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Microaggregate Counts in Frozen-preserved Erythrocytes: Effects of Washing in Three Blood Processors and Filtration

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Since microaggregates have been implicated in posttransfusion pulmonary insufficiency, their elimination has become an active concern in blood transfusion. Various types of filters, as well as frozen-preserved erythrocytes, have been used to provide blood relatively low in microaggregates. We have counted particles in frozen-stored blood before deglycerolization, after washing in each of three cell processing systems, and after filtration through a 40-μm filter. Washing frozen erythrocytes reduced the total particle counts by an average of 89%. Slight differences were found among the three blood processors with respect to particle removal. Passing washed blood through a 40-μm filter did not result in significant further reduction in particle counts. Hence, the use of such filters in a frozen-preserved blood system is not warranted.

THE MECHANISM of microaggregate formation of platelets, leukocytes, and fibrin in acid-citrate-dextrose (ACD) blood stored at 4°C for up to 21 days has been previously studied.[1,2] These microaggregates have been implicated in episodes of posttransfusion pulmonary insufficiency.[3] Since the standard 170-μm filters provided with transfusion kits have been shown to be inadequate in filtering the smaller particles, various other microfiltration methods have been tried with varying degrees of success. The use of a 40-μm filter has been shown to result in acceptable blood flow rates with no changes in the coagulation and fibrinolytic systems.[4] A significant alteration in filtration characteristics and blood flow rates has been demonstrated during the passage of multiple units of blood through 20-μm filters.

Washing erythrocytes has been suggested as a means of decreasing these microaggregates and at the same time circumventing the inadequacies of filtration. Goldfinger et al.[5] have shown that freezing, thawing, and deglycerolization reduces the number of microaggregates by 80%–90%. Since the Cook County Hospital Blood Bank converted to a primarily frozen-stored blood service in July 1973, we have been interested in evaluating the particle content of reconstituted frozen-preserved blood.

We have used three blood processing systems in washing previously frozen and thawed erythrocytes. A comparative study of the efficiency of these three processors in microaggregate removal, and an evaluation of the additional benefit of passing washed erythrocytes through a 40-μm filter have been done in an attempt to solve the microaggregate problem.

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MATERIALS AND METHODS

Three blood processing units are in use at our facility: the continuous-flow Haemonetics blood processor, model 15, with polycarbonate disposable bowl (Haemonetics Corp., Natick, Mass.); the continuous-flow automated Sorval Elutramatic with polyvinyl chloride bag (DuPont-Sorvall, Newtown, Conn.); and the batch washer, automated IBM blood processor with polyvinyl chloride sheeting (International Business Machines Corp., Princeton, N.J.). Twenty-seven units of glycerolized frozen erythrocytes were evaluated, nine prepared in each of the three blood processors by a modification of the technique of Meryman and Hornblower.[6] The number of particles per unit of blood was calculated from hematocrit values and total volumes for each unit immediately after thawing, after washing, and after filtration through a 40-μm filter (Ultipore, Pall Corp., Glen Cove, N.Y.). For comparison, particle counts were done on 15 units of packed red blood cells before and after filtration and on 15 units of whole blood at 0, 3, 7, 14, and 23 days of storage (3 units for each storage date). The same counting and calculation procedures were used as for glycerolized, thawed red blood cells.

The microaggregates were counted by a Coulter Counter, model ZBI with Channelyzer II (Coulter Electronics, Hialeah, Fla.) using a 280-μm aperture so that particles of 5.6 112-μm diameter could be counted. The instrument was calibrated with 18.04-μm latex particles (Coulter Diagnostics, Hialeah, Fla.).

Blood sample preparation for particle counting was as follows: aliquots of 0.8 ml were taken from each unit, and each was pipetted into a flask containing 100 ml of sterile isotonic saline solution and 75 mg of saponin at room temperature. The flask was gently swirled to mix the particles and lyse the erythrocytes. The hemolyzed suspension was drawn through the 280-μm aperture of the Coulter Counter and the number of particles at ten different sizes, from 5.6 to 112 μm, was counted in 5 ml of the suspension. Appropriate background counts of the saponin-saline were subtracted from the total. The average of triplicate results was used to determine the number of microaggregates in 1 ml of blood.

Microscopic study on Giemsa-stained buffy-coat smears was done on all 27 units under evaluation to determine the composition of the particles in unwashed, washed, and filtered units.

RESULTS

Table 1 shows detailed particle counts of blood cells in 27 units of blood after thawing, after washing in each of the three processors, and after filtration through the 40-μm microfilter. Since microfilters of 20 μm and 40 μm have frequently been used, the particle counts were divided into three ranges of size: 5.6-20 μm, 20-40 μm, and 40-112 μm. In freeze-preserved, washed

Table 1. Microaggregates in Frozen, Thawed, and Processed Red Blood Cells by Three Washing Systems

		IBM (N = 9)		Haemonetics (N = 9)		Elutramatic (N = 9)		Mean	
RBC	Particle Diameter (μm)*	Count $\times 10^4$/unit	Particles Removed (%)	Count $\times 10^4$/unit	Particles Removed (%)	Count $\times 10^4$/unit	Particles Removed (%)	Count $\times 10^4$/unit	Particles Removed (%)
Postthaw	5.6–20	3655		2777		3500		3310	
	20–40	49		63		64		59	
Total		3704		2840		3564		3369	
Postwash	5.6–20	413	89	473	83	207	94	364	89
	20–40	12	76	13	79	7	89	11	81
Total		425	89	486	83	214	94	375	89
Postfiltration	5.6–20	382	8	403	15	195	6	327	10
	20–40	11	8	9	31	4	43	8	27
Total		393	8	412	15	199	7	335	11

*Particles larger than 40 μm were rare and therefore were not included in this table.

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Table 2. Microaggregates in Liquid-Stored Whole Blood (N = 15) and Packed Cells (N = 15) Stored at 4°C up to 23 Days

Particle Diameter (μm) Days of Storage*	Packed Cells × 10^4/unit Count Prefilter	Count Postfilter	Particles Removed (%)	Whole Blood, Count × 10^4/unit Prefilter
5.6–20				
0 d	1069	910	15	988
3	2945	1659	44	2870
7	3704	3185	14	2751
14	3091	2171	30	3030
23	4343	3194	27	3542
20–40				
0	24	3	87	22
3	35	17	51	40
7	84	40	52	67
14	252	115	54	116
23	263	146	44	198
40–112				
0	5	0	100	4
3	3	2	33	3
7	4	1	75	11
14	8	1	87	7
23	6	1	83	10

*Study was done on 3 units of packed red cells and 3 units of whole blood on each day of storage.

erythrocytes particles larger than 40 μm in diameter were rare. Microaggregates 5.6–20 μm in diameter were present in the largest numbers before washing (mean 3310 × 10^4), but they were greatly decreased by washing in the IBM and Elutramatic systems—to roughly 11% and 6%, respectively, of their original count by removing 89% and 94% of total particles. An exception was noted with Haemonetics-washed cells, which, by comparison, contained more particles of this size—17% of the original amount, removing 83% of the total count. Filtration further removed postwash particles of this size—8% after washing in the IBM processor, 15% after washing by Haemonetics, and 6% after washing by Elutramatic.

While the number of 20–40-μm particles in the blood was far lower initially (mean 59 × 10^4), washing removed 76%, 79%, and 89% by the IBM, Haemonetics, and Elutramatic processors, respectively. Filtration with the 40-μm filter resulted in a further removal of microaggregates by 8%, 31%, and 43%, respectively, of the postwash counts.

Table 2 presents the findings on the 15 units each of liquid stored whole blood and packed red cells. As in the case of frozen red cells, the number of 5.6–20-μm particles predominated; they increased with increasing storage time at 4°C. The 20–40-μm particles also increased in number after storage at 4°C. Particles of 40–112 μm were present in very small numbers. Filtration brought about a variable reduction in the number of particles of all sizes.

Microscopic study of the Giemsa-stained buffy-coat smears prepared from prewashed, postwashed, and filtered samples showed a gradual decrease in the number of particles. The particles were larger and primarily composed of

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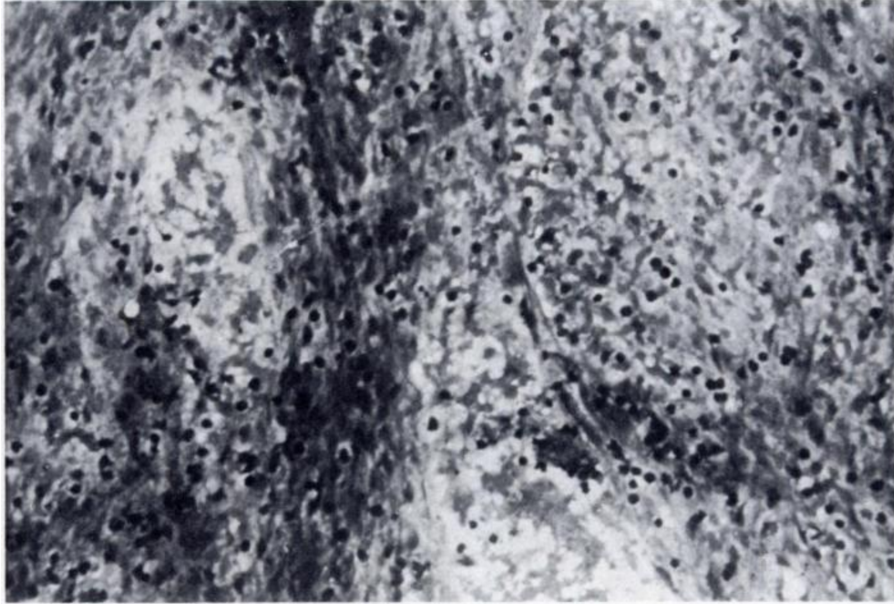

Fig. 1. Buffy-coat preparation of postthaw specimen. ×256.

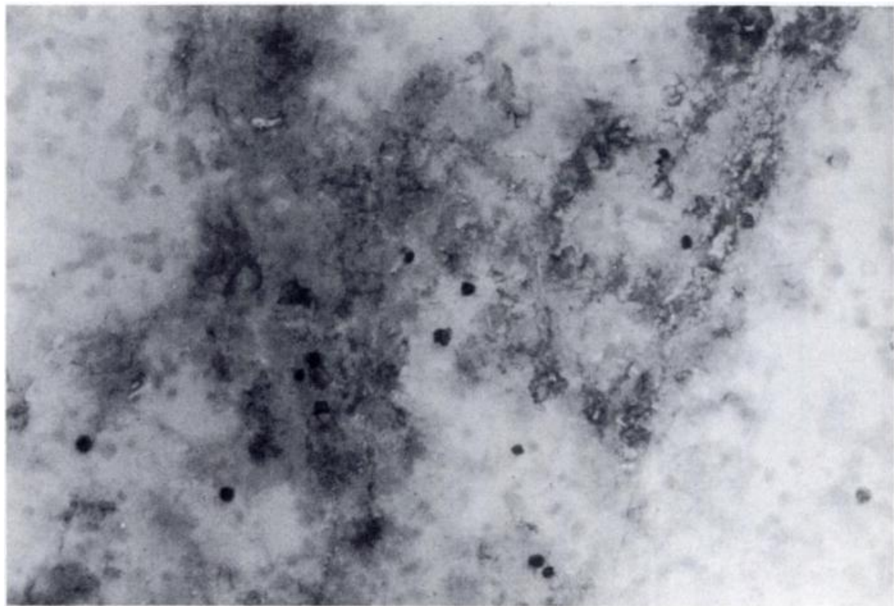

Fig. 2. Buffy-coat preparation of postwash specimen. ×256.

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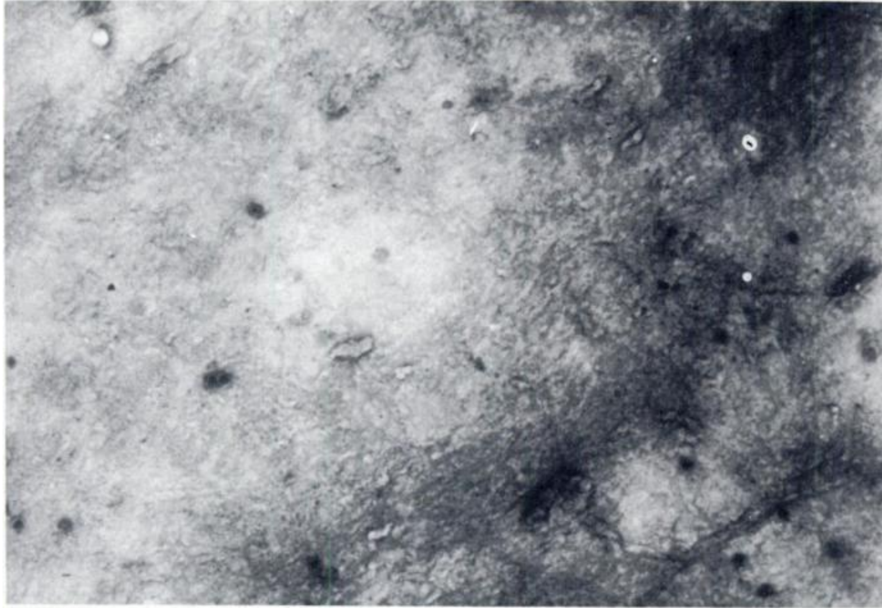

Fig. 3. Buffy-coat preparation of postfilter specimen. ×256.

tangled, well-preserved fibrin strands and masses of amorphous material with trapped, intact, and disintegrated white cells in the prewashed samples (Fig. 1). These particles were smaller and less cellular in the postwashed products (Fig. 2). In the filtered samples (Fig. 3), although there was further reduction in the amount of these cells and amorphous material, they were seen scattered throughout the smears.

DISCUSSION

Investigators working with liquid blood stored at 4°C for more than 1 wk have encountered particles of varying sizes composed of platelets, white cell aggregates, and fibrin less than 70 μm in size.[1] It has been suggested that transfusion of multiple units of such preserved blood produces pulmonary microembolization, a contributing factor in the development of irreversible posttraumatic respiratory insufficiency.[7,8] Patients with pulmonary disease with marked abnormalities of the ventilation-perfusion ratio, such as cases of emphysema, chronic bronchitis, recurrent pulmonary embolization, postperfusion syndrome, and acute adult respiratory distress syndrome, may develop acute respiratory failure because of additional reduction in the pulmonary vascular bed from microembolization.

Since the exact size and number of microaggregates that will occlude the microcirculation and produce irreversible damage have not been determined, their reduction to an absolute minimum should be the ultimate goal. Filters of varying pore sizes, quality, and design have recently become available to filter a larger portion of these microaggregates that are not eliminated by the conventional 170-μm filters included in blood administration sets. In the selection of micropore filters for large-scale use, important factors to be considered are

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filtration effectiveness, maintenance of blood flow, and effect on blood constituents. Because the efficiency of these filters decreases with respect to both particle elimination and rate of filtration as more blood is passed through them, often filters have to be changed every two to three transfusions.[9]

In this experiment we found that frozen-stored blood rarely contained particles 40–112 μm in size, whereas particles of this size were seen in small numbers in packed red cells and whole blood. As shown in Tables 1 and 2, the number of 20–40-μm particles in frozen-stored red cells was approximately the same as that found in packed cells and whole blood after 1 wk of storage. The greatest number of particles was in the 5.6–20-μm range, which evidently included single leukocytes and aggregates of white cells and aggregates with antigenic contents. Passage of blood through the 40-μm filter produced little additional reduction in the number of particles. This result was obviously due to the fact that the particle sizes in processed frozen-preserved blood were smaller than the filter mesh, making the use of any filter of 40 μm unnecessary in well-washed blood.

Since these residual particles are made up of varying numbers of platelets, leukocytes, amorphous debris, and fibrin strands, their elimination can be clinically important. This objective may be accomplished by using smaller filters or, possibly, prostaglandin,[10] a method recently advocated as being effective.

Our findings confirm that a frozen-preserved blood system is very efficient in reducing particulate debris, but it still falls short of eliminating the particles altogether. Use of a 40-μm filter does not produce sufficient reduction of particles in this system to justify the additional cost in the transfusion of frozen-preserved blood.

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