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# Asymmetric membrane filters for the removal of leukocytes from blood

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As part of a study on the mechanisms of leukocyte filtration, the influence of pore size distribution on filter efficiency was investigated. Conventional leukocyte filters are not suitable for model studies, as these filters are composed of tightly packed synthetic fibers, with a poorly defined porous structure. Therefore, open cellular polyurethane membranes with pore size distributions varying from approximately 15 to 65  $\mu\text{m}$  were prepared. Filtration experiments with stacked packages of these membranes showed that leukocytes are best removed (>99%) by filters with a pore size distribution of 11–19  $\mu\text{m}$ . These pore sizes approach the

size of leukocytes (6–12  $\mu\text{m}$ ). However, due to fast clogging, blood flow through these filters is rapidly reduced, which results in a low filter capacity. With an asymmetric membrane filter, in which the pore size decreases from about 65 to 15  $\mu\text{m}$  in the direction of blood flow, both moderate removal of leukocytes (>80%) and maintenance of flow ( $\sim 0.2$  mL/s) are obtained. This results in efficient leukocyte removal. From cell analysis of both filtrate and filter, it is concluded that adhesion rather than sieving is the major filtration mechanism. Thus, further optimization of the filter may be achieved by surface modification.

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## INTRODUCTION

Removal of leukocytes from blood before transfusion is considered to be important for various reasons. It is well known that the transfusion of leukocyte-containing blood components may induce alloimmunization against these cells, which cause immune reactions after repeated transfusions.<sup>1,2</sup> Non-hemolytic febrile transfusion reactions (NHFR) are often accompanied by fever, headache, nausea, and diarrhea and may sometimes be fatal. Moreover, alloimmunization may result in refractoriness against platelet transfusions.<sup>3</sup>

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Several authors have reported that these effects may be prevented by the use of leukocyte poor blood components.<sup>4-7</sup>

Since 1974, it is known that renal allograft survival is decreased in patients who have not been transfused previously, or who have been transfused with leukocyte poor blood, as compared to patients transfused with red blood cell concentrates.<sup>8</sup> Immunosuppressive effects, such as increased suppressor cell activity, are likely to account for this phenomenon.<sup>9</sup> It has been speculated that leukocyte-contaminated blood components may also reduce the immune response to cancer cells and thereby facilitate the growth of metastases and the recurrence of tumors after surgery.<sup>10,11</sup> When adequate proof for this effect is obtained, it is attractive to postulate that transfusion of leukocyte depleted blood is potentially beneficial for patients undergoing surgery for tumors, such as colorectal carcinoma.<sup>12</sup>

In addition, various blood-borne viruses are predominantly associated to leukocytes and may be transmitted during blood transfusion. Removal of leukocytes from blood proved to be effective in reducing the transfer of cytomegalovirus (CMV)<sup>13</sup>, human T-cell leukemia virus (HTLV-I),<sup>14</sup> or human immunodeficiency virus (HIV).<sup>15</sup>

Techniques which have been used for the removal of leukocytes from blood cell preparations include differential centrifugation, sedimentation, filtration, washing, and successive freezing and thawing of blood.<sup>16-18</sup> Although all these procedures have inherent advantages and limitations, filtration has become very popular because of its simplicity, clinical effectiveness, relatively low costs, and its use at the bedside.<sup>19-21</sup>

Removal of leukocytes from blood through filtration was already described in 1928,<sup>22</sup> but it took many years before a modification of this method led to its clinical use.<sup>23</sup> Large-scale application of leukocyte filters started in the early 1970s, after the introduction of the first commercially available blood filter, which consisted of a column filled with tightly packed cotton wool fibers.<sup>24</sup> Since that time, several modified blood filters have been developed and tested clinically.<sup>21,25-28</sup> Although high filter efficacies have been obtained with fibers of different synthetic materials, or by improvement of the nonwoven filter structure and fiber diameter, further optimization of leukocyte filters is still needed.<sup>29</sup>

Thus far, the optimization of filter materials is achieved rather empirically. The development of new filter materials would be served by a better understanding of the mechanisms causing depletion of leukocytes by the filter. Very little is known about these mechanisms so far. It has been suggested that leukocyte filtration is governed by both sieving and adhesion,<sup>16,30</sup> but the quantitative contribution of each factor is still unclear.<sup>18</sup>

To study the mechanisms of leukocyte filtration, the effects of mechanical sieving and cell adhesion during leukocyte filtration should be included. With regard to the mechanical aspects of leukocyte filtration, it has been reported that, due to differences in cellular deformability, most leukocytes will plug passages smaller than 5  $\mu\text{m}$ , whereas erythrocytes will easily pass pores larger than 3  $\mu\text{m}$ .<sup>31-37</sup> However, these studies employed either flow through small capillaries or flow through Nuclepore membranes, which may be re-

garded as screen filters with a narrow size distribution of tubular pores. Leukocyte filters, which are usually classified as depth filters, have distinct flow characteristics, which depend on the shape and the distribution of the pores. Moreover, the mechanisms of depth filtration and screen filtration differ significantly.<sup>38</sup> Consequently, the results obtained from rheological studies, cannot simply be applied to leukocyte filter optimization.

The aim of the study presented here is to investigate the contribution of the filter structure to the depletion of leukocytes. We have prepared several model filters by stacking microporous polyurethane membranes with varying pore size distributions. In this way, the pore size distribution can be adjusted throughout the filter. In order to investigate the usefulness in routine blood filtration, the effectiveness of our model filters was compared with several commercially available nonwoven leukocyte filters.

## MATERIALS AND METHODS

### Filter materials

Polyurethane membrane filters were prepared from medical grade Pellethane 2363-80A (Dow Chemical Nederland BV, Delfzijl, the Netherlands). Polyurethane was first extruded at 190–220°C to improve its solubility in organic solvents. In order to purify the polymer, a 10% solution of polyurethane in dimethylformamide was slowly added to a 10-fold excess of water which was stirred at high speed. The precipitate was collected by filtration, and dried *in vacuo* at 60°C.

Polyester nonwoven filter materials, used for the manufacturing of commercially available Cellselect® leukocyte filters, were obtained from the manufacturer (NPBI, Emmer-Compascuum, The Netherlands). Three different materials, i.e., a prefilter, a coarse main filter and a fine main filter, varying in thickness, fiber size, and porous structure, were used.

### Membrane preparation

Series of polyurethane (PU) membranes were prepared by two different salt suspension techniques. Both techniques were slightly modified compared with methods reported in the literature.<sup>39–44</sup> For instance, an alternative solvent system was used here. Table I gives a survey of the different conditions used to prepare the membranes.

Membrane types 7.3, 7.4, and 7.5 were prepared from solutions of PU in dimethylformamide (7 w/v-%) in which particles of sodium citrate (Janssen, Beerse, Belgium) with size distributions of 38–63  $\mu\text{m}$ , 63–106  $\mu\text{m}$ , and 106–212  $\mu\text{m}$ , respectively, were suspended. Particles with these distributions were obtained by sieving. For the respective membranes, the concentration of particles used was 88, 89, and 90 v/v% with respect to PU. After degassing, thin films (500  $\mu\text{m}$ ) were cast from these suspensions on clean glass plates, after which the PU was coagulated by immersing the plates in ethanol for 12 min.

TABLE I  
Major Process Conditions for the Preparation of Different Membranes

Code	Concentration PU (g/100 mL DMF)	Salt	Particle Size ( $\mu\text{m}$ )	Concentration Salt (vol % of solid)	Extraction Solvent
7.3	7.0	Sodium citrate	38-63	88.0	Water
7.4	7.0	Sodium citrate	63-106	89.0	Water
7.5	7.0	Sodium citrate	106-212	90.0	Water
8.1	9.5	MgCO <sub>3</sub>	Not fractionated	71.0	3M hydrochloric acid
8.2	8.0	MgCO <sub>3</sub>	Not fractionated	75.0	3M hydrochloric acid
8.5	5.5	MgCO <sub>3</sub>	Not fractionated	82.4	3M hydrochloric acid

The salt particles were finally washed out with water. This resulted in microporous membranes with a pore size distribution, mainly dependent on the size of the salt particles used.

Filter types 8.1, 8.2, and 8.5 were prepared from solutions of 9.5, 8.0, and 5.5 w/v% PU respectively in dimethylformamide in which particles of magnesium carbonate (Janssen, Beerse, Belgium) ( $\text{MgCO}_3$ ) were suspended. These particles were not fractionated before use. Salt particles were added in amounts of 71, 75, and 82.4 v/v%, respectively, with respect to PU. The suspensions were treated ultrasonically for at least 1 day to break down particle aggregates. The size of the remaining particles was found to be less than 1  $\mu\text{m}$ , as measured by scanning electron microscopy (SEM). Films of these suspensions were prepared and coagulated as described above. Thereafter, the salt particles were dissolved by extraction with 3M hydrochloric acid. It turned out that the amount of PU, dissolved in the casting suspension, was directly correlated to the pore size distribution in the resulting membranes.

After preparation, all membranes were washed with distilled water for at least 1 day, rinsed in ethanol, and finally dried in air. Membranes were kept in the dark at room temperature.

### Filter preparation

Filters were prepared by stacking membranes described in the membrane preparation section. First, thin layers with a thickness of 1.0 mm were cut from a stack of three to five membranes. Three of these layers, with a total thickness of 3 mm, were then mounted together in a special polycarbonate filter housing (Schleicher & Schüll, type FP 050/0). Two of these filter units were finally connected by a small polyvinylchloride tube, to achieve sufficient depth filter capacity.

In this way, both symmetric and asymmetric filters were prepared. Symmetric filters, with a total thickness of 6.0 mm, are composed of identical membranes, and have a uniform pore size distribution throughout the filter. Asymmetric filters are composed of a stack of different membranes, with a total thickness of 6.0 mm, in such a way that the average pore size decreases in the direction of blood flow through the filter. These filters contained six layers of 1.0 mm thickness, each composed of identical membranes, type 7.5, 7.4, 8.5, 7.3, 8.2, and 8.1 respectively.

Nonwoven filters were prepared in a similar way. Thus, symmetric nonwoven filters were composed of a stack 6.0 mm of identical filter layers. Asymmetric nonwoven filters contained three layers of 2.0 mm thickness, each composed of identical filter layers, prefilter, coarse filter, and fine filter, respectively.

### Characterization of filter materials

Surfaces and cross sections of each membrane were examined by SEM. Pore size distributions were determined *in duplo* by counting 200–300 open

passages observed in pictures of representative structures on both sides of the membrane, using a particle size analyzer. It is generally assumed that pores are Gaussian distributed, so that their distribution may be characterized by an average pore size and a standard deviation.

The overall average pore size of the membrane was determined by porometry. Membranes were soaked with a special inert solvent to fill all pores. A Coulter porometer was used to measure the pressure increase which is needed to remove the fluid from the sample. With the porometer the average pore size can be automatically calculated from the pressure curve. All measurements were carried out *in duplo*.

Cut-off values of the membranes were determined *in duplo* by filtration with glass beads. Depending on the pore size distribution of the membrane, either 10–70  $\mu\text{m}$  spheres or 0–20  $\mu\text{m}$  spheres were used. An amount of glass beads, matching about one monolayer on top of the membrane, was filtered with 100 mL of distilled water. Size distribution of glass beads in the filtrate were determined by counting particles observed on SEM micrographs. The arbitrarily chosen cut-off particle size means, that at least 98% of the particles in the filtrate are smaller.

The porosity, or pore volume fraction, of the membranes was determined *in duplo* from the weight and volume of dry samples. Sample volumes were calculated from the filter thickness, as averaged from five measurements (SEM). For calculating the porosity, the density of air was neglected compared to the density of the filter material, 1.13 g/mL for polyurethane, according to the manufacturer.

The characterization of nonwovens was carried out in a similar way as the characterization of membranes, as described above. A density of 1.38 g/mL for polyester fibers, according to the manufacturer, was taken to calculate the porosity of these materials.

## Blood cells

Blood from healthy human donors was collected in blood bags containing citrate-phosphate-dextrose-adenine (CPDA). After storage for 10–15 h at 20°C, the bags were centrifuged for 10 min (3600g at 20°C) to remove the plasma layer from the packed cell suspension. Filtration experiments were performed, within 16 h after blood collection, with pooled units of the packed cells, diluted with an aqueous solution containing 0.9% sodium chloride (saline) to a hematocrit of 60%.

## Filtration procedure

In a typical filtration experiment, filters were first rinsed with 200 mL of saline to remove air and to wet the filter surface. By varying the hydrostatic pressure over the filter, the initial saline flow was adjusted to 1.0 mL/s in every new experiment. Blood filtration was started by connecting the blood cell

reservoir to the filter. The hydrostatic pressure was kept constant throughout the entire filtration.

During filtration, samples of 5–10 mL of the filtrate were collected at different times, and analyzed for cell composition and concentration.

### **Analysis of filtrate**

The total leukocyte count in filtrate samples was determined with an electronic particle counting technique. Aliquots of 20  $\mu\text{L}$  were diluted with 10 mL electrolyte containing solution. Lysis was induced with two drops of a saponin containing electrolyte solution. Remaining particles were then counted in 0.5 mL lysate sample, using a Coulter counter, with a 100- $\mu\text{m}$  counting tube opening. Measurements were carried out *in duplo*.

The first part of the filtrate was usually diluted by saline, present in the prerinsed filter. To account for this dilution, in all lysates, used in the counting, an additional spectrophotometric hemoglobin determination was performed. The adsorbance was measured at 370, 415, 500, and 577 nm. The hematocrit was measured according to a standard procedure. Samples of the filtrate were centrifuged at 3600g for 10 min. The free hemoglobin in the supernatant, was determined spectrophotometrically, as described above.

Concentrations of granulocytes, monocytes and T-lymphocytes were determined in selected samples with a standard direct binding assay for leukocyte subpopulations (DBA), which has been described in detail elsewhere.<sup>45</sup> This assay uses <sup>125</sup>I-labeled antibodies directed against specific structures on the membranes of different cell types.

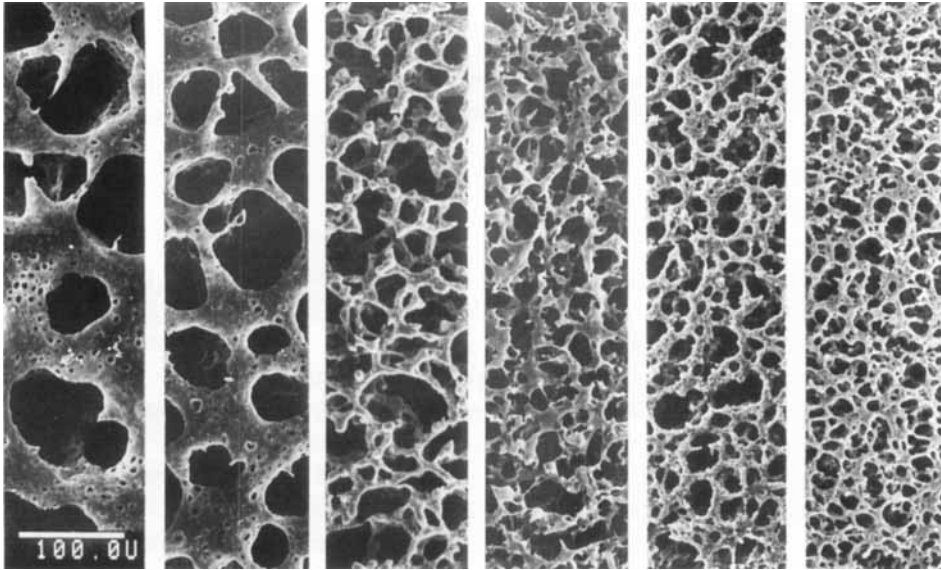
### **Analysis of the filter materials after filtration**

After leukocyte filtration some membrane filters were used for further evaluation. Small parts, about 1 cm<sup>2</sup>, were cut from the filter and fixed with formaldehyde containing solution, as described elsewhere.<sup>46</sup> Fixed samples were embedded in methacrylate resin, according to standard procedures. Sections of 7  $\mu\text{m}$  were cut with a microtome. After staining with hematoxylin solution, the preparates were studied with a light microscope.

## **RESULTS**

### **Characterization of filter materials**

SEM micrographs of membranes (Fig. 1) demonstrate that, using the salt suspension technique, a series of membranes with different pore size distributions can be prepared. All membranes show an open and cellular pore structure, with a relatively narrow pore size distribution. Although some structural differences exist between the top and the bottom of membranes,



**Figure 1.** SEM micrographs of layers of the asymmetrical membrane filter. From left to right, the micrographs show membrane type 7.5 (top side), type 7.4, type 8.5, type 7.3, type 8.2, and type 8.1 (bottom side).

the pore size data (Table II) indicate that each single membrane has an almost symmetrical structure. Pore sizes determined with the Coulter porometer, which represent the average values throughout the membranes, are in reasonable agreement with the average pore sizes measured at the membrane surfaces, according to SEM. It also appears from Table II that the specific filter surfaces of the membranes, as calculated from a simple morphological model, adopted from literature,<sup>47</sup> differ largely. This results directly from variations in pore sizes, as the porosity of the membranes is roughly comparable.

SEM micrographs of commercial nonwoven filter materials (Fig. 2) show the large structural differences of these materials as compared to the membrane structures from Figure 1. Nonwoven filters have an irregular and roughly shaped porous structure, with a relatively broad distribution of pore sizes (Table III). Although large structural differences exist between membrane filters, the specific filter surface of various nonwoven filters is comparable to the specific filter surface of membrane filters.

Using the pore size data from Tables II and III, asymmetric filter compositions may be illustrated schematically by their pore size distributions throughout the filter as shown in Figures 3 and 4. These figures represent an idealized pore size distribution with a continuous decrease in pore size, while in practice there is a stepwise decrease of pore sizes throughout the filter.

### Blood filtration

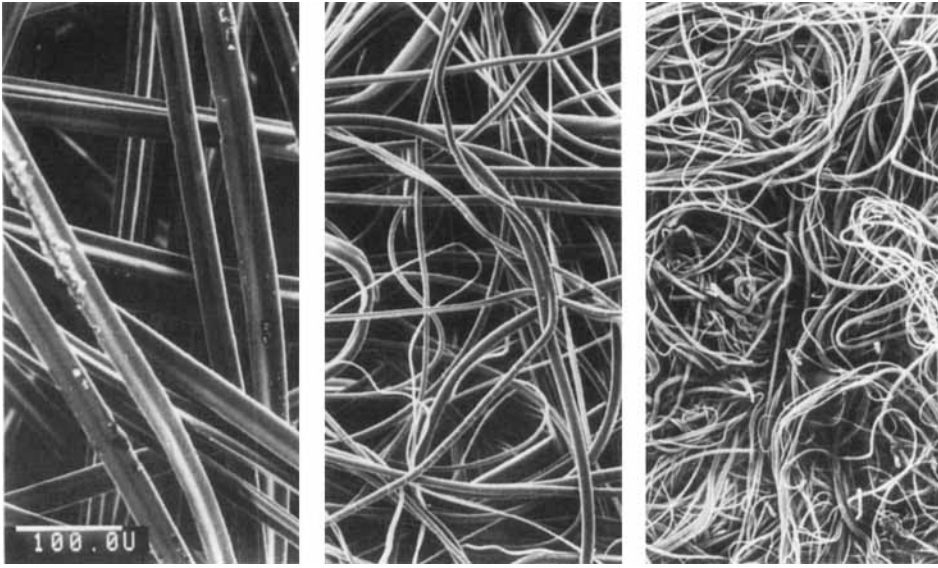
Both flow and leukocyte concentrations in the filtrate were measured as a function of time for each filter. From these measurements, differential leuko-



TABLE II  
 Characteristics of Membranes Used in Filtration Experiments

Code	Thickness ( $\mu\text{m}$ )	Porosity (%)	$d_{\text{av}}$ (porometer) ( $\mu\text{m}$ )	$d_{\text{bottom}}$ (SEM) ( $\mu\text{m}$ )	$d_{\text{top}}$ (SEM) ( $\mu\text{m}$ )	Cut-Off ( $\mu\text{m}$ )	Spec. Surface <sup>a</sup> ( $\text{m}^{-1}$ )
8.1	210	83	12	15 $\pm$ 4	23 $\pm$ 9	19	267.000
8.2	220	85	19	19 $\pm$ 7	27 $\pm$ 12	23	211.000
7.3	230	92	21	24 $\pm$ 10	40 $\pm$ 17	26	167.000
8.5	200	89	30	31 $\pm$ 12	37 $\pm$ 15	31	129.000
7.4	290	93	38	50 $\pm$ 15	66 $\pm$ 37	35	80.000
7.5	280	93	43	65 $\pm$ 15	92 $\pm$ 43	51	62.000

<sup>a</sup>The available specific filter surface ( $S$ ) is calculated from the average pore size ( $d_{\text{av}}$ ), using a "cubic lattice" model, in which  $S = 4/d_{\text{av}}$  (see ref. 47).



**Figure 2.** SEM micrographs of the layers of the asymmetrical nonwoven filter. From left to right, the micrographs show a prefilter layer (top side), a coarse main filter and a fine main filter (bottom side).

cyte retention and total leukocyte uptake by the filters were calculated. Figures 5(I) and 6(I) show the fraction of leukocytes which passed through the filters as a function of filtration time. A high leukocyte removal was obtained for relatively small pore size filters. These latter filters also gave rise to a fast drop of the initial flow [Figs. 5(II) and 6(II)]. From the removed fraction of leukocytes and the flow through the filter, the leukocyte uptake can be calculated. Figures 5(III) and 6(III) show the results. Figures 5(IV) and 6(IV) are obtained by time integration of the data from Figures 5(III) and 6(III). These figures show that filters with small pores do not have the largest leukocyte uptake capacity, although they remove these cells most efficiently. Filters with large pores do also have a small uptake capacity. The data in Figures 5(IV) and 6(IV) demonstrate that asymmetric filters are most efficient in the uptake of leukocytes.

The occurrence of hemolysis, caused by filtration of blood through membrane filters, was measured in samples taken after filtration of approximately 50 mL of blood (Table IV). Filtration through filters with small pores was accompanied by an increased hemoglobin level in filtrate samples. The hematocrit did not change significantly at higher rates of red cell destruction.

### Composition of the filtrate

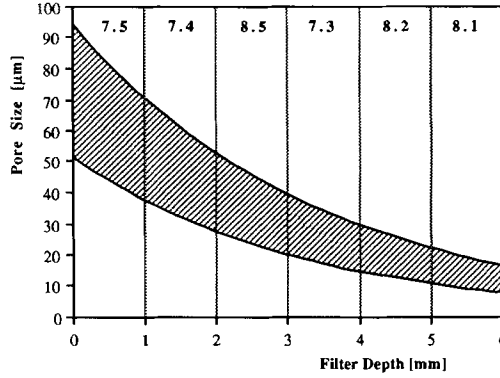
Because of differences in size, concentration and functions,<sup>48</sup> various leukocyte populations may behave differently during filtration. Therefore, concentrations of granulocytes, lymphocytes and monocytes were measured in a selected number of filtrates, derived from blood. Both an asymmetric type

TABLE III  
 Characteristics of Fibrous Filter Layers Used in Filtration Experiments

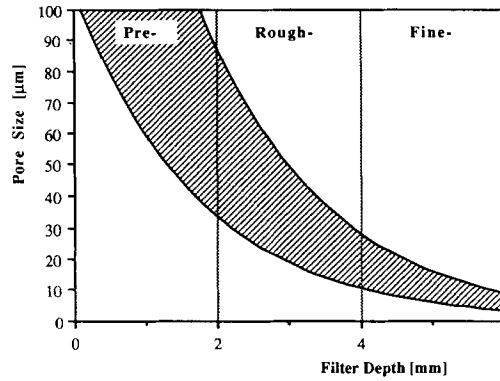
Type	Thickness ( $\mu\text{m}$ )	Porosity (%)	$d_{av}$ (porometer) ( $\mu\text{m}$ )	$d_{surface}$ (SEM) <sup>a</sup> ( $\mu\text{m}$ )	Cut-Off ( $\mu\text{m}$ )	Spec. Surface <sup>b</sup> ( $\text{m}^{-1}$ )
Fine	490	85	9	13 $\pm$ 6	7	300,000
Rough	550	86	26	24 $\pm$ 11	37	93,000
Pre	2360	91	179	127 $\pm$ 56	61	11,000

<sup>a</sup>No distinction was made between top side and bottom side of the filter layer.

<sup>b</sup>The available specific filter surface ( $S$ ) is calculated from the average pore size ( $d_{av}$ ) and the filter porosity ( $\epsilon$ ), according to the derived equation  $S = 4(1 - \epsilon)/d_{av}$ .



**Figure 3.** Idealized scheme of the pore size gradient in the asymmetrical membrane filter. The marked area represents the distribution of pore sizes as a function of filter depth.



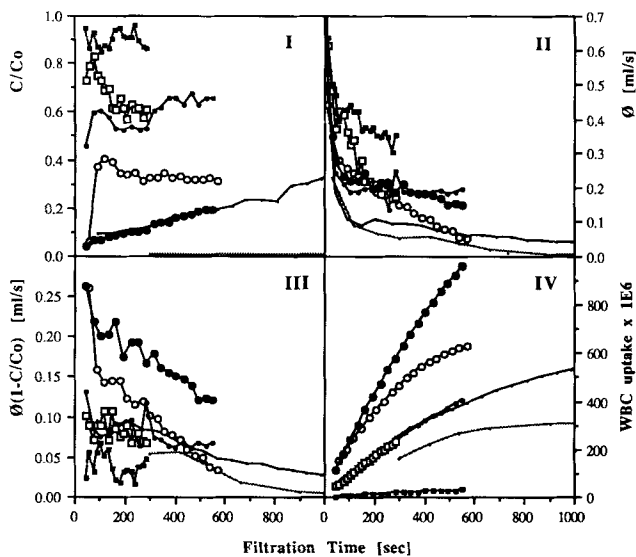
**Figure 4.** Idealized scheme of the pore size gradient in the asymmetrical nonwoven filter. The marked area represents the distribution of pore sizes as a function of filter depth.

filter and a symmetric type filter, composed of type 7.3 membranes, were used for this study.

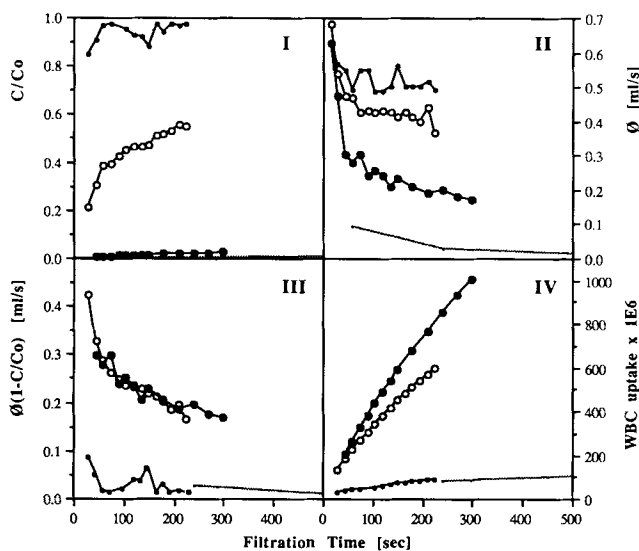
Figure 7 shows the fractions of leukocyte populations which passed the filters as a function of filtration time. Both filters showed large differences between the different kinds of leukocyte populations. Lymphocytes passed relatively easily through both filters, whereas monocytes, and to a less extent also granulocytes, were well retained by the filters. For each type of leukocyte, the asymmetric filter shows superior removal as compared with the symmetric filter.

**Histological analysis of the filter after blood filtration**

Typical pictures of stained cross sections of the top section, the intermediate section, and the bottom section of an asymmetric filter and a sym-



**Figure 5.** Filtration characteristics of membrane filters; filter 8.1 (~~~~), filter 8.2 (—), filter 7.3 (○—), filter 8.5 (●—), filter 7.4 (□—), filter 7.5 (■—), and an asymmetrical filter (●—). Plots in the graphs show relative output of leukocytes (I), flux (II), differential leukocyte uptake by the filter (III), and total leukocyte uptake by the filter (IV) as a function of filtration time.



**Figure 6.** Filtration characteristics of fibrous filters; fine filter (~~~~), rough filter (○—), prefilter (●—), and an asymmetrical filter (●—). Plots in the graph show relative output of leukocytes (I), flux (II), differential leukocyte uptake by the filter (III), and total leukocyte uptake by the filter (IV) as a function of filtration time.

TABLE IV  
Hemolysis after Filtration of 50 mL Blood

Filtration	Free Hemoglobin (mg/L)	Hematocrit (%)
Not filtered	407	61
8.1	>10000	61
8.2	1126	60
7.3	1094	60
8.5	1071	59
7.4	757	62
7.5	571	61
Asym.	2200	60

metric filter are shown in Figure 8. Large amounts of platelets, most of them aggregated, were retained at the top sections of both filters [Figure 8(Ia) and 8(IIa)]. Within these aggregates, a considerable number of leukocytes, mainly granulocytes, were trapped. In the intermediate sections of the filters however, most of the blood cells are erythrocytes, as can be seen from Figures 8(Ib) and 8(IIb). These sections contain only a small number of leukocytes. Most distinct differences between both filters are found in the bottom sections. The bottom section of the symmetric filter [Fig. 8(Ic)] is almost ineffective in the removal of leukocytes. In contrast, the bottom section of the asymmetric filter, with much smaller pores compared to the symmetric filter section, significantly contributes to the retention of leukocytes. Figure 8(IIc) shows that large numbers of mainly lymphocytes are captured in the pores of this section.

## DISCUSSION

In order to study the influence of pore size distributions on the efficiency of leukocyte filtration, the use of well defined model filters is a prerequisite. Conventional filter structures, composed of packed fibers, have a broad pore

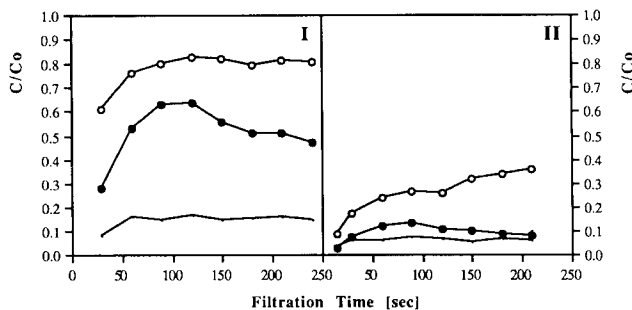
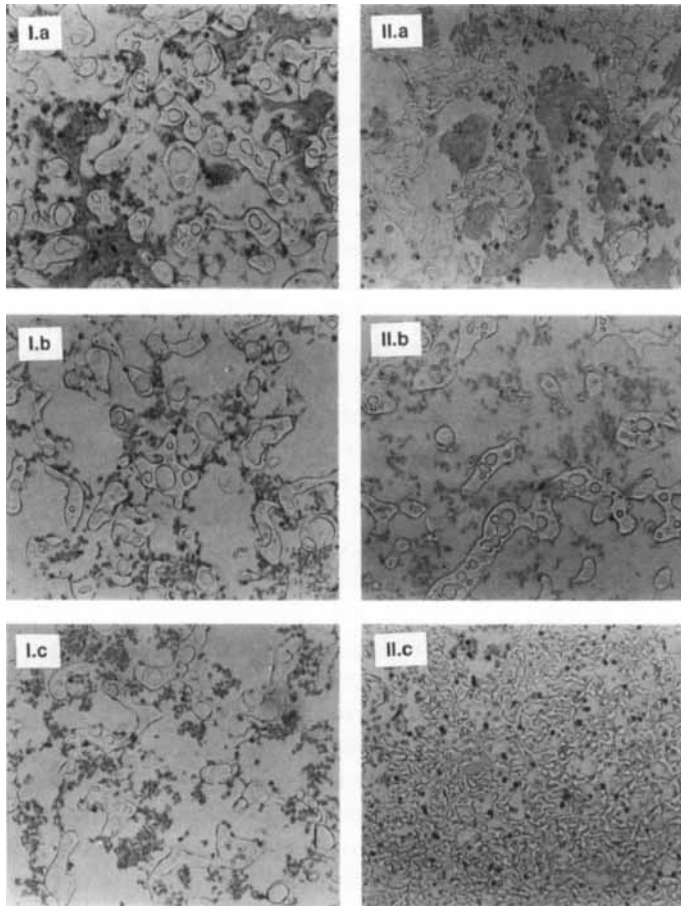


Figure 7. Filtration characteristics for different leukocyte subpopulations; monocytes (—), granulocytes (—●—), and lymphocytes (—○—), after filtration through a symmetrical membrane filter type 7.3 (I) and after filtration through an asymmetrical membrane filter (II).



**Figure 8.** Stained cross sections of membrane filters after filtration of blood. Cross sections of a symmetric filter (I), and those of an asymmetric filter (II). In each series, from top to bottom, are shown top side (A), mediate section (B), and bottom section (C), respectively (magnification  $\times 250$ ).

size distribution, which is poorly defined. In contrast to nonwovens, membranes are composed of a continuous porous matrix, usually with a well defined structure. Most of the techniques to prepare membranes offer unique possibilities to adjust the pore structure. Open cellular structures, with a narrow pore size distribution may be obtained. A purpose of this study was to optimize the porous structure of leukocyte filters, with respect to their filtration efficiency, for which membrane structures offer several advantages.

A range of membranes, differing in pore size, were prepared by the use of salt suspension techniques. These membranes and fibrous material used in commercially available leukocyte filters showed a number of structural differences. Membrane structures are very regular, which is demonstrated by the relatively narrow pore size distributions according to SEM measurements. In contrast to these membrane structures, it is impossible to define an absolute pore size in the fibrous structures. In particular the apparent pore size of nonwovens will be largely dependent on the characterization method

used. This is illustrated by the results presented in Table III, which show relatively large differences between cut-off values and average pore sizes as determined with SEM and porometry.

The results of membrane filtration experiments demonstrate that with filter type 8.1, with a pore size ranging from 11 to 19  $\mu\text{m}$ , it is possible to remove almost all leukocytes from whole blood. However, clogging in an early stage rapidly stops the blood flow through this filter, thus causing a low uptake capacity. Another disadvantage of this filter is the high rate of hemolysis (Table IV), caused by a high shear stress exerted on the blood cells during filtration. It can thus be concluded that this small pore filter was not very suitable for leukocyte removal from blood. When larger pore size filters are applied, for instance filter type 7.5 with a pore size about 50–80  $\mu\text{m}$ , the degree of hemolysis decreases significantly. However, these larger pore filters are also inefficient when the leukocyte uptake capacity is considered. This is caused by a relatively large leakage of leukocytes through the filter. However, blood flow is not impaired during the filtration.

With respect to filtration efficiency, asymmetric filters will have advantages over symmetric filters. Early stage clogging may be prevented by the partial removal of particles at the top section of the filter which contains layers with relatively large pores, whereas most of the remaining particles will be removed at the bottom section, containing layers with small pores.<sup>49</sup> The results of our experiment (Fig. 5) show both a moderate leukocyte removal and a modest blood flow impairment for the asymmetric filter type. Both properties contribute to an optimal leukocyte uptake during filtration of blood. The same appears applicable for fibrous filters. For the study of the filtration properties of these filters, asymmetric structures have been rather arbitrarily chosen. It may be expected that the efficiency of asymmetric filters can be further improved by optimization of the pore gradient structure in the filters.

It appears that our membrane filters show a low capacity to remove leukocytes from blood, as compared to the nonwoven filters tested. Although SEM pictures of fibrous filter layers suggest a less efficient porous structure of the nonwoven filter, the asymmetric nonwoven filter removed almost twice as many leukocytes as the asymmetric membrane filter. This result may be attributed to a more efficient asymmetric structure of the nonwoven filter, as may be deduced from Figure 4. It is also very likely that the filter material plays an important role in the retention of leukocytes. It is well known from literature that the adherence of leukocytes to polymer surfaces is largely dependent on the surface free energy of the material.<sup>50,51</sup> In general, cell adhesion increases when the polymer surface is more hydrophilic.<sup>52–54</sup> In this regard, polyurethane (PU) from the membranes or polyester (PET) from the fibers may behave differently towards leukocytes. As PET has a higher surface free energy than PU, the former are expected to retain more leukocytes, which is in agreement with our results.

The results of a histological study of the cell content of the membrane filters after filtration also demonstrate the contribution of leukocyte adherence in the filtration process. Leukocytes were found to stick to the filter surface



and sometimes even spread, which suggests a very strong interaction with the filter material. In this respect, not only the smaller pores are capable to capture leukocytes, but the larger pores are susceptible to leukocytes as well. This explains the capacity of large pore filters to remove a small fraction of leukocytes. We observed that a large number of leukocytes was trapped by platelet aggregates, present in the top sections of the filter. Such interaction between platelets and leukocytes, which was also observed by Steneker in leukocyte filters for routine use,<sup>46,55</sup> may be explained in two ways. First, the adhesion and aggregation of platelets at the top section of the filter will locally reduce the apparent pore size. As smaller pores are more efficient in leukocyte retention compared to larger pores, leukocytes will easily be captured by the pores which are already partially clogged by platelets. Secondly, there is evidence that leukocyte adherence is promoted at surfaces covered with adherent platelets.<sup>56</sup> This effect has been attributed to the release of adhesive proteins like fibrinogen, fibronectin, and von Willebrand factor by activated platelets.

After most of the platelets have been removed at the top section of the filters, the middle filter sections are hardly effective with regard to the removal of leukocytes. This is indeed demonstrated in Figures 8(IIa) and 8(IIb), which only show a few leukocytes in the middle filter section. The bottom section of the symmetric filters functions even worse. In contrast, the bottom section of the asymmetric filter is rather efficient in the removal of leukocytes, due to the much smaller pores of this section. As can be seen in Figure 8(IIc), mainly lymphocytes are captured by these pores. Whereas most of the granulocytes are most probably retained at the filter top sections, by means of adhesion to the filter material, most of the lymphocytes are probably removed by sieving through the small pores at the filter bottom section.

This important distinction between different leukocyte subpopulations is also demonstrated by cell analysis of filtered blood. Large differences were found between amounts of monocytes, granulocytes, and lymphocytes in the filtrate. This is not surprising, as leukocyte subpopulations may significantly differ in size. Granulocytes and monocytes may be captured easier by an arbitrary filter pore than lymphocytes, which are somewhat smaller in size.<sup>48</sup> Moreover, lymphocytes are in general less reactive towards polymer surfaces,<sup>57-62</sup> whereas monocytes will adhere very easily to foreign surfaces.<sup>60-65</sup> It is generally known that lymphocytes are difficult to remove, compared to other leukocytes.<sup>66,67</sup> This is also confirmed by our results.

## CONCLUSIONS

Polyurethane membrane filters were prepared to study the influence of pore size distributions on the mechanisms involved in leukocyte filtration. From our experiments we conclude that the pore size distribution has a considerable effect on the filtration efficiency. Two cases have to be distinguished.

First, when significant adhesion of leukocytes occurs, such as the adhesion of granulocytes onto polyurethane filter surfaces, the filter efficiency may be

improved by increasing the filter surface, available for leukocyte adhesion. In our approach, this can indirectly be achieved by decreasing the pore size. In addition, smaller pores may also promote the contact between leukocytes and filter material and thus enhance adhesion. However, small pore size filters induce excessive adhesion of leukocytes to pores at the top section of the filter, which leads to rapid clogging of the filter. Clogging may be avoided by applying a gradual decrease of the pore size in the direction of the blood flow. We demonstrated that these asymmetric leukocyte filters show optimal filtration characteristics.

Second, when adhesion of leukocytes is less pronounced, as in the case of lymphocytes onto polyurethane filter surfaces, increasing the filter surface is of no use. In this case the pore size may play a direct role because lymphocytes are captured by the sieving action of small pores. As asymmetric filter structure to avoid clogging may also be applied here.

Thus, in this study we have demonstrated that both adhesion and sieving mechanisms are involved in leukocyte filtration, although cell adhesion play a predominant role.

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