Red Cell Depletion on Bone Marrow/Peripheral Blood Collections Utilizing PrepaCyte®- CB Solution

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Background

The aim of this study is to test a new reagent to salvage red cells in grafts obtained from bone marrow donors in order to minimize the risk of severe anemia following bone marrow harvest. Additionally, this method is also important in the prevention of ABO incompatibility when there is an ABO mismatch between donor and recipient.

Several approaches have been used to prevent severe anemia in adult patients who undergo bone marrow collection such as: pre-harvest autologous blood donations, and the use of erythropoietin with or without iron supplementation. In pediatric patients, because of the size of the donors, the degree of anemia can be quite severe, and because of their age they are not candidates for autologous blood donations prior to the harvest. Some investigators have used pre harvest erythropoietin to increase their hematocrit prior to collection, others have done nothing except to give the donors iron supplementation.

For the past 15 years at our Institution we have salvaged the red cells from donors after processing and reinfused them back to the donor thus preventing anemia. Unfortunately the device used has been discontinued and has forced us to look for other alternatives.

PrepaCyte®–CB (BioE, Inc) is a cell processing system use for the separation of white blood cells, including hematopoietic stem progenitor cells from umbilical cord blood units. This product is a sterile closed system and has a Device Master File (DMF) with FDA for use under IND for cord blood processing only. Most blood cell isolation procedures involve the removal of red blood cells by density gradient sedimentation using starch methodologies, but with a significant loss of progenitor cells especially in the case of umbilical cord blood. PrepaCyte®–CB is a non-density based one step reagent designed to aggregate red blood cells while leaving the white cells in the supernatant suspension. The supernatant is expressed out into a transfer bag, and the white cells including the hematopoietic stem cells are recovered with very little red cell contamination.This process therefore eliminates the red cells which are undesirable for transplantation, but could be reinfused into the bone marrow donor. The yield and cell recovery of the hematopoietic cells is greatly enhanced as well with this procedure.

Without a system to perform red cell depletion in bone marrow hematopoietic cells collected during a bone marrow harvest wherein the product may contain a large amount of red cells (~25-35% of the component volume). We intend to assess this method in bone marrow harvest, allowing us to collect an optimal number of hematopoietic progenitor cells with minimal cell loss and to reinfuse the red blood cells that are separated using the PrepaCyte®-CB processing system.

Our preliminary data was obtained using small volumes of bone marrow or peripheral blood progenitor cells spiked with O blood type red cells to demonstrate and evaluate the process.

Materials and Method

BM/PBSC samples were used to spike blood type O red cells in volumes ranging from 60 to 600 ml that included the spiked sample and added plasmalyte A solution. PrepaCyte®-CB solution was added to the sample mixture at a 1:1 ratio. After addition of reagent the bag was gently mixed for 3-5 minutes by hand at room temperature, then allowed to stand upright for 30 minutes at room temperature for aggregation and precipitation to occur. After the specified time, a leukocyte-rich supernatant layer and a red cell layer was observed. The stem cell rich supernatant was expressed using a plasma expressor into a transfer bag. An aliquot sample was tested for cell count, CD34⁺ enumeration by flow cytometry, clonogenic assay, viability by trypan blue exclusion test, and sterility test. Pre and post cell counts were compared using t test and column statistics using Prism software. Results are presented in table below.

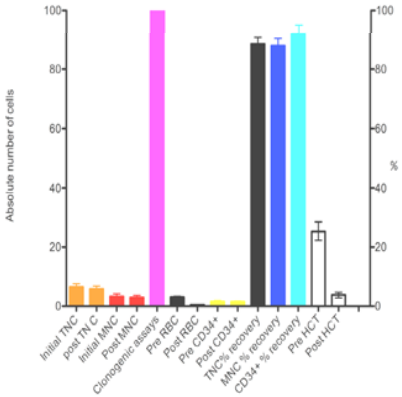
Table

Sample	1	2	3	4	5	6	7	8	9	10	Median	P Values
Initial TNC ($x10^9$)	5.3	3.9	4.7	7.3	13.3	9.9	5.6	2.9	1.26	9.9	5.5	
Post TNC ($x10^9$)	4.2	3.6	4.3	6.9	11	9.7	4.7	2.4	1	9.7	4.5	0.1
Initial MNC ($x10^9$)	2.34	1.7	1.8	3.7	10.7	2.7	4.7	2	0.6	2.6	2.5	
Post MNC ($x10^9$)	1.9	1.6	1.7	3.5	8.8	2.6	3.8	2	0.5	2.6	2.3	0.7
Pre-Hct (%)	41.3	17.3	29	28	11.7	28	8.8	35.8	27	27.9	28.0	
Post-Hct (%)	6.6	8.4	8	3.6	4.2	1.2	1.2	1.8	1.5	1.2	2.7	<0.0001
Pre-RBC ($x10^6$/uL)	4.47	1.89	3.14	2.89	1.97	3.45	1.01	3.96	3.52	4.17		
Post RBC ($x10^6$)	0.73	0.33	0.88	0.93	0.59	0.68	0.11	0.19	0.15	0.08		<0.0001
TNC% Recovery	79	91	91	95	83	98	84	86	79	98	88.5	
MNC% Recovery	83	94	96	95	82	96	81	83	78	96	88.5	
Initial Volume	90	80	60	350	570	172	793	492	88	172		
Vol + PrepaCyte	180	160	120	700	1140	344	1586	984	176	344		
Pre-CD34⁺ ($x10^6$) (((($x10^6$)	2	1.6	0.9	3.3	1	1	0.7	1.2	1.5	2.3		
Post- CD34⁺ ($x10^6$)	2.1	1.3	0.8	2.9	0.9	1.1	0.7	1.6	1.1	2.4		0.4
Clonogenic Assay	49	109	63	153	68	83	71	92	87	246		

Results

There is no statistical difference between the pre and post counts for TNC (p=0.7) and for MNC (p=0.1), however a statistically significant difference in the pre and post hematocrit (p=<0.0001) and the pre and post red cell count (p=<0.0001) was observed. Recovery of cells on TNC was 88.5% ± 2.7; on the MNC 88.5% ± 3

Figure 1. This figure represents a comparison of the absolute number of TNC, MNC and CD34⁺ cells pre and post on the left axis and on the right axis the % recovery of TNC, MNC and CD34⁺ cells as well as the decrease in Hct and pre and post red blood cells.



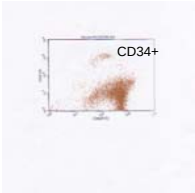


Figure 2. Representative CD34⁺ sample post process

Conclusion

The use of PrepaCyte^R-CB (BioE, Inc.) to deplete red cells from BM/PBSC preparations was very effective with minimal cell loss.The future direction is to use this method prospectively to process bone marrow for transplantation in children.

References

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