Novel Cell Washing System Using Spinning Membrane Filtration[1]

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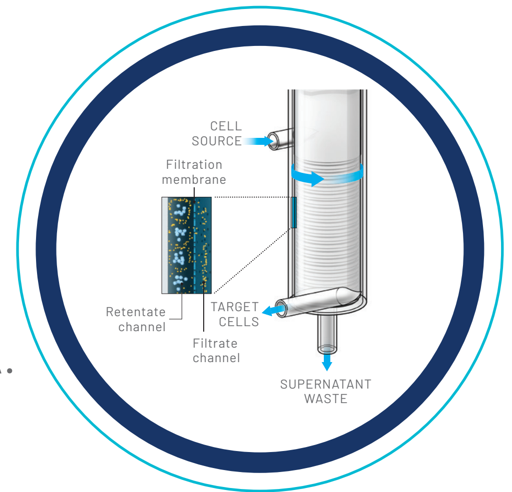

Background

Several automated cell washing and/or volume reduction products exist that incorporate centrifuge-based technologies into a closed-set system. These systems cause cell pelletization requiring manual resuspension. Spinning membrane filtration is currently used in other automated blood cell separating technologies (i.e. plasmapheresis) and leverages the same benefit of these technologies (i.e. non-pelletized blood cell products) that may be useful in cell therapy applications. In this study, an automated cell washing protocol was performed on apheresis-derived mononuclear cell (MNC) products using a closed-system, spinning-membrane separation system (Figure 1, Figure 2).

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Figure 1: Depiction of the spinning-membrane cell washing instrument.

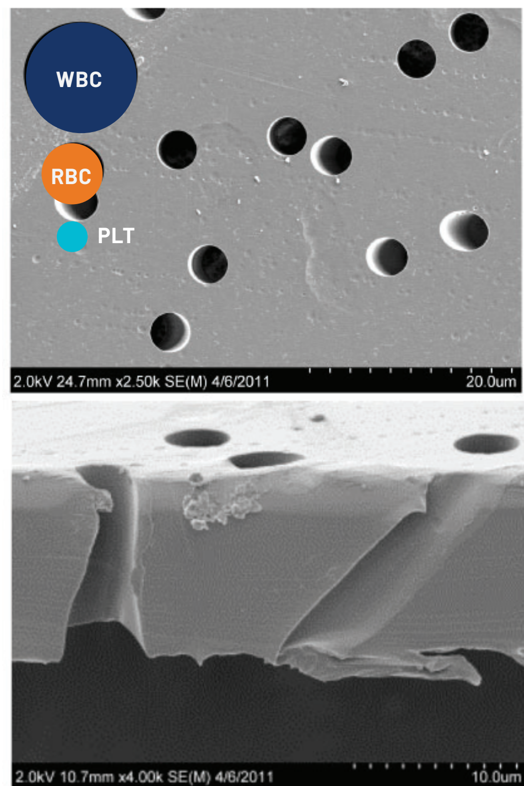

Figure 2: Spinning-membrane separation disposable component used for wash protocol (top). Face and cross-section (second image from top) SEM images of the polycarbonate filter membrane used in protocol.

Methods

A total of 22 MNC products were collected from healthy donors using a commercially available leukapheresis device. MNC products were suspended in 150mL of autologous plasma. Within 2 hours of collection, products were sampled for baseline cell counts and connected to a closed, disposable tubing set containing a spinning membrane separation device. Cells were then separated from supernatant without pelletization, producing a 2-log supernatant-reduced cell concentrate, suspended in 0.9% NaCl solution (final product volume of ~100–200mL). Washed cell products were immediately sampled for calculation of cell recovery. A subset of these products (n=5) were sampled for pre- and post-wash leukocyte apoptosis levels on Day 0 and through 3 days of culture at 5% CO2 and 37°C in RPMI 1640. In another subset of products (n=4), lymphocyte proliferation was measured on Day 3 after stimulation with PHA.

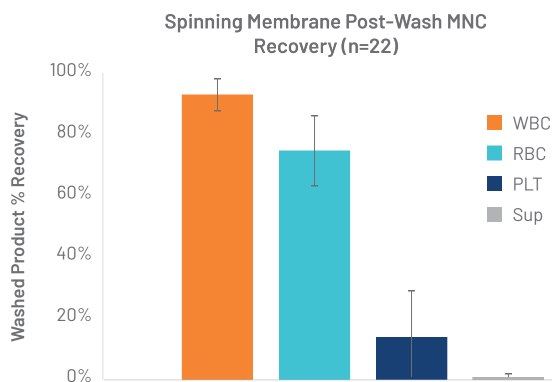

Figure 3: Post-wash cell recoveries from a 2-log supernatant reduction protocol using non-pelletizing spinning-membrane technology. Recoveries are shown as percent (±st. dev.) of source cell suspension recovered in washed product.

Results

Washed cell products showed average WBC, RBC, and Platelet recoveries of 92.3% (±4.6%), 74.4% (±11.1%), and 14.2% (±14.3%), respectively (Table 1, Figure 3). The washing procedure achieved a 98.8% (±0.8%) average supernatant removal. Apoptosis levels in pre- and post-wash samples were not significantly different (p=0.66). Lymphocyte proliferation did not appear to be affected by the wash (p=0.14) (Figure 4). Duration of the washing procedure averaged less than 7 minutes (range 5.7-10.6 minutes).

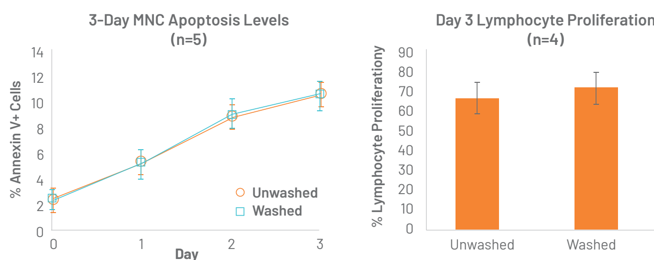

Figure 4: Three-day apoptosis levels for washed and unwashed cells measured as the percentage of Annexin V+ cells using flow cytometry (left). Day three lymphocyte proliferation of washed and unwashed cells measured by Dim-CFSE FITC-A (right).

Conclusion

The use of spinning-membrane filtration for cell washing protocols generates a platelet-reduced WBC product with good viability and supernatant removal, while avoiding pelletization. The resultant washed cells show no decrease in viability or proliferation.

[1]Data on File at Fresenius Kabi USA

Table 1: Pre- and post-wash fluid volumes, cell counts, and supernatant amounts for 22 fresh MNC products measured by Dim-CFSE FITC-A (right).

	Pre Wash MNC				Post Wash MNC							
	Volume	WBC	RBC	PLT	Volume	WBC		RBC		PLT		SUP
	(mL)	($x10^9$)	($x10^9$)	($x10^9$)	(mL)	($x10^9$)	(%Pre)	($x10^9$)	(%Pre)	($x10^9$)	(%Pre)	(%Pre)
Mean	203.3	2.33	36.7	51.9	183.0	2.14	92.3%	27.0	74.4%	6.65	14.2%	1.3%
Std Dev	62.6	0.87	20.4	77.7	55.1	0.77	4.6%	15.6	11.1%	9.13	14.3%	0.8%

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