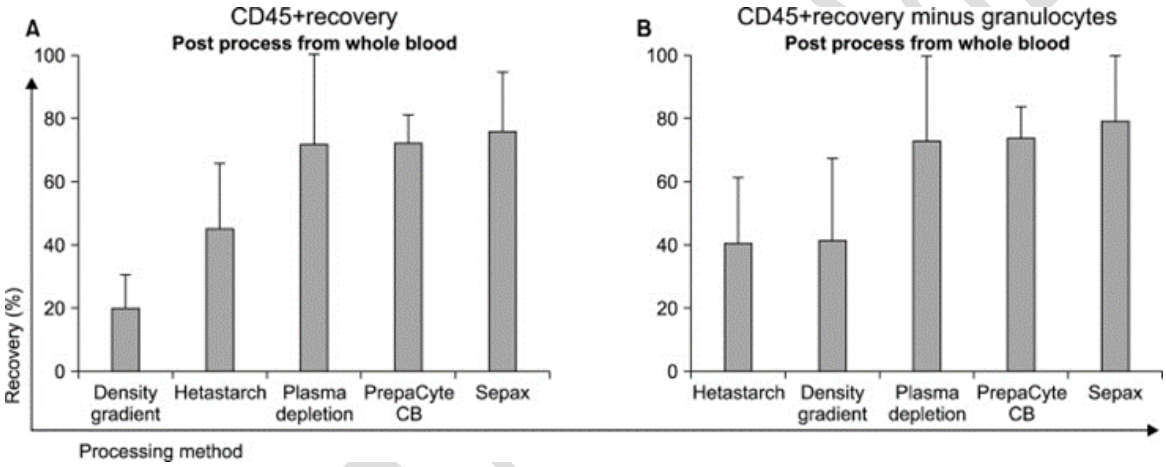

Figure 3: Rate of Recovery of Umbilical Cord Blood Nucleated Cells

Source: Basford *et al*.[9]

Basford *et al*.,[9] also studied the recovery of haematopoietic stem cells of three different developmental stages and their results showed that PrepaCyte-CB offers the best methodology for optimum HSC numbers from all three stages (Figure 4A, 4B and 4C), although again it is worth mentioning that Sepax recovery for both CD34+ and TNC is diminished as the volume of the UCB increases. The benefit of Sepax is that it is a fully automated system which allows for the mass processing of samples, which is suitable for larger cord blood banks.

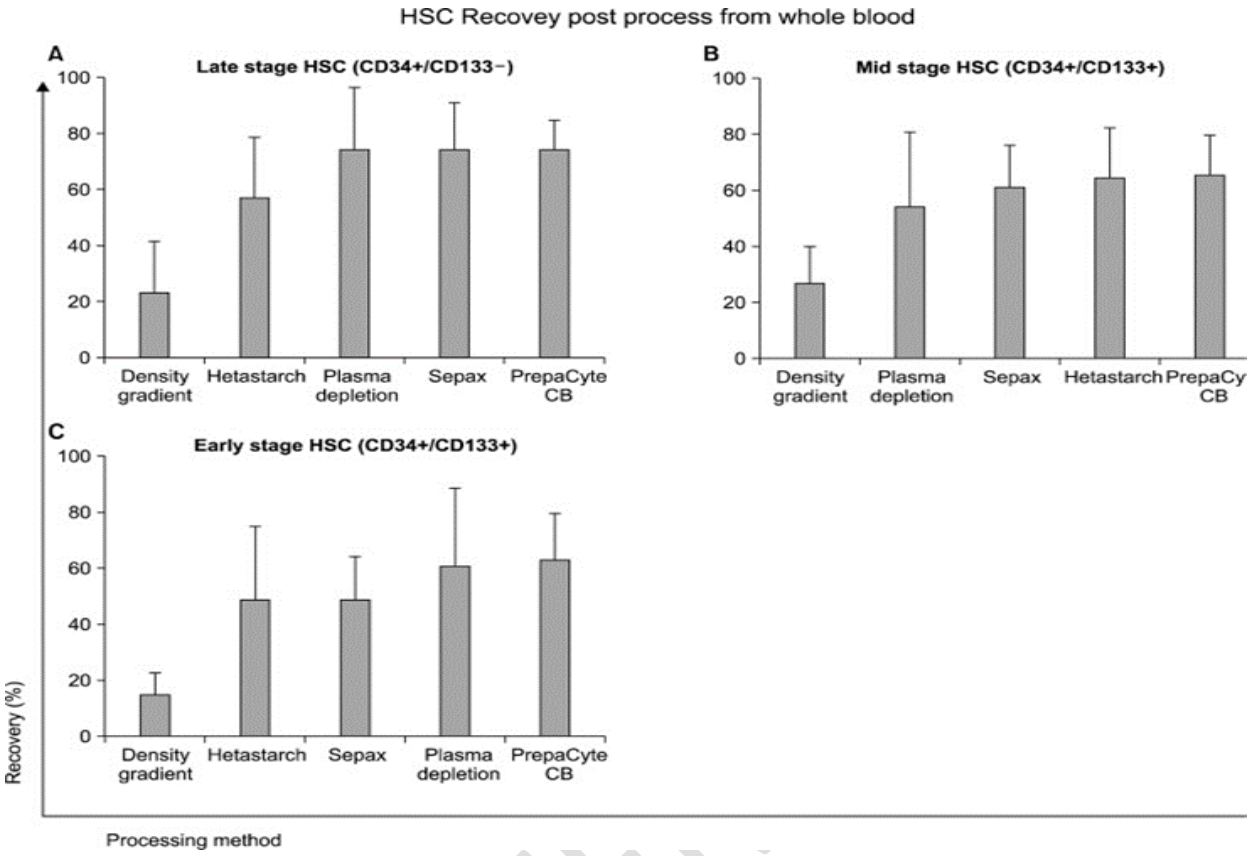

Figure 4: Recovery of Haematopoietic Stem Cells of Three Different Developmental Stages

Source: Basford *et al.*[9]

The recovery of some immune cells: T and B cells were also examined by Basford *et al.*,[9]. During this study Basford *et al.*, found superior recovery of CD45+/CD3+ T lymphocytes with PrepaCyte- CB which also gave the best results for CD10+ B cells (Figure 5A and 5B). Not much is known about whether these cells play a role in engraftment in humans but some mice models have shown that increased numbers of T cells transplanted can increase bone marrow reconstitution and therefore haematopoiesis and also eliminate residual leukemic disease in the transplanted mice[9].

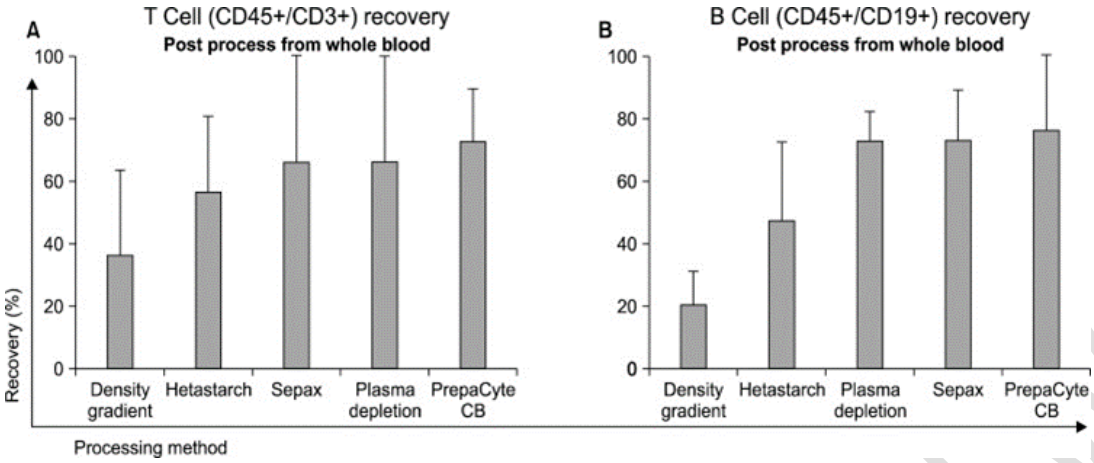

Figure 5: Comparison of T Cell and B Cell Recovery Rate for Different Umbilical Cord Blood Processing Methods

Source: Basford *et al*.[9]

Basford *et al*., examined the effectiveness of the different processing methods on cord blood volume reduction. For red blood cell removal, density gradient separation was the most effective method. The average number of red blood cells per ml of whole blood was 2.92×10^6 cells. After processing with density gradient methods the number of red blood cells was reduced to 0.03×10^6 cells/ml of blood. This reduction was significantly greater than with PrepaCyte-CB, plasma depletion, HES and Sepax (Figure 6A). The same condition applied in the removal of haemoglobin (Figure 6B). They also examined whether initial collected cord blood volume had an effect on RBC reduction but their data revealed no correlation. Initial collected volume of UCB also had no correlation on RBC reduction according to Basford *et al*.[9]

When looking at the volume reduction of the physical size of the unit, it would seem that plasma depletion would be of particular benefit, as a smaller volume reduces the space needed for storage[9] and also means less DMSO is added to the sample in preparation for cryopreservation[34]. This means that it could even save the need to wash samples before infusion (for haematopoietic transplant only) as it has been previously shown that TNC recovery after cryopreservation is greater without a wash step[35]. However, if volume reduction is measured as the ability to remove

RBC and haemoglobin; then it is actually a simple and economic density gradient separation which is the most efficient.

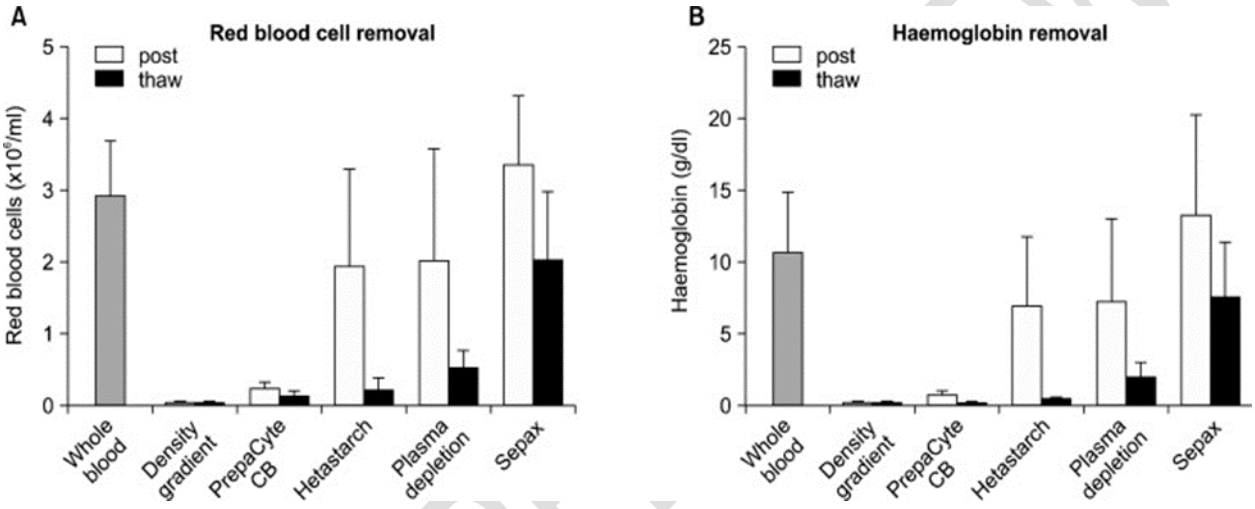

Figure 6: Comparison of Red Blood Cell Removal for Different Umbilical Cord Blood Processing Methods

Source: Basford *et al*.[9]

Basford *et al*.,[9] examined the clonogenic potential of the UCB units which was measured by the CFU assay using the different clinical processing techniques. Traditionally, this is the most important test, since it gives the best possible readout for potential of the cord blood to be useful if used therapeutically. PrepaCyte-CB performed best in this test, not only post processing but also after cryopreservation and the subsequent thawing (Figure 7). The significance of post thaw CFU is critical for future therapeutic uses of UCB units as it is necessary to know that they will still be able to engraft after storage[36]. This could be because PrepaCyte-CB is the second most efficient method for removing RBC. A reduction in RBC numbers has previously been shown to have an advantageous effect on CFU[37].

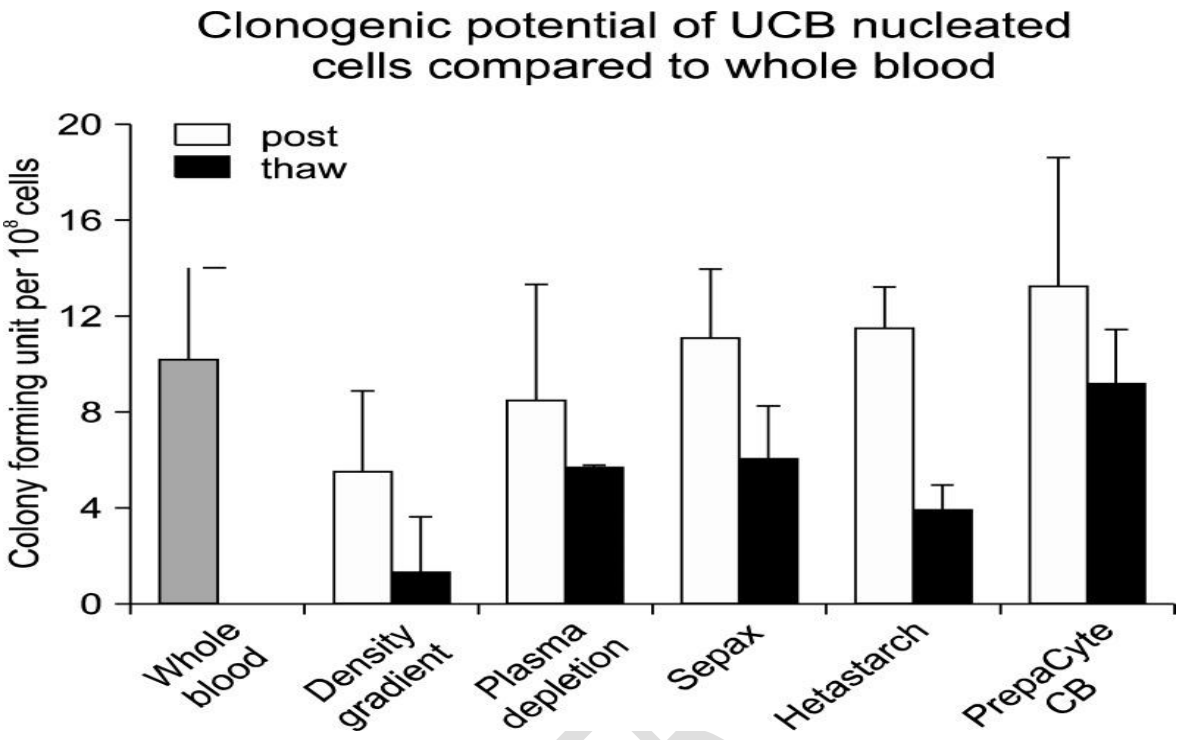

Figure 7: Clonogenic Potential of UCB Units Using the Different Clinical Processing Techniques.

Source: Basford *et al*.[9]

Badowski and Harris[31] compared Total Nucleated Cells (TNC) and Mono Nucleated Cells (MNC) recovery rate of Hespan and SEPAX against AXP processing methods. His work showed that both Hespan and SEPAX reproducibly recover greater than 95% of the cord blood stem cells in a typical collection and result in a reduced final volume of approximately 20 cc for final storage (Figure 8). AutoXpress allows for greater throughput with fixed personnel numbers (increasing the economy of operations) and is a Food and Drug Administration (FDA)-cleared, functionally closed system which is capable of processing cord blood collections of any volume.

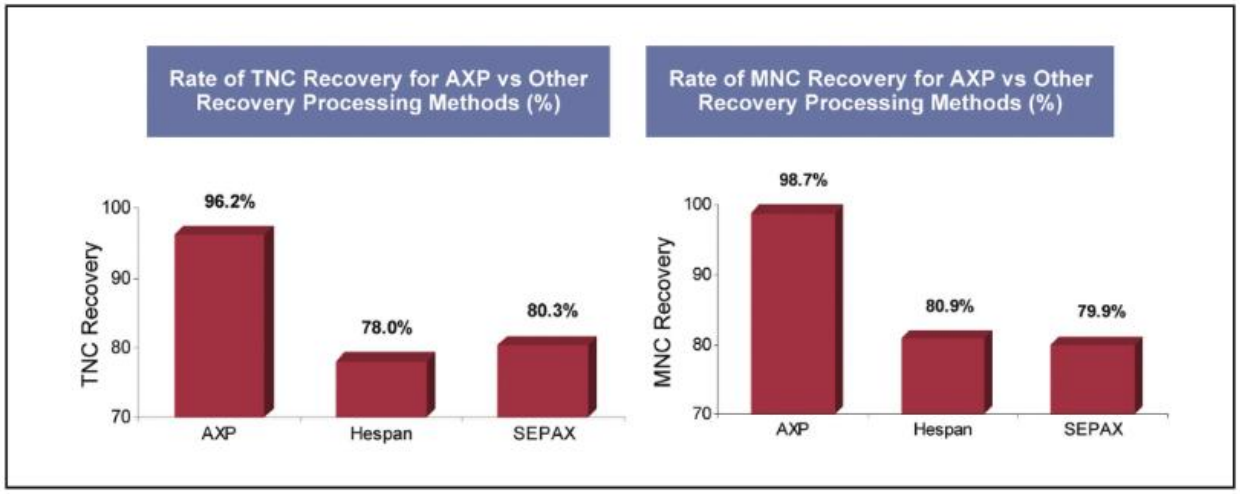

Figure 8: Rate of TNC and Mono Nucleated Cell Recovery for Different Processing Methods

Source: Badowski and Harris.[31]

Chow *et al*.,[2] examined the volume reduction methods of processing umbilical cord blood and classified them into zero, first, second and third generations (Table 3). The most common method for processing CB units today appears to be the Hetastarch or HES RBC reduction (RCR), whether manual or automated.

Table 3: Technical Comparison of Some of the Most Popular First Generation Cord Blood(CB) Processing Techniques and the Proprietary Second and Third Maxcell CB Processing Technologies

	Nature of UCB	**Manual/automated Processing**	**RBC reduction**	**Plasma depletion/reduction**
Zero generation	Whole cord blood	Manual	None	None
First generation Hetastarch	Red cell reduced	Manual SEPAX* or AutoXpress*	Yes	Yes
First generation PrepaCyte-CB	Red cell reduced	Manual	Yes	Yes

First generation Top & bottom Operations ll	Red cell reduced	Manual	Yes	Yes
First generation Ficoll	Red cell reduced	Manual	Yes	Yes
MaxCell (MC) Technologies				
Second generation	Plasma depleted/reduced	Manual SEPAX	NO	YES
Third generation	MaxCord RBC Reduced + RBC Replete	Manual SEPAX AutoXpress	Yes/No	Yes

Source: Chow *et al*.[2]

These have been called first generation (1st Gen) cord blood processing techniques. It should be noted that these methods are often erroneously referred to as red cell reduction when all of these techniques retain considerable RBCs and do not deplete, but only reduce the number of RBCs[38]. Unfortunately, all 1st Gen RCR processing methods lose significant numbers of nucleated cells, stem cells, and progenitor cells as measured by CD34+ cells or colony forming unit's enumeration, with an approximately 25% nucleated cell loss on average among various reports in the literature[3, 5,26,39-43].

4. Conclusion

First problem of cord blood banking is that banking a sufficiently large number of cryoprotected CB units requires vast amounts of costly storage space in liquid nitrogen (LN). It was for this reason that volume reduction processing of CB units was developed, with associated reduction of the bulk of the Red Blood Cells (RBC) and depletion of plasma.

The processing of UCB has developed over the last three decades. The primary purpose of UCB processing is to reduce the volume of the whole blood through red cell reduction (RCR) or plasma depletion (PD) or both and separate stem cells from the cord blood so that the sample produced concentrates the stem cells and can be used safely.

The five most popular processing techniques are Plasma Depletion, Density Gradient, Hetastarch, PrepaCyte-CB and Automated Centrifugal Machine (Sepax). Most methods involve centrifugation, sedimentation and/or filtration for reducing the red cell content, plasma volume, or both

Many workers have compared the performance of the different processing techniques in achieving the set goals.

PrepaCyte-CB and Hespan record similar success of neutrophil engraftment. PrepaCyte-CB however has faster engraftment time than the four other UCB processing methods. This can translate to less time in the hospital and less stress and worry for both patient and care-giver. PrepaCyte-CB recovers significantly more viable stem cells than other processing methods.

In terms of recovery of nucleated cells crucial for successful engraftment Sepax depletion gives a high recovery of nucleated cells crucial for successful transplantation but recovery using Sepax is reduced as the size of unit processed increases. Hetastarch, density gradient and plasma depletion separation were also affected in this way, but PrepaCyte-CB processing was not affected by the initial volume of the collected unit, and recovery of both TNC and CD34+ progenitor cells was as efficient with smaller volumes as it was with larger units. Density gradient separation showed a reverse correlation: as UCB volume increase, so does recovery. The benefit of Sepax is that it is a fully automated system which allows for the mass processing of samples, which is suitable for larger cord blood banks.

PrepaCyte-CB offers the best methodology for optimum HSC numbers from all three developmental stages of stem cells. Words on the comparison of the effectiveness of common UCB processing methods found superior recovery of CD45+/CD3+ T lymphocytes with PrepaCyte CB which also gave the best results for CD10+ B cells. For red blood cell removal, density gradient separation has been found the most effective method.

The clonogenic potential of UCB units have been measured by the CFU assay using the different clinical processing techniques. PrepaCyte-CB performed best in this test, not only post processing but also after cryopreservation and the subsequent thawing. Hespan and Sepax reproducibly recover greater than 95% of the cord blood stem cells in a typical collection and result in a reduced final volume of approximately 20 cc for final storage.

It should be noted that Hespan and Sepax are often erroneously referred to as red cell depletion when all of these techniques retain considerable RBCs and do not deplete, but only reduce the number of RBCs.

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