# The Mechanisms of Leukocyte Removal by Filtration

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CONVINCING evidence exists that blood transfusions are associated with deleterious effects caused by residual leukocytes in blood and blood components. These side effects include alloimmunization to histocompatibility antigens, transmission of viruses, immunosuppression and graft-versus-host disease (GVHD). Leukocyte depletion of blood components may prevent or ameliorate some of these harmful effects. Among the various methods to remove or reduce leukocytes in blood components, filters have been shown to be most efficient. However, the mechanism of leukocyte depletion by such filters is not completely understood, which limits the development of improved, cost-effective, and clinically applicable filter materials.

The aim of this review article is to discuss the development of leukocyte filters, the various mechanisms of leukocyte filtration, and mathematical models to describe the process of leukocyte filtration.

## CLINICAL APPLICATION OF LEUKOCYTE-DEPLETED BLOOD COMPONENTS

The prophylactic use of red blood cell transfusions containing fewer than $5 \times 10^6$ leukocytes per transfused unit has been shown to prevent or delay the acquisition of nonhemolytic febrile transfusion reactions (NHFTR) in patients who are transfusion dependent.[1-3] Leukodepletion is also an effective method to abolish recurrent NHFTR to red blood cell concentrates. NHFTR are preceded by the formation of antibodies directed against HLA antigens on donor cells. HLA immunization may also be the cause of refractoriness to random donor platelet transfusions.[4-6] However, the evidence that leukocyte depletion in transfused components can prevent the development of a refractory state is limited.[7] Newly diagnosed patients with severe aplastic anemia, who are potential recipients of bone marrow transplants, are candidates for leukodepleted red blood cell and platelet concentrates. Once such patients become sensitized to HLA antigens, there is a higher risk of graft rejection.

Viruses that are cell-associated such as cytomegalovirus (CMV), Epstein-Barr virus (EBV), and human T-cell lymphotropic viruses (HTLV) may be transmitted by blood transfusion. The removal of leukocytes from blood is effective in reducing the risk of leukocyte-associated virus transmission.[8-10] Leukodepletion of red blood cell and platelet concentrates to less than $5 \times 10^6$ cells per unit has been shown to be effective in preventing CMV transmission.[11,12]

Although leukocyte reduction through filtration has been applied in a variety of other conditions, the evidence of clinical benefit awaits further studies. Notably, improvements in clinical outcome, in terms of morbidity and mortality, have not been investigated adequately. The use of leukocyte-poor blood components has been recommended to prevent immune modulation in neonates and surgical patients, which might lead to a decreased rate of postoperative infection.[13] Similarly, cancer patients undergoing surgery might benefit from leukodepletion of blood components because immunomodulation by transfusion is prevented and cancer recurrence would be lower. The indications for leukodepletion of blood components are at present classified as only possible, until more definitive studies have been performed.

GVHD in patients undergoing organ transplantation can be induced by transfused leukocytes. In theory, depletion of leukocytes could be a way to prevent this complication. However, gamma-irradiation of components has already been shown to be effective.[14] Removal of leukocytes from blood may also prevent the formation of microaggregates. Microaggregates are clumps of degenerating leukocytes, platelets, and fibrin that are formed spontaneously during the storage of leukocytes containing blood.[15] During cardiopulmonary

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bypass surgery and massive transfusion, microaggregates may induce the formation of microemboli in the patient's pulmonary circulation, which is probably associated with a large number of unexplained postoperative reactions such as acute respiratory distress syndrome (ARDS).[16-18] The removal of microaggregates from stored blood[18] and the removal of leukocytes from blood before storage[19,20] may prevent these complications.

*Techniques to Prepare Leukocyte-Poor Blood*

A variety of techniques have been developed to prepare leukocyte-poor blood components.[21-25] Although many methodological variations and combinations of these techniques exist, five basic methods can be distinguished; (1) differential centrifugation, (2) sedimentation, (3) cell washing, (4) freezing and thawing, and (5) filtration.

*Differential centrifugation.* Differential centrifugation was the earliest and is still one of the most frequently used techniques for the production of leukocyte-poor blood.[21] When blood packs are centrifuged, blood cells will sediment according to their specific size and density (Table 1). Thus, after centrifugation, whole blood can be separated into three fractions; (1) packed red blood cell concentrate, (2) cell-free plasma, and (3) a leukocyte- and platelet-rich layer (buffy coat) that separates plasma from the red blood cell concentrate. By removal of the buffy coat approximately 70% to 90% of the leukocytes can be depleted, with red blood cell loss ranging from 10% to 40%.[23,24,44,45] However, complete separation of the various cellular components cannot be obtained by centrifugation. The depletion of leukocytes can be improved by a second centrifugation step after the addition of an isotonic saline solution to the packed red blood cells, but this procedure causes a substantial loss of red cells.[26]

The widespread use of centrifugation for the preparation of leukocyte-poor blood can thus be attributed to its simplicity, adaptability, production efficiency, and ability for large-scale production. Shortcomings of the technique are the limited removal of leukocytes, the appreciable loss of red blood cells, and the relatively long processing time.[21,25]

*Sedimentation.* Spontaneous sedimentation of red blood cells, ie, without the use of centrifugation, can be enhanced by the addition of agents that promote the formation of red blood cell aggregates, known as rouleaux formation.[21] High molecular weight dextran has been frequently used for this purpose.[45-47] After sedimentation, the supernatant and the buffy coat can be removed, resulting in the depletion of more than 80% of the leukocytes. The loss of red blood cells is very low compared with other methods. Eventually, red blood cell concentrates can be subjected to a second sedimentation procedure to improve the removal of residual leukocytes without significant loss of red blood cells.[46,47]

The advantages of dextran sedimentation are its low cost and the independence from special equipment. However, the technique is time consuming, labor intensive, and when performed in an open system, susceptible to the risk of bacterial contamination, thus shortening the expiration date of the product. This technique is no longer widely used.[19]

**Table 1. Concentration and Physical Properties of Human Blood Cells**

| Cell Types | Concentration* ($\mu L^{-1}$) | Density† ($g/cm^3$) | Diameter‡ ($\mu m$) | Deformability§ | Adhesiveness‖ |
|---|---|---|---|---|---|
| Erythrocytes | 4,000,000-6,000,000 | 1.090-1.110 | 7-8 | ●●●● | ● |
| Platelets | 150,000-400,000 | 1.054-1.062 | 2-3 | ● | ●●●● |
| Granulcoytes | 2,000-6,000 | 1.080-1.084 | 5-8 | ●●● | ●●● |
| Lymphocytes | 1,500-4,000 | 1.060-1.072 | 4-8 | ●● | ●● |
| Monocytes | 200-800 | 1.055-1.062 | 4-10 | ●● | ●●●● |

* Blood cell counts in adult whole blood, according to Baker and Silverton.[26]

† Blood cell density according to Roos and de Boer.[27]

‡ Leukocyte diameters in isotonic salt solution (pH 7.4), measured by transmission electron microscopy (TEM),[28] other cell diameters according to Baker and Silverton.[26]

§ Relative deformability of cells (more dots, ie, more deformable), as defined by their ability to passively flow through the pores of a Nucleopore filter.[29-35] Deformability of platelets according to Brånemark and Lindström.[36]

‖ Relative adhesiveness of cells to solid surfaces (more dots, ie, more adhesive), arbitrarily classified, and based on various reports in which the adhesion of specific blood cells to solid surfaces is compared.[37-43]

*Cell washing.* Red blood cell washing combines differential centrifugation and continuous dilution of the cells using isotonic saline solution.[25] A variety of automatic cell washers is currently available for this purpose. The centrifugation process in these machines is performed in a disposable container of defined size and geometry, which allows the various blood components to be removed in the order of their specific densities. However, the separation efficiency is limited by the small differences between the densities of the blood cells (Table 1). Depending on the process conditions, automatic cell washers can remove 70% to 95% of the leukocytes from whole blood, whereas the loss of red blood cells is approximately 15%.[23,24,48,49]

The major advantage of this method is its efficiency in removing more than 95% of the plasma from whole blood.[48,49] Plasma removal may be beneficial in reducing the risk of virus transmission.[21] Disadvantages of the method are the high cost, the long processing time, the open-system handling, and the logistical problems with regard to the supply of cellular products.[21] Red blood cell washing is therefore used only occasionally for the routine preparation of leukocyte-poor blood.

*Freezing and thawing.* The freezing method was developed originally for the long-term preservation of red blood cells. To protect them against freezing, a cryoprotective agent, eg, glycerol, which is avidly taken up by red blood cells, is added to the blood.[50] On freezing, ice crystals that rupture the cell membranes during thawing are formed in the leukocytes. The subsequent washing procedure removes both glycerol and leukocyte-rich stroma.[51] With this method usually more than 95% of the leukocytes are removed, whereas red blood cell loss is less than 10%.[22-24,52] However, it should be noted that red blood cell concentrates may contain residual leukocyte fragments that may cause the same posttransfusion complications as previously described for intact leukocytes.[51]

Because of its efficiency in depleting leukocytes from blood, the freeze-thaw method has long been considered as the optimal method for the preparation of leukocyte-poor blood.[19,25] However, several logistic difficulties such as the requirement for expensive facilities for the freezing and storage of cells, the limited availability of products on a routine basis, and the short expiration period because of open-system handling have led to the displacement of this method by other approaches.

*Filtration.* Filtration as a means to remove leukocytes from blood became routine practice after the development of microaggregate filters. Removing aggregates from blood to prevent formation of emboli in recipients after transfusion was accomplished with the use of these microaggregate filters.[18,53,54] Although originally not intended for this purpose, microaggregate filters have been reported to have an appreciable leukocyte-removal capacity.[24,55,56] Over the last decades, microaggregate filtration has evolved into a method to produce leukocyte-poor blood. Currently available special leukocyte filters do not require microaggregate formation before filtration, because their function is based on differences in deformability and adhesiveness between different cells (Table 1). These filters generally remove more than 95% of the total number of leukocytes in one unit of blood, whereas the red blood cell loss typically is less than 10% (Table 2).

Nowadays, leukocyte filtration is the most commonly used method to prepare leukocyte-poor blood. The procedure is simple, fast, clinically effective, and does not require expensive equipment.[1,11,12,58] Moreover, the process does not require open-system handling, which favors the shelf life of the products. Nevertheless, leukocyte filters can be further improved by the optimization of leukocyte-depletion capacity, red blood cell recovery, processing time, and cost.

### *Development of Leukocyte Filters*

Filtration as a means to remove leukocytes from blood was first described in 1926 by Fleming, who used a column filled with cotton wool to prepare small quantities of leukocyte-poor blood.[66] In subsequent years, a number of filters of different materials were developed,[16,37] but it took until 1962 before Greenwalt et al reported a filtration method for use in blood bank practice.[67] When heparinized blood was filtered over columns filled with Orlon (Du Pont de Nemours, Geneva, Switzerland), Dacron (Du Pont de Nemours), Teflon (Du Pont de Nemours), or nylon fibers, it was found that leukocyte removal was most effective when nylon fiber filters were used. With these filters granulocytes were completely removed, whereas most of the lymphocytes were recovered in the filtrate. Although leukocyte removal was only partial, the incidence of NHFTR in recipients was significantly reduced when granulocyte-poor blood was used for

**Table 2. Survey of Currently Available Filters to Remove Leukocytes From Blood**

| Trade Name | Manufacturer | Filter Material | Depletion WBC (%) | Recovery RBC (%) | References |
|---|---|---|---|---|---|
| Imugard IG500 | Terumo, Tokyo, Japan | Cotton wool | 93-98 | 85-97 | 1, 3, 22-24, 56-59 |
| Cellselect* | NPBI, Emmercompascum, The Netherlands | Cellulose acetate fiber | >99 | 75-88 | 3, 57, 60 |
| Erypur* | Organon, Boxtel, The Netherlands | Cellulose acetate fiber | 96-98 | 90-99 | 1, 56, 57, 59, 61 |
| Miropore | Miramed, Mirandole, Italy | Cellulose acetate fiber | 95-99 | >92 | 3, 58 |
| Leukoseize | Dideco, Mirandole, Italy | Cellulose acetate fiber | >95 | >92 | 58 |
| Sepacell R500 | Asahi, Tokyo, Japan | Polyester fiber | 95-98 | 8-96 | 3, 57, 58, 60-64 |
| Optima | NPBI, Emmercompascum, The Netherlands | Polyester fiber | 95-99 | 86-92 | 58, 60, 62, 63 |
| RC100 | Pall, Glen Cove, NY | Polyester fiber | 98-99 | 75-94 | 57, 60, 62, 63 |
| Leukostop | Miramed | Polyester fiber | 95-98 | 88-92 | 58, 60 |
| Leukopak† | Travenol, Thetford, UK | Nylon fiber | 42-54 | 90-99 | 22, 56 |
| Ultipor SQ40S‡ | Pall | Polyester mesh | 13-57 | 93-95 | 53-56, 65 |
| 4C 2423‡ | Fenwall, Glendale, CA | Polyester wool | ~44 | ~66 | 53, 54, 56 |

Note: It should be noted the data in columns headed Depletion WBC and Recovery RBC were obtained from different laboratories and that different filtration methodologies may have been used. For example, some investigators have filtered whole blood, whereas others have used red blood cell concentrates, ie, blood from which plasma and/or buffy coat was removed by centrifugation.

* In some countries, the Cellselect filter is marketed by Organon, The Netherlands, under the trade name Erypur.

† The Leukopak filter was originally designed to harvest granulocytes from heparinized blood.[22]

‡ The Ultipor and 4C 2423 filters were originally designed to remove microaggregates from stored blood, but are known to have appreciable leukocyte removal capacity.[55,56]

transfusion.[67] Filtration over nylon fibers later evolved into a technique to isolate lymphocytes from blood[68] and was also used to purify granulocytes by a technique known as filtration leukapheresis.[69,70]

The leukocyte filtration technique was substantially improved in 1972 by Diepenhorst, who developed a prototype leukocyte filter consisting of a column filled with tightly packed cotton wool.[71] Using this filter, more than 95% of all leukocytes in whole blood could be removed, whereas red blood cell loss was less than 10%. Two years later, the first disposable leukocyte filter for routine use in blood banks became available.[72] A similar filter, the Imugard IG-500 (Terumo Corp, Tokyo, Japan), based on the Diepenhorst prototype was introduced in 1978.[73] Several clinical studies have shown that such filters are effective in the preparation of leukocyte-poor blood (Table 2).

Soon after the introduction of the cotton wool fibers, other types consisting of cellulose acetate fibers were developed. The replacement of natural by synthetic fibers led to less pyrogenic filters[59] and constant quality of the filter material,[94] resulting in safer products. Cellulose acetate filters are still widely used to remove leukocytes.

More recently, the technology to prepare leukocyte filters has advanced considerably. The observation that leukocyte removal by filters was accomplished, at least in part, by adhesion of cells to the filter fibers led to the preparation of filters with larger internal surfaces,[75] which can be achieved through the use of small-diameter fibers (Fig 1). A melt-blowing process, in which a molten polymer is cast into fibers by a high-velocity stream of gas and collected as a nonwoven web, is used to prepare webs of fibers with an average fiber diameter of less than 2 μm.[75,76] Such thin fibers have been reported to be more effective for leukocyte depletion as compared with fibers with a larger diameter.[77] Current leukocyte filters (Table 2) consist of different layers of nonwoven polyester fibers that can easily be processed by melt blowing. Polyester fiber filters have been shown to be very effective in the preparation of leukocyte-poor blood, as is shown by the removal of up to 99.9% of leukocytes.[60,61,78,79] Recently, the development of a filter that removes 99.9999% of leukocytes was reported.[80]

Although most currently used leukocyte filters show a high efficiency, further optimization is still desirable.[61] Filters that can produce red blood cell concentrates that are completely free of leukocytes have not yet been developed but may be beneficial for the various clinical situations discussed previously. Another characteristic to be optimized is the filter capacity. Increased leukocyte removal capacity may lead to a reduced filter size and thus to a

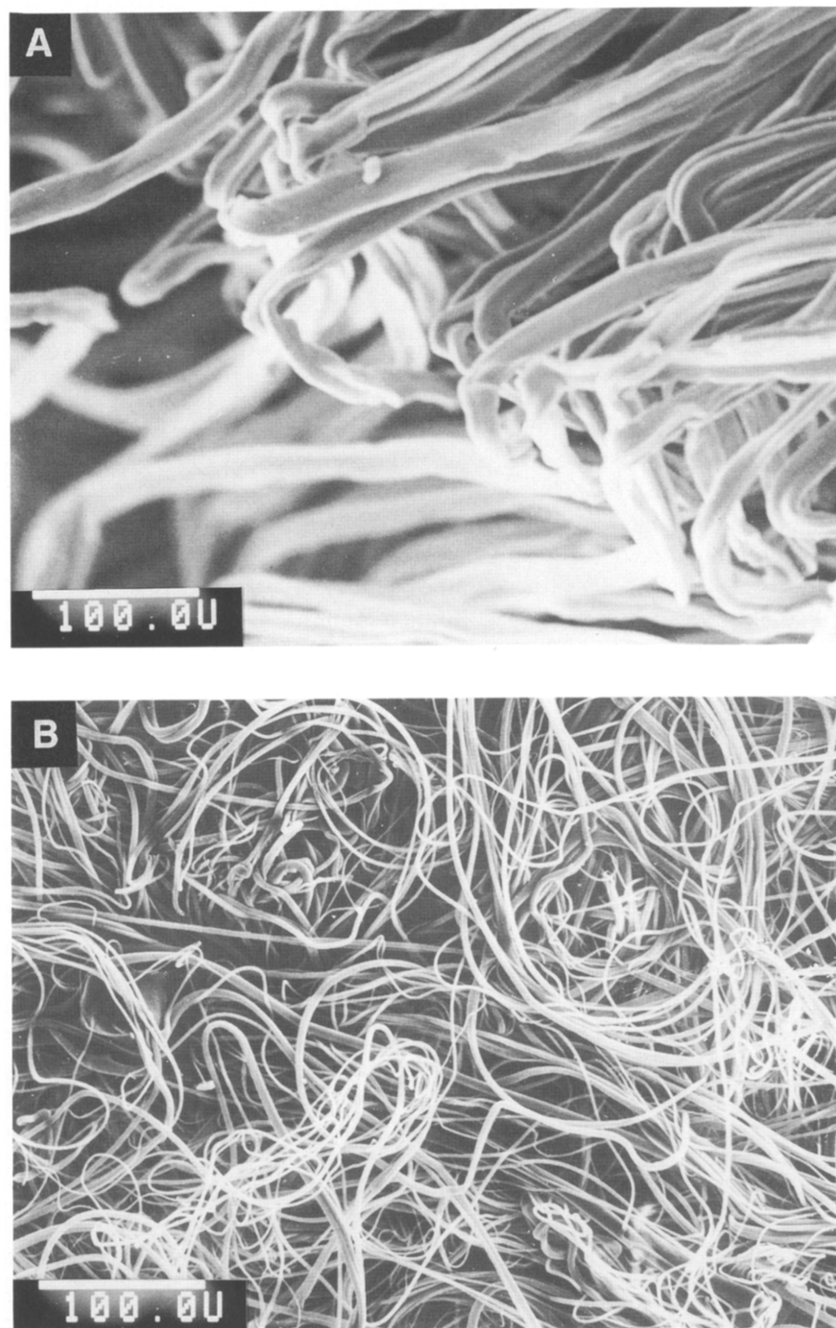


**Fig 1. Typical example of the different diameters of fibers, used in (A) traditional cellulose acetate fiber filters (Cellselect; NPBI) and (B) current polyester fiber filters (Optima; NPBI).**

higher yield of purified red blood cells, which is desirable for both clinical[1] and logistical purposes.[23] Filter optimization may also reduce the cost and length of filtration and lead to easier filtration procedures.[1] Overall, leukocyte filtration is still a relatively expensive method to prepare leukocyte-poor blood, as compared with other methods such as differential centrifugation and washing.[23] Reduction of the processing time is advantageous for the rapid provision of leukocyte-poor blood[60] and for the application of filtration directly at the bedside.[79]

Thus far, the optimization of filter materials has been achieved largely by trial and error. The de-

velopment of new filters would benefit from a better understanding of the mechanisms causing leukocyte depletion. So far, these mechanisms have not been studied systematically, although it has been suggested by several investigators that leukocyte filtration is governed by sieving and adhesion.[21,22,60,63,78,81] However, the quantitative contribution of each of these factors has never been rigorously investigated.

## LEUKOCYTE FILTRATION MECHANISMS

Filtration processes are usually divided into three categories; surface filtration, cake filtration, and depth filtration.[82,83] Surface filtration is the process in which particles larger than a given size cannot pass through the filter surface, causing a complete separation of these particles from the suspension medium. Because the retained particles will rapidly clog the filter, surface filtration is only possible when the particle concentration is low. During cake filtration the filtered particles form a porous layer, a cake, on top of the filter. As long as flow through this filter cake is possible, the layer itself will contribute to the retention of more particles. For depth filtration, the retention of particles is not restricted to the filter surface. In general, depth filters have an open porous structure, with a wide distribution of pore sizes throughout the filter matrix. This specific structure allows the retention of particles at any place inside the filter.

The filtration of leukocytes from blood by means of leukocyte filters can be regarded as a depth filtration process.[65] Depth filters usually consist of a bed with tightly packed granular or fibrous material,[84,85] and are most frequently used for the purification of waste water[82,84,86,87] and gases.[88] Although membranes are often regarded as surface filters,[82,89] open cellular foams resembling membranes have rarely been used for depth filtration.[90-92] The theory developed to explain the mechanism of depth filtration has been almost exclusively applied for the analysis of deep-bed sand filtration.[83] The same theory could be used to explain leukocyte filtration but this has never been reported.

Several elementary mechanisms are known to play a role in the retention of particles in depth filters.[86,87,93,94] The mechanisms that are probably involved in leukocyte filtration include blocking or

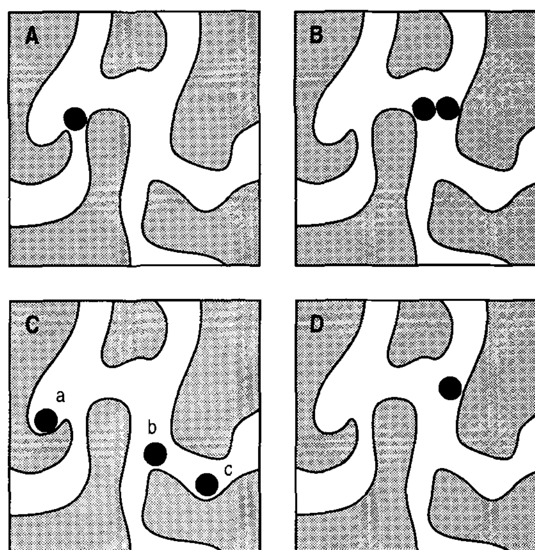


**Fig 2. Schematic diagrams of elementary mechanisms of particle retention in depth filters; (A) blocking, (B) bridging, (C) interception, either dead end (a), labile (b), or stable (c), and (D) adhesion.**

straining, bridging, interception, and adhesion (Fig 2). Combinations of the afore-mentioned mechanisms may also occur. Blocking may occur when the particle size is larger than the diameter of the pore that has to be passed. Bridging becomes significant at high particle concentrations[95] and occurs when two or more particles simultaneously flow through a relatively large pore and become entrapped as an aggregate. Interception is the process in which particles are mechanically trapped at filter sites other than small pores. Interception may be stable, labile, or occur at dead ends (Fig 2). Adhesion of particles to the filter material becomes important when the ratio of particle to pore diameter[93,94] is about $10^{-4}$ to $10^{-1}$. When adhesion occurs, the action of mechanical forces such as gravity, hydrodynamic pressure, or fluid flow is not necessarily a prerequisite to retain the particle in the filter. Because depth filtration is generally concerned with the removal of particles that are many times smaller than the pores of the filter, the effects of blocking, bridging, and interception are often neglected because adhesion is the predominant mechanism.[87,96]

An alternative approach to explain depth filtration is to distinguish mechanical entrapment (sieving) from physico-chemical entrapment (adhesion). The pore size of the filter then determines if

sieving becomes important. Based on particle/filter dimensions, it has been assumed that sieving occurs when particles are larger than 30 μm and that adhesion occurs when particles are smaller than 1 μm.[85,86] When the particle size is between 1 and 30 μm, as occurs during leukocyte filtration, both processes likely occur simultaneously.

*Cell Sieving*

Because blood cells differ both in size and deformability, sieving should be considered as a possible mechanism in the filtration of leukocytes (Table 1). Very little is known about the role of sieving in cell filtration processes, although the literature in the field of biorheology and microvascular research provides some information about possible effects of porous matrices on blood cell separation.

*Pore size effects.* The degree of deformability of leukocytes is approximately one thousand times less than red blood cells. This factor is determined by the resistance of a blood cell to flow through a small capillary,[31,97] and depends on the viscoelastic properties of the cytoplasm, the plasma membrane, the cell shape, and the cell surface area/volume relationship.[32] The extent of cell deformability is generally reduced[98] in the presence of $Ca^{2+}$, and therefore dependent on the anticoagulant used.[99]

As a result of differences in deformability, leukocytes have difficulty passing pores with a diameter less than 5 μm, whereas red blood cells will easily pass 3-μm pores[29,100,101] (Fig 3). Thus, when blood cell mixtures are filtered through a filter with pores of 3 to 8 μm, the leukocytes will selectively obstruct small pores by a process known as pore plugging. The time-dependent increase in pressure, which is required to continue the flow of red blood cells through the filter, has been mathematically described by a complex function of several factors, such as cell deformability, pore size, pore size distribution, pore length, blood cell composition, and stirring.[102,103] Simplified expressions are available for filtration through Nuclepore filters which have well-defined capillary pores,[31,97] but the relevance of these mathematical calculations with respect to leukocyte filters in which the porous structure is very irregular is probably low.

It should be noted that in addition to the pore size of the filter, the hydrostatic pressure applied also determines whether cells will flow through pores. Hemolysis may occur when the stress on the red blood cell membrane reaches a critical value, which is defined by both the filter pore size and the applied pressure[100] (Fig 3). In some cases, ie, when the pores are very small and the pressure is low, the flow of red blood cells through the filters can be completely stopped.[100]

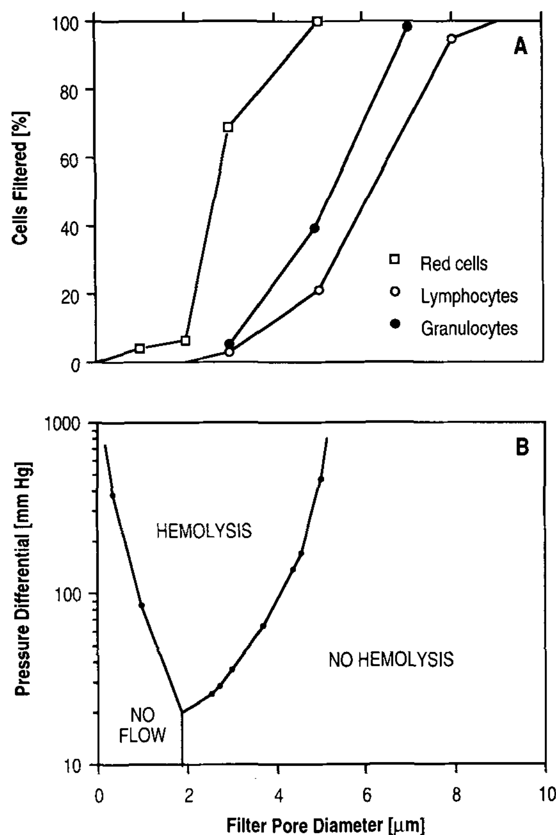


**Fig 3. Effect of filter pore size on the possibility for blood cells to flow through pores (A) and hemolysis thresholds (B), at room temperature. Experimental data adapted from Lichtman[29,30] and Blackshear.[100]**

The effects of pore size in leukocyte filters have been studied by Bruil et al[104,105] Using well-defined model filters composed of membranes, they found that leukocytes were only successfully removed when the filter pore size approached the size of leukocytes (~10 μm). However, because of clogging, blood flow through such filters rapidly decreased, resulting in low filter capacity. With asymmetric membrane filters, in which the pore size decreased from about 65 to 15 μm in the

direction of blood flow, both moderate removal of leukocytes and maintenance of flow were obtained. The clogging phenomena were also encountered by Callaerts et al, who reported similar observations with nonwoven fiber filters in which the average pore size ranged from 7.3 to 14.2 μm.[106]

*Pore-branching effects.* Because of relatively small deformation during flow, the axial velocity of leukocytes in capillaries is generally lower than that of red blood cells.[107] For example, in a capillary with a diameter of 6.8 μm the ratio of leukocyte to red blood cell velocity is 0.88 ± 0.06.[34] This difference causes a nonhomogeneous distribution of cells and plasma in the capillary, because slowly moving leukocytes will retard the movement of red blood cells.[107,108] At branching points it is likely that leukocytes will continue to move in those capillaries with the largest diameter, but with time the flow rate will decrease, with the red blood cells continuing to flow in the other capillaries.

The results of theoretical simulations have confirmed that blood cells can be nonuniformly distributed over two divergent capillary branches.[108] This deviation from a uniform distribution of cells increases as the diameter of the capillaries becomes smaller.[109] The effects of capillary branching on blood cell separation become significant when capillaries have a diameter of approximately 10 μm[32] (Fig 4).

*Margination effects.* When blood flows through small capillaries, the red blood cells will generally assume the axis of the capillary.[33,110,111] This effect is caused by the flexible and biconcave shape of red blood cells or by the formation of red blood cell aggregates, making these cells very susceptible to radial migration in a flowing fluid. Because the flow rate is highest in the center of vessels, red blood cells are driven to flow in the axis of the capillary. As a result, leukocytes, which are stiff and round and therefore less susceptible to flow, will be pushed to the capillary wall.[111-113] This process, known as margination, may stimulate leukocytes to adhere to the capillary wall.

Although the effects of margination on cell adhesion increase as the diameter of the capillary becomes smaller,[113] the adhesion of leukocytes in small blood vessels is generally inhibited by high fluid shear stresses at the capillary wall, which become predominant in small capillaries.[113] In this respect it should be kept in mind that products released by vascular cells may affect the behavior of blood cells, whereas synthetic materials lack this property. The mechanical trapping of leukocytes by interception (Fig 2) is probably promoted by their margination in blood filters.

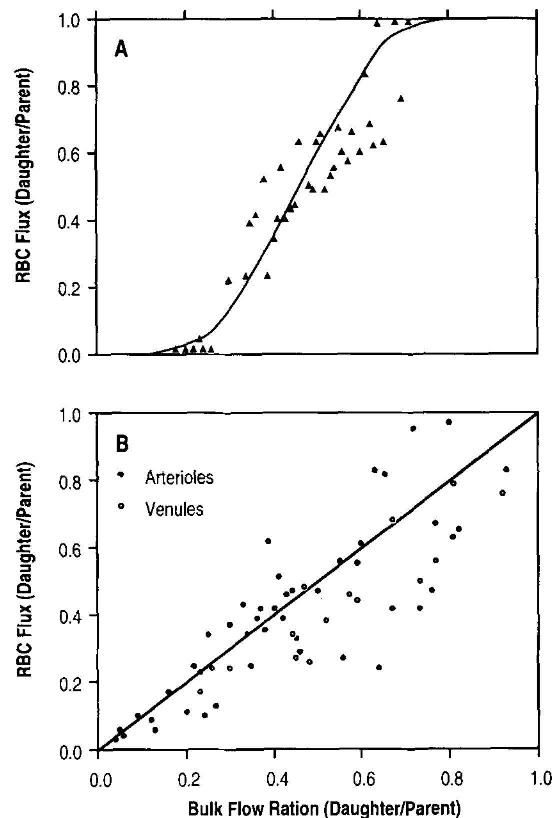


**Fig 4. Effect of parent capillary branching on the distribution of red cells (RBC) into daughter branches, either for capillaries with a diameter of 10 μm (A) and arterioles or venules with a diameter of 20 to 90 μm (B). Experimental data from Chien.[32]**

## *Cell Adhesion*

Adhesion of blood cells to solid surfaces is a complicated process, and the underlying mechanisms are poorly understood. The cell membrane should be regarded as a heterogeneous surface, composed of a phospholipid matrix that contain clusters of glycoproteins.[114] These glycoproteins, which are often receptors, regulate cell behavior, including the adhesion of the cell to a given substrate.[115-117] Specific leukocyte adhesion receptors such as LFA-1, Mac-1 and p150,95,[118-120] which

are related to a larger family of Arg-Gly-Asp (RGD) receptors,[120-122] are often located on the cell membrane. Although several investigators have explored receptor-mediated cell adhesion during cell affinity chromatography,[115,117,123,124] the relevance of these studies to leukocyte filtration is not clear.

The nonspecific adhesion of leukocytes to surfaces has been extensively described in the literature. A survey of the best-recognized factors known to be important follows.

*Surface chemistry.* Several investigators have tried to relate the extent of cell adhesion to the chemical composition of the substrate surface, but very few specific studies using leukocytes are available. Curtis et al have studied the adhesion of leukocytes and baby hamster kidney (BHK) cells to modified polystyrene surfaces. They found that adhesion of both cell types was promoted by hydroxyl groups, and inhibited slightly by carboxylic acid groups.[125-127] Bruil et al reported that the adhesion of granulocytes and lymphocytes to polyurethane surfaces increased substantially after modification with amine groups but only slightly after modification with carboxylic acid groups.[128,129]

Other investigators have studied distinct cell types with respect to adhesion. When reviewing such data, it should be kept in mind that the adhesion of different cells may differ greatly. Thus, surfaces bearing hydroxyl groups,[125-127,130,131] carbonyl groups,[132] and amine groups[133,134] have been reported to enhance cell adhesion, whereas sulphonate groups[125,134-136] are known to inhibit adhesion. The effect of carboxylic acid groups is controversial. Ramsey et al reported increased adhesiveness of monkey kidney cells to polystyrene plates after the introduction of carboxylic acid groups by oxygen gas plasma treatment.[137] Similar results were found by Klemperer and Knox, who reported rapid attachment of BHK cells and rat liver cells on chromic acid–treated polystyrene dishes, which was attributed to the introduction of carboxylic acid groups.[135] On the other hand, other investigators found that the adhesion of BHK cells on carboxylized polystyrene surfaces was inhibited only slightly as compared with carboxylized polystyrene surfaces on which these cells were blocked by acetylation[125] or methylation.[127]

It should be emphasized that the introduction of specific chemical groups at the substrate surface not only changes the surface chemistry but may also alter the physical surface properties such as charge, wettability, and microstructure. Moreover, the physico-chemical properties of the surface may readily be altered by the adsorption of proteins from the cell suspension medium. As each of these factors has inherent effects on cell adhesion, as will be discussed subsequently, it is difficult to study the effect of a single parameter.[138] This may explain why the results of adhesion studies reported by different investigators do not always agree.

The effect of various surface chemical groups on cell adhesion in leukocyte filters have been studied by Bruil et al.[139] With the use of a surface modification technique, they were able to prepare model filters that were structurally identical but which differed with respect to the presence of surface amine groups. When filtration was performed using purified leukocytes in the absence of red blood cells, platelets, and blood plasma, the number of cells removed by amine group–modified filters was significantly greater than unmodified filters. However, no significant differences between the filters were found when the filtration was performed using whole blood. It was postulated that the physico-chemical properties of the filter surface were overshadowed by other characteristics affecting leukocyte behavior during filtration.

*Surface charge.* Like most mammalian cells, leukocytes have a net negative surface charge[140-142] caused by the presence of anionic groups such as phosphate, sialic acid, and carboxylic acid groups on the cell membrane.[142,143] Electrostatic forces, either repulsion or attraction, may consequently influence cell adhesion.[141,144] It has been reported that whereas negatively charged substrates reduce cell adhesion,[134,145,146] positively charged substrates promote it.[134,145-148] These findings are in general agreement with the previously discussed effects of ionizable functional groups, which may easily affect the charge of solid surfaces.

The Derjaguin, Landau, Verwey, and Overbeek theory, which describes the free energy of a colloidal particle as a function of the inter-particle distance, has often been used to predict the rate of cell adhesion to surfaces with different surface charges.[145,149,150] This theory defines the deposition rate of cells to a solid surface by the presence and height of an energy barrier, which depends on

electrostatic interactions and London-van der Waals interactions between the surface and the cells. It follows from this theory that the chance for cells to adhere is smaller when a surface is more negatively charged.

However, leukocyte adhesion is not necessarily inhibited by negatively charged surfaces. Leukocytes often form pseudopods,[151] which easily penetrate the electrical double layer of the negative substrate, thus facilitating close contact and the molecular interactions between the cells and the surface.[141] Groth et al have reported that the number of leukocytes adhering to polyurethane surfaces increases when the surface becomes more negative.[152]

*Surface wettability.* There exists a widely accepted concept that wettable, or hydrophilic, surfaces promote the adhesion of leukocytes as compared with nonwettable, or hydrophobic, surfaces.[40,125,141,153] However, contradictory results were reported by Lim and Cooper, who used a series of polyurethane surfaces differing in wettability and found that most granulocytes adhered to the most hydrophobic surface.[154] Van Kampen et al found large numbers of adherent leukocytes in dogs on implants consisting of both hydrophobic and hydrophilic polyaminoacids.[155] Some investigators have used the hydrophobic interactions of blood cells with hydrophobic substrates to separate blood cells by affinity chromatography.[156,157]

To explain the increase of leukocyte adhesion to surfaces with increased wettability, Neumann et al used a thermodynamic model in which the change in free energy resulting from adhesion was related to the surface free energy of the solid surface,[158,159] which in turn is related to the surface wettability.[160] This model has also been used to relate the adhesion of platelets[158,161] and red blood cells[159,162] to the wettability of the solid surface and to determine if the efficiency of microaggregate filters depends on the wettability of the filter material.[163] In the latter study it was shown that the number of leukocytes and platelets retained by these filters was proportional to the surface free energy of the filter material. The same theory could probably be used to explain the different efficiencies of the different leukocyte filters. However, it should be emphasized that the model does not account for effects of surface charge on cell adhesion.

*Surface microstructure.* Synthetic substrates are generally nonhomogeneous and sometimes show a microstructure, ie, the surface consists of small microdomains differing in chemical composition. Because the membrane structure of most cells may easily rearrange during adhesion,[114] it is likely that cell adhesion is influenced by the microstructure of the substrate surface, ie, a structure consisting of small microdomains differing in chemical composition. Kataoka et al have studied the adhesion of blood cells onto materials with various microphase-separated structures, composed of neutral-charged,[164,165] hydrophobic-hydrophilic,[166,167] or crystalline-amorphous[168,169] domains. The degree of cell adhesion to these materials, which is influenced by the size and type of the microdomain at the solid surface, was in general low compared with surfaces without such a microstructure. Moreover, it has been reported by Okano et al that different plasma proteins selectively adsorb to either hydrophilic or hydrophobic microdomains,[170] thereby forming protein-coated microstructures, with similar effects on cell adhesion as described previously for uncoated microstructures.

Several frequently used block copolymers such as polyurethanes[171,172] are known to have a microphase-separated surface structure. Cell adhesion to such materials may be substantially reduced compared with materials with a homogeneous surface structure.

*Surface morphology.* Certain surface morphological features such as porosity, curvature, and texture are known to influence cell adhesion to extracellular substrates.[40] Furthermore, the adhesion of platelets[173] and red blood cells[174] to porous surfaces is often diminished during perfusion of the suspending fluid through the pores of the surface. However, fibroblasts adhere more firmly to porous surfaces compared with smooth surfaces.[175] Surface roughness is also known to influence cell adhesion, as was shown by the pattern of erythrocyte adhesion to a number of substrates.[176] Macrophages and platelets adhere preferentially to rough surfaces compared with smooth surfaces under conditions of flow.[177,178] Guidoin et al observed larger numbers of adherent leukocytes and platelet aggregates in blood filters with an irregular and imperfect surface texture compared with filters having a smooth surface.[65]

The effect of surface roughness on cell adhesion was attributed to the presence of air nuclei at rough surfaces, which may enhance cell adhesion.[179] Air nuclei at rough surfaces, formed particularly in hydrophobic substrates, are known to activate the complement system,[180] which in turn may mediate the adhesion of leukocytes to artificial surfaces.

*Complement activation.* The human complement system is a complex group of at least 25 plasma proteins that, after activation, interact with each other in a sequential manner to produce biological effector molecules.[181,182] One of the physiological functions of the complement system is the regulation of the immunological response to bacterial or viral infections via the so-called classical pathway. Complement activation is also known to be involved in the adhesion of leukocytes to artificial surfaces.[183-185] Activation of the $Ca^{2+}$- and $Mg^{2+}$-dependent alternative pathway generates C3a and C5a fragments, which are known to mediate the adhesion and aggregation of leukocytes.[185-187]

The extent of complement activation depends on the physico-chemical properties of the artificial surface.[188,189] Protein preadsorption, IgG in particular,[190] and the presence of air nuclei at the material surface[180] alter the effect of complement. The complement activation potential of some commonly used polymers is shown in Table 3. Negatively charged surfaces are known to have a relatively low propensity to activate complement, probably because of adsorption (ie, inactivation) of the cationic C5a.[191] Nucleophilic groups such as amine[190,192] and hydroxyl[186,193] have been reported to activate the complement system. In this respect, it should be noted that functional groups of denaturated plasma proteins preadsorbed onto the material surface rather than the material itself may cause complement activation.[188,189]

*Protein adsorption.* One of the first events to occur when materials are exposed to blood, even before cells can adhere, is the adsorption of plasma proteins to the surface. During this process, there is competition between the various proteins present in the plasma. The initially adsorbed small proteins, which have a high diffusion coefficient and occur in a high concentration, are sequentially displaced by larger proteins, which have a higher affinity toward the surface but are present in a lower concentration and have a lower diffusion coefficient. Gradual displacement of adsorbed proteins, known as the Vroman effect, occurs on hydrophilic surfaces in the following sequence; albumin, IgG, fibrinogen, fibronectin, high molecular

**Table 3. Survey of Polymer Materials for Complement Activation and Leukocyte Adhesion Potential**

| Materia(s)/Common Name | Complement Activation* | Leukocyte Adhesion† | References |
|---|---|---|---|
| Polyacrylonitrile | • | ••• | 194-196 |
| Polycarbonate | • | • | 194, 195, 197 |
| Polyethylene | • | •• | 158, 159, 188, 198-200 |
| Polypropylene | • | •• | 189, 195, 201 |
| Polytetrafluoroethylene/Teflon | • | • | 158, 159, 180, 195, 202 |
| Polystyrene | • | • | 126, 158, 159, 189, 195, 203 |
| Polysulfone | • | • | 194, 195, 201 |
| Polyvinylchloride | • | • | 189, 195, 200 |
| Cellulose acetate | •• | ••• | 60, 194, 195, 198 |
| Polydimethylsiloxane/Silicon rubber | •• | •• | 158, 180, 188, 199, 201 |
| Poly(ethylene terephthalate)/Dacron | •• | ••• | 60, 158, 195, 202 |
| Poly(methyl methacrylate) | •• | ••• | 185, 189, 194, 195, 198 |
| Polyurethanes | •• | •• | 129, 153, 188, 189, 195, 204 |
| Cellulose/Cellophane | ••• | ••• | 180, 194, 195, 201, 204 |
| Polyaramids/Nylons | ••• | ••• | 158, 185, 195 |
| Poly(hydroxyethylene methacrylate) | ••• | • | 186, 203 |
| Polyvinylalcohol | ••• | • | 198, 204, 205 |

* Relative potential to activate complement (more dots, more activation), arbitrarily classified according to hemolytic activity or C3a/C5a generation. Results taken from various studies by different investigators.

† Relative susceptibility to leukocyte adhesion (more dots, more adhesion), arbitrarily classified after comparison of results from various studies by different investigators.

weight kininogen, and factor XII.[206-209] The rate and amount of protein adsorption are dependent on the physico-chemical properties of the material surface.[209-210] In general, proteins adsorb stronger and to a larger extent to hydrophobic surfaces than to hydrophilic surfaces.[211-213]

Leukocyte adhesion to surfaces is largely influenced by preadsorbed proteins. It is well known that albumin has an inhibitory effect on the adhesion of leukocytes to solid surfaces,[40,126,158,214,215] whereas globulins enhance the adhesion of leukocytes.[203,214,215] Fibronectin (Fn)[216,217] acts as a bridging molecule between the cells and the substrate in many cell-adhesion processes.[218,219] Although the concentration of Fn in plasma is low, it has been reported that Fn is a normal constituent of the leukocyte membrane, which may be secreted on activation of the cells.[220] Several investigators have used the cell adhesive properties of fibronectin,[221] and the RGD peptide sequence,[122,205] which is regarded as the active site of fibronectin,[222,223] to enhance cell adhesion to artificial surfaces.

The plasma concentration level required to achieve optimal protein adsorption for optimal leukocyte adhesion during filtration is not known. The results of experiments with leukocytes reported by Bruil et al favor the use of filtration conditions in which the concentration of plasma is reduced.[224] However, Shimizu et al found that the leukocyte depletion efficiency of polyester fiber filters did not alter when the cells were stored is a plasma-poor medium.[225]

*Platelet adhesion.* There is evidence that adherent platelets promote subsequent adhesion of leukocytes. It was reported in 1961 by Garvin that the extent of adhesion of polymorphonuclear leukocytes to siliconized glass wool was dependent on prior adherence of platelets.[37] Similar effects were reported by other investigators.[226,227] Bruil et al have studied the adhesion of granulocytes to modified polyurethane surfaces under flow conditions and found that the presence of platelets in the cell suspension medium led to a significant increase in the number of adherent cells.[224] Steneker et al showed that the break-through point during filtration of leukocytes was achieved earlier in the absence of platelets in the cell suspension than in the presence of platelets.[228] It has also been reported that activated platelets can release adhesive proteins such as fibrinogen, fibronectin, and von Willebrand factor, which may promote leukocyte adhesion.[227] Alternatively, activated platelets may express a receptor generally known as PADGEM (platelet activation-dependent granule-external membrane protein; GMP-140), to which leukocytes may adhere.[229-231]

The physiological aspects of platelet-leukocyte interactions have been well documented,[232] but the significance of these interactions with respect to leukocyte filtrations has received less attention. Swank studied the attachment of cell aggregates to glass wool filters by a microscopic technique and concluded that adhesion of platelets to leukocytes is essential for effective filtration.[16] Steneker et al have recently performed an electron microscopic examination of leukocyte depletion in leukocyte filters in which the adhesion of granulocytes to platelets was observed.[233] Similar observations were also reported by Bruil et al.[104] It thus seems that the adhesion of granulocytes to platelets is an important factor for the depletion of leukocytes by leukodepletion filters.

*Composition of blood.* The composition of the blood may influence leukocyte adhesion in many aspects. Because each of the leukocyte subpopulations has a different tendency to adhere to artificial surfaces (Table 1), the overall degree of leukocyte adhesion depends on the concentration and composition of the leukocyte population. The number of platelets is important with respect to the effects already discussed. Red blood cells may influence leukocyte adhesion under conditions of flow, because these cells are known to promote the migration of leukocytes to the substrate surface.

Several investigators have reported that leukocyte adhesion is reduced in the presence of plasma.[40,201,215,224] Forrester et al found that leukocyte adhesion to glass slides was inhibited when the cells were suspended in autologous plasma[215] probably because of the adsorption of albumin to the substrate. Bruil et al found that the number of granulocytes that adhered to modified polyurethane films from saline was approximately four times high compared with the number that adhered from plasma in saline.[224] However, some investigators have reported that plasma does not inhibit but sometimes even promotes leukocyte adhesion to glass surfaces.[38,234]

Divalent ions, $Ca^{2+}$ and $Mg^{2+}$ in particular, are

generally known to promote leukocyte adhesion.[37,40,215,218,220,224,234] Hoover et al[235] reported that the extent of leukocyte adhesion to endothelium is maximal in the presence of $Mn^{2+}$, followed by $Zn^{2+} > Ni^{2+} > Mg^{2+} > Ba^{2+} > Ca^{2+}$. With respect to leukocyte filtration, it should be noted that not only the adhesiveness but also the deformability of leukocytes is affected by divalent ions.[98,99]

The effect of anticoagulants on leukocyte adhesion is primarily caused by the lowering of the concentration of divalent ions,[37] except for heparin, which does not bind $Ca^{2+}$ and $Mg^{2+}$. In citrate-anticoagulated blood the concentration of $Ca^{2+}$ and $Mg^{2+}$, although low, is still enough to permit leukocyte adhesion.[37] When ethylenediaminetetraacetic acid (EDTA) or oxalate is used as anticoagulant, the concentration of free $Ca^{2+}$ and $Mg^{2+}$ is negligible and leukocyte adhesion is inhibited.[37,39,236]

*Mathematical Models*

Mathematical models to describe the leukocyte filtration process, based on current knowledge of filtration mechanisms, may be helpful to explain results obtained with leukocyte filtration and to optimize filters and filtration procedures. Only a few attempts to describe mathematically the leukocyte filtration process are known. Diepenhorst evaluated a mathematical model, originally derived to explain the removal of ferrous hydroxide particles from ground water through sand beds, to describe the filtration of leukocytes through cotton wool filters but did not succeed in explaining his experimental results.[237] More recently, Prins developed a computer model to explain the depletion of leukocytes in filters composed of filter segments with different leukocyte-trapping efficiencies.[238] However, a shortcoming of the model was that its parameters were based on empirically derived probability factors, and therefore the theoretical value of the model is minimal. Moreover, the statistical basis of the model reduces its adaptivity in process engineering and product design.

More sophisticated filtration models have been derived to optimize applications in the field of chemical engineering.[239-242] Bruil et al[243] have adapted the basic theory from these studies to describe the leukocyte filtration process. With the use of this theory it was possible to compare quantitatively the filtration characteristics of different filter materials and to calculate the dimensions of a leukocyte filter composed of a specific material.

The model is based on the general hypothesis of depth filtration, which states that the particle concentration decreases per unit of filter length ($\partial c/\partial h$) and is proportional to the concentration of the particles in suspension ($c$)[83,239,244]:

$$-\frac{\partial c}{\partial h} = \lambda c \quad (1)$$

The filtration coefficient $\lambda$ is a measure of the efficiency of the filter and was first introduced by Iwasaki[245] in 1937. The filtration coefficient may be regarded as the particle capture probability, ie, the fraction of particles that is captured per unit of filter length. During filtration, $\lambda$ changes as a function of time, because the amount of material already deposited in the filter, $\sigma$, influences the filtration of the other particles present. It is assumed that a limited number of retention sites ($\sigma_{max}$) in the filter is accessible for the capture of particles. Therefore, the filter efficiency decreases on occupation of these retention sites during filtration. Hence, a linear decrease of $\lambda$ with $\sigma$ is often assumed[90,94,241]:

$$\lambda = \lambda_o\left(1 - \frac{\sigma}{\sigma_{max}}\right) \quad (2)$$

In equation (2) $\lambda_o$ is the filtration coefficient of the noncontaminated filter. As the value of $\lambda_o$ depends purely on the properties of the filter material, $\lambda_o$ can be described as function of the filter properties. Some investigators have postulated that $\lambda_o$ is proportional to the specific surface area ($S$) of the filter when adhesion is the predominant mechanism for particle depletion.[244,246] Assuming that $S$ is inversely proportional to the filter pore size $d$,[104] it follows that:

$$\lambda_o \sim \frac{1}{d} \quad (3)$$

The $\lambda_o$ values of model leukocyte filters, composed of membranes with variable average poor sizes, were found to agree reasonably well with this relation.[243]

During leukocyte filtration the filter efficiency

may increase on clogging of the filter. Deposited platelets may enhance the retention of leukocytes from the blood cell suspension.[224,226-228] Moreover, material deposited in the filter may reduce the apparent pore size in the filter, thus improving the filter efficiency.[104-106] Some investigators have proposed a liner expression for $\lambda$ as a function of $\sigma$, in which a factor $\xi$ accounts for the positive effect of particle deposition in the filter on filter efficiency[87,245]:

$$\lambda = \lambda_o\left(1 + \xi\frac{\sigma}{\sigma_{max}}\right) \tag{4}$$

Several other relations between $\lambda$ and $\sigma$ have been reported in the literature.[84,86,244] It was postulated by Bruil et al that the filtration coefficient in leukocyte filters can be expressed as a linear combination of Equations (2) and (4)[243]:

$$\lambda = \lambda_o\left(1 - \frac{\sigma}{\sigma_{max}}\right)\left(1 + \xi\frac{\sigma}{\sigma_{max}}\right) \tag{5}$$

This expression includes both the effects of filter properties (first term; $\lambda_o$), filter saturation (second term) and cell-cell interaction in the filter (third term). In this respect, the constant factor $\xi$ can be defined as a cell-cell interaction parameter, which accounts for the positive effects of deposited cells on the retention of other cells.

The amount of material deposited in the filter as a function of time ($t$) can be calculated from the mass balance for particles in a filter element with thickness $\partial h$, which can be approximated by[86]:

$$\frac{\partial\sigma}{\partial t} + w\frac{\partial c}{\partial h} = 0 \tag{6}$$

Here, the fluid flow rate through the filter, $w$, is expressed in unit distance per unit time. The volume flow rate can eventually be calculated by multiplication of $w$ with the planar filter surface area $A$. Hence, the leukocyte filtration process can be mathematically described by a differential system of the three Equations (1), (5) and (6). However, this is not an easy task. Only few cases of the differential system can be solved analytically. When $\xi = 0$, ie, when cell-cell interaction is not considered to be important in leukocyte filtration, equation (5) simplifies to equation (2), and an analytical expression for the time-dependent purity of the filtered product ($c/c_o$) can be obtained[86,94,243]:

$$\frac{c}{c_o} = \frac{1}{1 + (\exp(\lambda_o h) - 1)\exp(-\lambda_o w c_o t/\sigma_{max})} \tag{7}$$

Another simplification can be made when $\xi = 1$. In this case, the analytical solution of the differential equation becomes[86,243]:

$$\frac{c}{c_o} = \frac{\left(\dfrac{1 + \exp(2\lambda_o w c_o t/\sigma_{max})}{\exp(2\lambda_o w c_o t/\sigma_{max}) - 1}\right)}{\sqrt{1 + \left(\left(\dfrac{1 + \exp(2\lambda_o w c_o t/\sigma_{max})}{\exp(2\lambda_o w c_o t/\sigma_{max}) - 1}\right)^2 - 1\right)\exp(2\lambda_o h)}} \tag{8}$$

In the general case, in which the value of $\xi$ is variable, the differential system cannot be solved analytically. However, a numerical solution has been obtained and applied to explain the effects of cell-cell interaction in leukocyte filtration.[243] If followed from this theory that the filtration breakthrough point, ie, the filtered volume after which an increase of the leukocyte content in the filtrate can be measured, is shifted toward higher volumes when the value of $\xi$ is increased.

*Filtration characteristics.* To describe the results of filtration experiments by means of the general mathematical formulation for the filtration process,[243] at least three filtration parameters, $\xi$, $\sigma_{max}$, and $\lambda_o$, should be solved independently. Because theoretical relations for these parameters with the properties of the filter are not yet well developed, this can only be achieved by trial and error. However, the problem is simplified when the effects of cell-cell interaction in the filter are negligible compared with the leukocyte retention by the filter material itself. In this case, when $\xi \approx 0$, the filtration process is described by Equation (7), and the parameters $\sigma_{max}$ and $\lambda_o$ can be determined from the results of filtration experiments according to a method described by Heertjes and Zuideveld.[94] Leukocyte filtration data reported for different types of leukocyte filters[59,71] fit well with this theory (Fig 5). When the filtration parameters $c_o$, $h$, $A$, and $w$ are accurately known, the values of $\sigma_{max}$ and $\lambda_o$ can be determined precisely and a

quantitative comparison of the filtration characteristics of different filter types is possible.

*Filter design.* Another application of the model is to calculate the optimal dimensions of a leukocyte filter composed of a specific uniform material. For exp $(\lambda_o h^*) \gg 1$, the nominal filter thickness $h^*$ required to prepare a leukocyte-poor suspension with volume $V^*$ and a permissible leukocyte concentration $c^*$, can be derived from Equation (7),[243] resulting in:

$$h^* = \frac{1}{\lambda_o} \ln \left(\frac{c_o}{c^*}\right) + \frac{c_o}{A\ \sigma_{max}} V^* \qquad (9)$$

Thus, when the filter parameters $\lambda_o$ and $\sigma_{max}$ as well as the leukocyte concentration $c_o$ in the suspension applied to the filter are known, the dimensions of the filter, $A$ and $h$, can be optimized by using Equation (9). The values of $\lambda_o$ and $\sigma_{max}$ can be determined by curve fitting to obtain the filtration characteristics of different filters (Fig 5).

## CONCLUSIONS

The evolution of leukocyte filters to prepare leukocyte-depleted red blood cell products has been reviewed. Currently available leukocyte filters remove more than 99% of the leukocytes from whole blood, whereas red blood cell loss is only small. Although current filter performances are acceptable, optimization is still desirable. However, further improvement of leukocyte filters has been hampered by a lack of knowledge about the mechanisms causing the depletion of leukocytes by the filters.

According to the literature, many factors, many of which are interrelated, may be involved in the function of the leukocyte filters. Although most of these factors have been intensively studied, very little is known about their relevance in the leukocyte filtration processes. Considering the complexity of the structure of current leukocyte filters and the composition of the blood to be filtered, it seems that the leukocyte filtration process can be defined by a complex interaction of the above mentioned factors, instead of by one single factor, as was originally suggested by several investigators.[19,21,25,64,81,106] Although many details about the leukocyte filtration mechanisms are still to be elucidated, the filtration process can be described by a mathematical model. The use of this model may be helpful to design novel leukocyte filters.

Recognition of the complexity of leukocyte filtration mechanisms is important in many respects. First, knowledge of the mechanisms causing the depletion of leukocytes by leukocyte filters is essential for further development and optimization of filter materials. Second, understanding the rela-

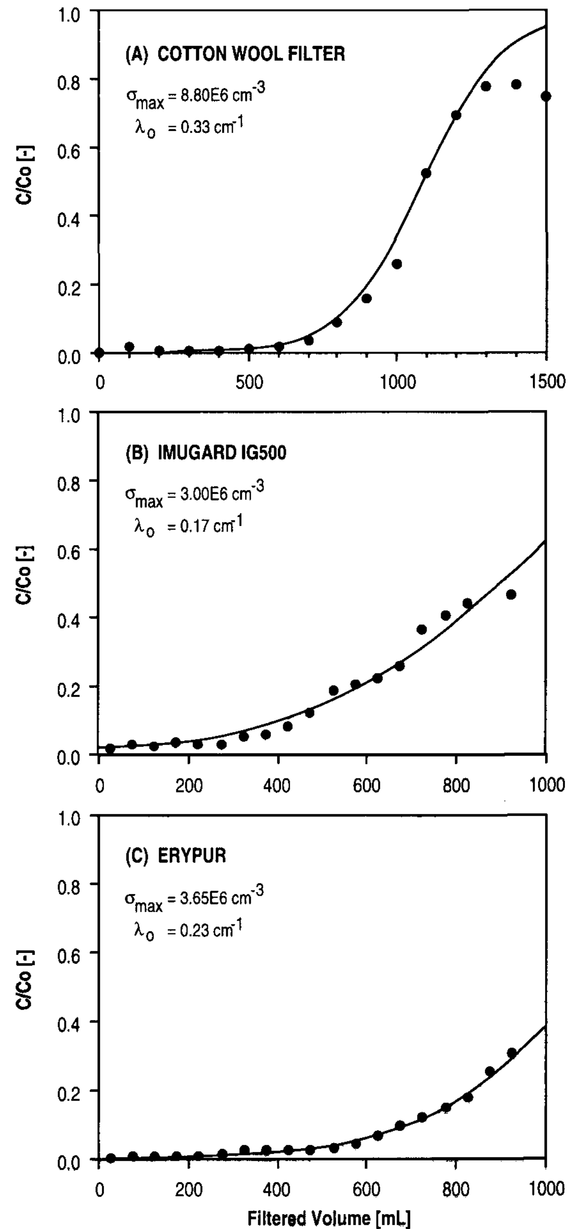


**Fig 5. Theoretical fit of a leukocyte filtration curve obtained by filtration of a blood cell suspension through an experimental cotton wood filter (A), and Imugard IG500 filter (B), and an Erypur filter (C). Experimental data from Diepenhorst et al.[71] (A) and Reesink et al.[59] (B and C). Fixed parameters were set at $\xi = 0$, $h = 25$ cm, $A = 25$ cm$^2$, $w = 0.03$ cm $\times$ s$^{-1}$, and $c_o = 5 \times 10^6$ leukocytes $\times$ cm$^{-3}$ (A) or $2.1 \times 10^6$ leukocytes $\times$ cm$^{-3}$ (B and C).**

tions between filter efficiency and blood cell functionality supports the standardization of protocols for blood collection, blood storage, and component preparation. Third, awareness of the critical steps during the leukocyte filtration process may stimulate blood bankers to respect and obey the directions for leukocyte filtration procedures given by filter manufacturers. This may eventually lead to a safer and more efficient preparation of leukocyte-depleted blood products.

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