Journal of Applied Polymer Science

Poly(butylene terephthalate) Electrospun/Melt-Blown Composite Mats for White Blood Cell Filtration

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ABSTRACT: In this research, poly(butylene terephthalate) (PBT) electrospun nanofibrous mats were prepared for white blood cell (WBC) filtration after the blood was collected from donors. The feasibility of using PBT electrospun mats used as WBC filtration materials was studied. The average fiber diameter and mean pore diameter of the PBT electrospun mats were much lower than those of melt-blown mats, and such properties were found to be beneficial for the functions of filtration and cell absorbency. A layer of PBT electrospun mats was added before the exit of filter; this formed a kind of compound material with different pore diameters and fibers diameters in the direction of blood flow. The WBC filtration efficiency of the newly designed filter and a traditional filter were also compared. The results show that filters with electrospun PBT fibers reduced the number of WBCs to 10^4/L, whereas the remaining WBCs after flow through the traditional filter was about 10^5 L^{-1}. The existence of electrospun fibers increased the filtration resistance at the same time, and this led to a longer filtration time. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 128: 3652–3659, 2013

KEYWORDS: biomaterials; nanofiber; polyesters; fibers

Received 31 March 2012; accepted 1 August 2012; published online 8 October 2012
DOI: 10.1002/app.38423

INTRODUCTION

In human blood, there are two kinds of components: cellular and noncellular (plasma). The cellular part includes white blood cells (WBCs), red blood cells (RBCs), and platelets. It has been proven that during blood transfusion between donors and patients, the removal of WBCs can reduce the occurrence if many adverse reactions, including allogeneic and febrile reactions.[1,2] Meanwhile, viruses that may propagate from donors to patients during blood transfusion are also carried by WBCs; this leads to the concern that WBCs should be removed as completely as possible before transfusion, especially for patients with lower immunity.

Until now, filtration remains to be the most effective method for removing WBCs. Three mechanisms have been found in the filtration process.[3,4] First, WBCs are captured or blocked in the pore formed by fiber materials; this is called mechanical sieving. Second, a few WBCs adhere to the filter fibers directly. In this stage, a larger specific surface is good for adhesion as it can increase the connection chance between the WBCs and filter fibers. Third, some platelets are activated and adhere to the filter fibers during filtration. Activated platelets adhere indirectly to the filter fibers as they can absorb some part of the WBCs. On the basis of the previous mechanisms, it is reasonable to conclude that the pore size and surface structures of filter materials are key parameters in the design of WBC filters with high efficiencies.

Melt-blown nonwovens are the most commonly used material for WBC filters. The pores of nonwovens are irregular, and their average diameter ranges from 1 to 5 μm, depending on the manufacturing parameters. The irregular shape and small size formed by microfibers can enhance the adhesion of WBCs. Usually, the number of WBCs is about 10^9 L^{-1} before filtration, whereas after filtration by melt-blown filters, the number decreases to 10^5 L^{-1}.

Over the past decade, electrospinning technology has drawn great attention because of its convenience in obtaining nanofiber mats. Various types of materials have been successfully electrospun into fibers. The nanoscaled fiber diameter, accompanied by a high porosity and large specific surface area, make electrospun nanofibers ideal substrates in the applications of filtration.[5–7] Several filtration applications of electrospun nanofibrous mats based on melt-blown nonwovens have been studied; these include water filtration, suspended particles in air, and so on.[8–10] The results all show that electrospun nanofibrous mats are conducive to increasing the filtration efficiency.

In this study, we selected poly(butylenes terephthalate) (PBT) as the starting material to form nanofibrous mats, as these would meet the demand that polymers used in blood filtration should have good machinability and the surface of materials should do no harm to the components of blood.[11] The nanofibers were directly electrospun on PBT melt-blown nonwovens to form a composite nano–micro structure. Their filtration performance was evaluated through analysis and comparison of the testing data with those of original filters using melt-blown nonwovens only.

EXPERIMENTAL

Materials

PBT pellets (intrinsic viscosity = 0.8, Sinopec Yizheng Chemical Fibre Co., Ltd., Yizheng City, China) were used for electrospinning. Trifluoracetic acid (TFA) and dichloromethane (DCM; analytically pure grade, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) were chosen as solvents. The mixture ratio of TFA to DCM was 50/50 v/v for the dissolution of PBT.

PBT melt-blown nonwovens (Shanghai Textile Industrial Co., Shanghai, China) were used as collective materials for electrospinning. The surface density of the PBT nonwovens was 163 $\pm$ 13 g/m^2. The thickness was about 1.00 $\pm$ 0.04 mm (as measured by a digital fabric thickness gauge, YG141N, Nantong Hongda Experiment Instrument Co., Ltd., Nantong City, China). In addition, they were also used directly as the traditional WBC filter in this study.

Electrospinning of PBT

PBT (20% w/v) was dissolved in a mixture of TFA and DCM. Before electrospinning, the PBT solution was stirred at room temperature until the polymer was completely dissolved. The solution was placed into a syringe, which was mounted on a syringe pump (multisyringe pump TS2-60, BaoDing Longer Precision Pump Co., Ltd., Baoding City, China); a high-voltage supplier (supplied by high-voltage direct-current power supply, BGG6-358, BMEI Co., Ltd., Beijing, China) was connected to the syringe needle.[12,13] The experiments were operated in a laboratory with a constant temperature (20–25°C) and a constant humidity (30–40%). The detailed parameters of electrospinning were as follows: high voltage $\approx$ 20 kV, inner diameter of the blunt-end needle $\approx$ 0.6 mm, feed rate of the PBT solution $\approx$ 0.8 mL/h, and distance between needle tip of the syringe and the collector = 13 cm. The speed rate and electrospinning time were kept consistent, and a great number of electrospun mats were produced. The relatively uniform mats were selected for filtration tests.

To completely remove the residual solvent, the electrospun materials were placed in a vacuum dryer at room temperature for more than 24 h.

Physical Characterizations

Scanning electron microscopy (SEM; JSM-5600, JEOL Co., Ltd., Tokyo, Japan) was used to observe the morphology of the PBT fibers. On the basis of the SEM images, the average fiber diameter and distribution of the fiber diameters were acquired by the software Image J (University Health Network Research, Toronto, Canada).

The pore sizes and pore distribution of the PBT electrospun mats and PBT melt-blown nonwovens were tested with a capillary flow porometer (CFP-1100-Al, Porous Materials, Inc, New York, USA). Before testing, the samples were first immersed into a liquid with a surface tension small enough to completely fill all the pores. Bubble-point theory is based on the hypothesis that all the pores or channels are regular cylinders, and the diameter is calculated according to the air flux. The air flux through the test samples at different air pressures were tested, and the air pressure increased progressively in a specific range. The pore sizes were obtained through analysis of the differences of the air permeability values at every air pressure when the test sample was filled with liquid and when it was not. The pore size can be expressed as shown in eq. (1):[14]

$$D = \frac{4\gamma \cos\theta}{\Delta P} \qquad (1)$$

where D is the pore diameter, γ is the surface tension of the wetting liquid, θ is the contact angle, and ΔP is the differential pressure.

The contact angle was used for the evaluation of the surface tension. It was tested with a contact angle tester (OCA15EC, DataPhysics Instruments GmbH, Filderstadt, Germany). Water (3 μL) was extruded from a minitype quantitative injector to the surface of the testing samples. When the shape of the droplet was stable, the contact angle could be calculated immediately with the image analysis software.

Safety of the PBT Mats

The safety of the PBT electrospun materials was evaluated with the hemolysis rate, which expresses the rate of broken RBCs after filtration.

The testing procedure for the hemolysis rate is described in brief. The filtration materials (5 g in weight) were first cut into a standard shape and were then immersed into 100 mL of normal saline for 2 h in a water bath at 37°C. When the lixivium of fiber mats were obtained, 0.20 mL of blood (hematocrit $\approx$ 12%) was dropped into 10 mL of sample lixivium, 10 mL of normal saline, and 10 mL of distilled water, respectively. After they were warmed in water baths at 37°C for 60 min, the previous mixtures were centrifuged to deposit RBCs with complete shapes at a speed of 2500 rpm for 10 min. The absorbency of the top liquid after centrifugation was tested to evaluate the rupture degree of the RBCs. Absorbency values were obtained at a wavelength of 545 nm.

The hemolysis rate was calculated with eq. (2):

$$\textit{Hemolysis rate} = \frac{A - B}{C - B} \times 100\% \qquad (2)$$

where A is the absorbency of the top liquid of the mixture obtained from blood mixed with the sample lixivium after centrifugation, B is the absorbency of the top liquid of the mixture obtained from blood and mixed with normal saline after centrifugation, and C is the absorbency of the top liquid of the

Figure 1. (a) Product picture of the WBC filter with blood bags and (b) enlarged drawing of the WBC filter part. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

mixture obtained from blood and mixed with distilled water after centrifugation.

Manufacturing of WBC Filters

WBC filters were made of filtration materials and poly(vinyl chloride) cover bags. Figure 1(a) shows the practical product in market together with blood bags, and Figure 1(b) shows an enlarged drawing of a PBT melt-blown filter.

To study the filtration performance of PBT electrospun materials, they should be manufactured into filters. In this study, the filtration materials were sealed up in a poly(vinyl chloride) bag, as shown in Figure 1(b). The structure of the filter in sectional views is shown in Figures 2 and 3. Figure 2 shows the traditional WBC filter (filter A), which was made with only six layers of melt-blown PBT fibers after hydrophilic finishing.

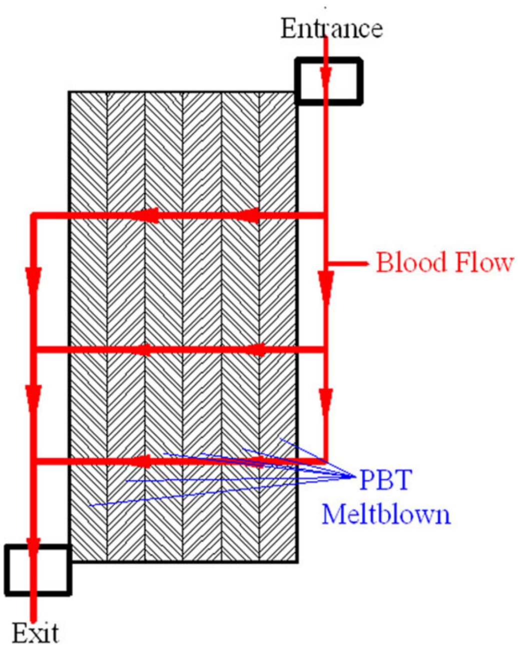

Figure 2. Structure of the traditional WBC filter (filter A) with PBT melt-blown mats as the only filtration material. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

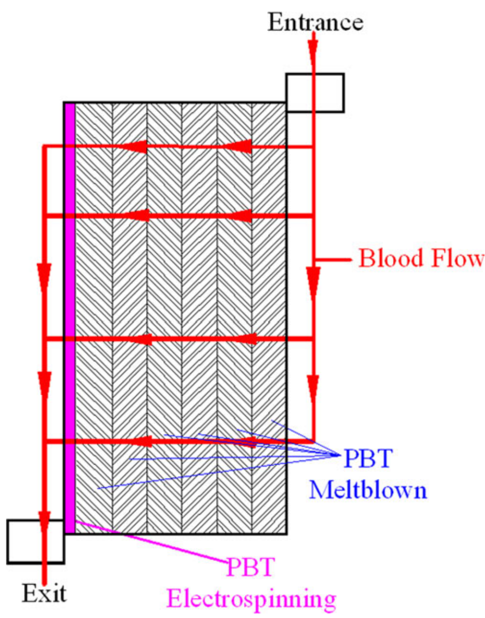

Figure 3. Structure of the WBC filter with the electrospun PBT nanofibers (filter B). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

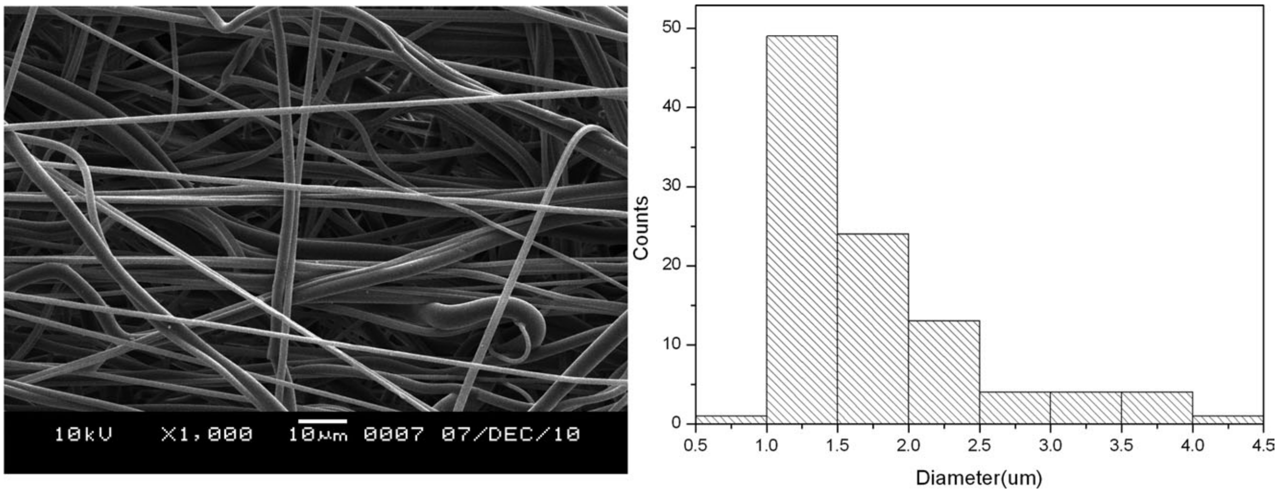

Figure 4. SEM image of the melt-blown mats and the distribution of the fiber diameter.

In this research, a layer of PBT electrospun mats was added near the exit of the filter, as illustrated in Figure 3. A new kind of WBC filter (filter B) was formed by the combination of the PBT melt-blown mats with the electrospun nanofibers. The melt-blown mats in the newly designed filter B had two functions. First, there was a conflict between the breaking strength and filtration resistance for filters made with electrospun mats only. This is also the main problem that restricts the application of electrospun mats. If the electrospun mat is too thin, it is too easily broken in the process of filtration. On the contrary, the filtration resistance is too large and the WBCs are easily blocked by the pores of electrospun materials because of the compact structure of the electrospun materials. Second, in the compound filter (filter B), larger WBCs could be filtered by the layer of melt-blown mats, and then the smaller ones could be further captured when the blood flowed through the electrospun layer. In this design, the combination of melt-blown and electrospun mats could reach the objective of further filtration, whereas the increase in the extent of the filtration resistance could be reduced to a large extent.

Filter B contained six layers of PBT hydrophilic melt-blown mats like filter A and one layer of electrospun PBT fibers. A relatively uniform electrospun mat was selected to manufacture filter B, and its thickness (measured with a micrometer) was 0.09 ± 0.02 mm.

WBC Filtration Tests

Before the filters were used for blood filtration, all of them must were sterilized. All of the operations were performed under hermetic conditions.

The WBC filtration efficiency was obtained through analysis of the number of WBCs before and after filtration. The number of blood cells before filtration was tested with a hemocytometer (p0cH-100i, Sysmex Corporation, Kobe, Japan). The testing blood sample was picked up from the blood bags after they were shaken to a homogeneous state. An amount of 1 mL of each blood sample was needed. After the blood sample was put into a testing groove, the number of blood cells was measured automatically.

After filtration, the counting of RBCs and platelets was done by a hemocytometer, but the number of WBCs was much lower than the measuring range of the hemocytometer (10^9 L^{-1}). So, we tested it by the method of direct counting under a microscope. Crystal violet was used to dye the WBCs so that they can be identified under the microscope. Blood diluted 10 times was used for cell counting. During the test, blood diluent was placed on a Nageotte blood count plate (a special glass slide with an H-shape groove to store specific volumes of blood and scale marks for counting, Brand, Wertheim, Germany), and then, the number of WBCs in a specific area was measured. The testing procedure was conducted under China Medicine standard YY0329-2002.

Whole blood bags (400 mL) were supplied by Shanghai Transfusion Technology Co., Ltd., Shanghai, China. To make sure that the population of blood cells before filtration was highly comparable, each whole blood bag was divided into four 100-mL blood bags for the WBC reduction tests of filters A and B. Two groups of blood filtration tests were performed, and different results were obtained.

RESULTS AND DISCUSSION

Morphology and Fiber Diameter

Two types of filtration materials were involved in this study: melt-blown nonwovens and electrospun mats. Filter A was made of melt-blown mats only, and filter B was made by a combination of melt-blown and electrospun mats.

Figures 4 and 5 show the SEM images of two kinds of filtration materials and their distribution of fiber diameters, respectively. It was clear that the diameters of the electrospun mats were much smaller than those of the melt-blown mats. Using Image J for analysis, we determined the diameter of the melt-blown mats to be 1.76 ± 0.72 μm, whereas that of the electrospun mats was 468 ± 161 nm. According to Barbe et al.,[15] the amount of fibers is a factor that influences leucocyte adhesion on synthetic fibers. Because the fiber diameter of electrospun

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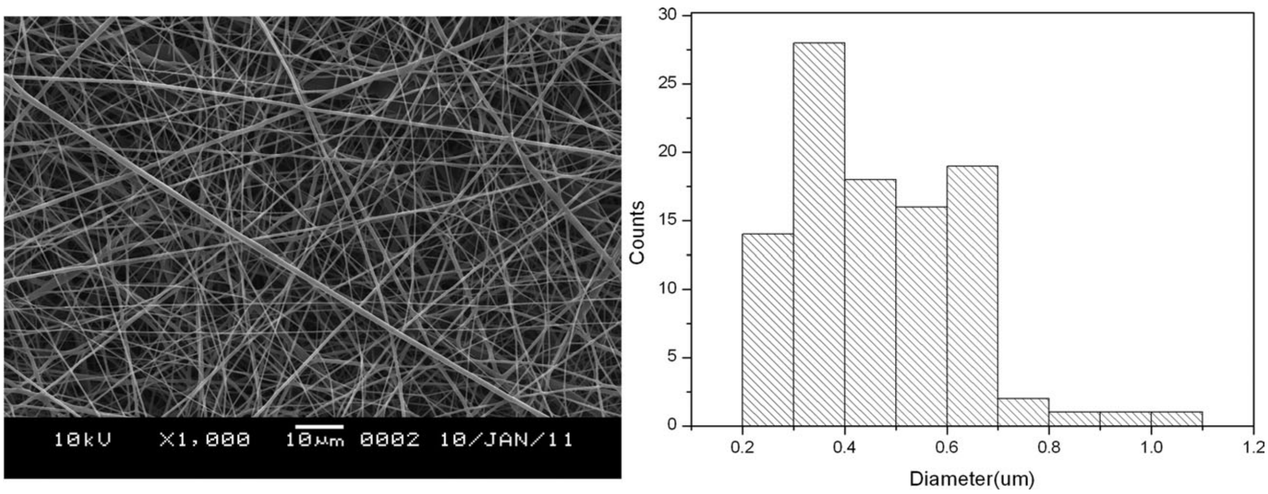

Figure 5. SEM image of the electrospun mat and the distribution of the fiber diameter.

mats was much smaller than that of the melt-blown nonwovens, the number of electrospun fibers was larger than that of the melt-blown nonwovens at the same weight. Thus, when we added electrospun mats with a high surface area to volume, the leucocyte adhesion of filter increased. What is more, we observed in the histogram that the uniformity of the electrospun PBT fibers was much better than that of the PBT melt-blown fibers. This was reasonable as the majority of PBT fibers from electrospinning had a narrow diameter distribution, from 200 to 700 nm.

Surface Tension

As previous research on WBC filtration has shown, the filtration materials with a high surface tension were good at reducing the filtration time because of their nice wettability.[16] So, in this study, water contact angle measurement was used to evaluate the surface tension of the filtration materials.

The water contact angle of the PBT melt-blown nonwovens after hydrophilic finishing was about zero; this indicated that the surface was completely hydrophilic. However, the wettability of the PBT electrospun mats was much lower. From the test, the water contact angles of the 10 samples were 101.03 ± 7.56°; this could be explained by the fact that the electrospun PBT nanofibers deposited on the PBT melt-blown mats experienced no hydrophilic finishing. As shown in Figure 6, the contact angle (CA) reached 102.30°.

Normally, the surface tension of hydrophilic materials is comparatively low. When blood flows through hydrophilic filtration materials, it can be easily spread out on the surface, and this results in a decrease in wetting time. Otherwise, the filtration resistance increases, and the wetting time is enhanced.

Pore Size Properties

As previous studies have shown, three mechanisms of WBC reduction occurred during the filtration process: mechanical sieving, direct adhesion, and indirect adhesion. The last two functions depend on the surface structure and surface-specific tension, whereas mechanical sieving is mainly altered by the pore size of the filtration materials. Therefore, the pore sizes of the materials, which largely depend on the bulking intensity and thickness of materials, is the key parameter that may directly affect the accuracy grade. The pore in the bubble-point test method indicates the channel unblocked through the top to the bottom of material. So, the bubble-point method was used to evaluate the pore characteristic of the component filter. Although the pores of both the melt-blown and electrospun mats were irregular, bubble-point theory is based on the hypothesis that all the pores or channels are cylindrical. That is a limitation of the bubble-point method. However, its test results are still meaningful in comparison with the approximate pore size of two different materials.

Five samples of each kind of material were tested. Table I lists the pore size parameters of the filter A materials, electrospun mat, and filter B materials, whereas Figures 7–9, show the pore diameter distribution. As expected, the mean pore diameter of

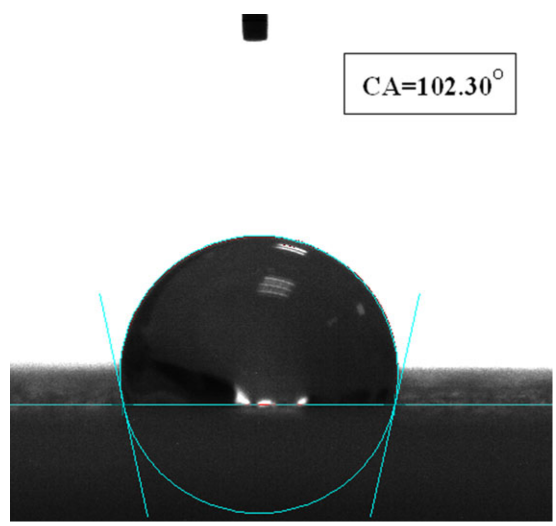

Figure 6. Photograph of a 3-μL water droplet spreading on the surface of the electrospun PBT materials. CA, contact angle. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table I. Pore Size Parameters of Filters A and B

	Bubble-point pore diameter (μm)	Mean pore diameter (μm)
Melt-blown mats only (filter A)	13.65 ± 2.78	5.36 ± 2.54
Electrospun mat only	1.84 ± 0.21	0.73 ± 0.17
Melt-blown/electrospun mat (filter B)	6.36 ± 1.63	2.18 ± 0.97

the electrospun mat was much smaller than that of melt-blown mats. A smaller deviation of the pore diameter was also observed for the electrospun mat. The average pore size and bubble pore size of filter B were bigger than those of the single electrospun mat; this was because the pore in filter B was an irregular channel, which contained the top part (from the melt-blown mat with a bigger pore diameter) and the bottom part (from the electrospun mat with a smaller pore diameter). The test of the composite pores was based on the hypothesis that they were cylindrical channels with equal flow.

Characteristics of blood cells are shown in Table II. WBC is a kind of cell with karyon, and RBC is akaryote; therefore, the size of WBCs is mainly invariable, whereas RBCs can easily travel through pores that are even smaller than themselves. The pore diameter of the pure electrospun mats was too small to allow passage of any blood cells, so it could not be used for WBC filtration because it blocks RBCs and platelets that are useful for blood. This is another reason that we combined the melt-blown and electrospun mats for the filter. Comparing the materials used in filters A and B, we found that the bubble-point pore diameter (the diameter of the biggest pore) of filter B was much smaller than that of filter A, so the small-sized WBCs (e.g., lymphocytes) could be captured effectively. Thus, an obvious increase in accuracy degree was observed in filter B.

Hemolysis Rate

Hemolysis is defined as the effusion of hemoglobin from RBCs; in other words, RBCs are broken when hemolysis occurs. *In vitro*, this usually happens when blood meets some substance,

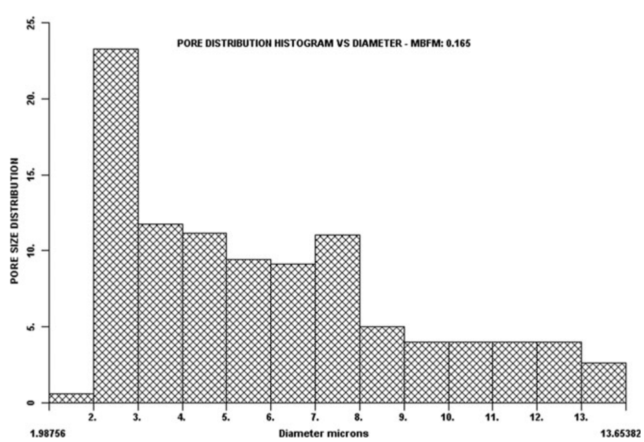

Figure 7. Pore diameter distribution of the melt-blown mats in filter A. MBFM, melt-blown fibrous mat.

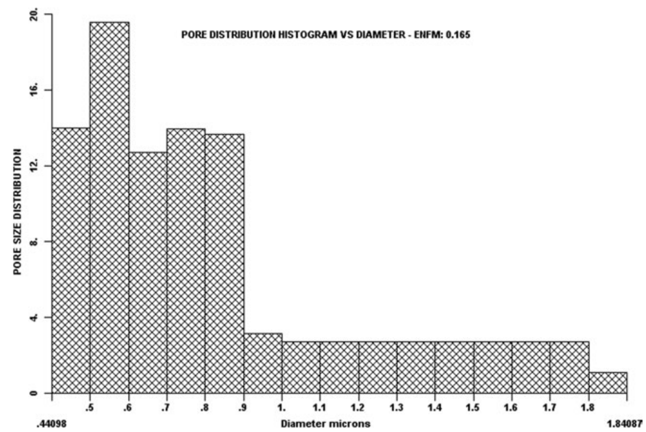

Figure 8. Pore diameter distribution of the electrospun mat. ENFM, electrospun nanofibrous mat.

such as a hypotonic solution, peracid, or alkali. If blood is frozen rapidly or suffers from heavy oscillation, hemolysis will also occur.

Because RBCs are one of the most valuable components in blood, hemolysis should be prevented as much as possible. Because TFA and DCM are used as solvents for electrospinning, it is important to make sure that the as-spun mats are free of solvent residues before they are used for blood filtration.

According to a China Medicine standard for blood filtration materials, the hemolysis rate of WBC filtration materials should be lower than 5%. As the melt-blown parts in filters A and B were made of the same material, which met the standard, it was necessary to evaluate the hemolysis rate of the electrospun PBT mat, which was 3.67% and was lower than the that of the standard requirement. Such a result demonstrated that the addition of electrospun PBT fibers to the original filter posed no risks for the mat's use as a blood filtration material (Table III).

WBC Filtration

In normal human blood, the number of WBCs is around 10^9 L^{-1}. The American Association of Blood Banks specified that

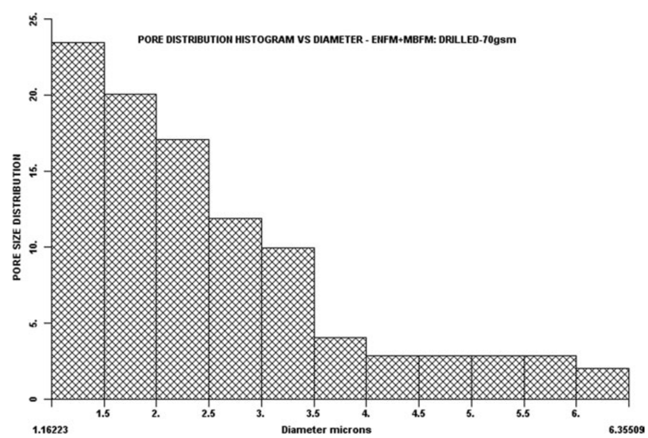

Figure 9. Pore diameter distribution of the melt-blown/electrospun mat in filter B. MBFM, melt-blown fibrous mat; ENFM, electrospun nanofibrous mat.

Table II. Characteristics of Cells in the Blood[17]

Blood component		Size (μm)
RBCs		8–9
WBCs (with karyon)	Lymphocyte	6–18
	Monocyte	12–20
	Neutrophile	10–15
	Eosinophil	10–15
	Basophil	10–15
Platelets		2–3

WBCs counts after filtration should be less than 5×10^6 L^{-1}, and the value was further restricted to lower than 1×10^6 L^{-1} by a standard of the Council of Europe.[18,19]

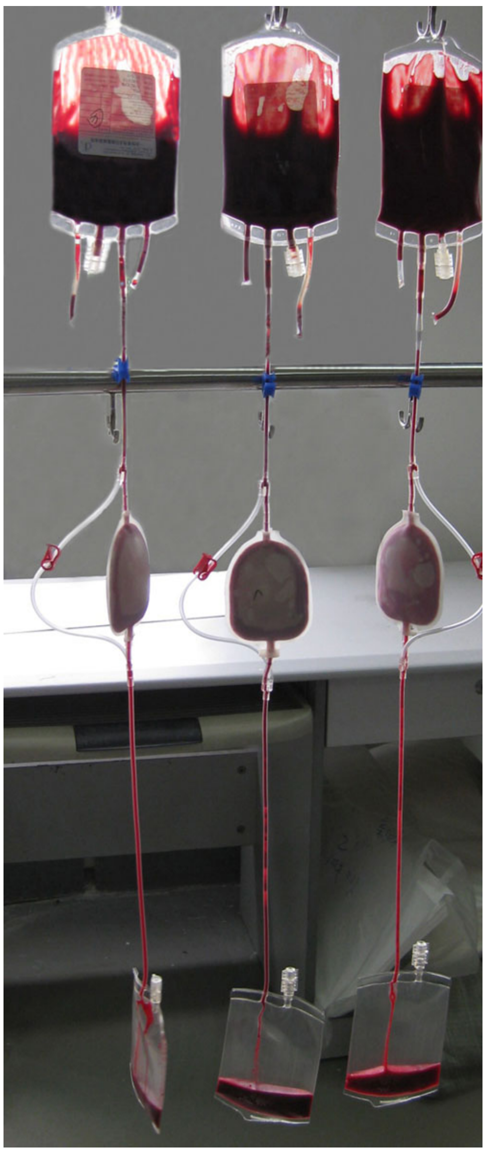

Figure 10. WBC filtration of whole blood. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table III. Hemolysis Rates of the Electrospun Mats in Filter B

	Absorbency
A (samples)	0.0026 (average of three results)
B (normal saline)	0
C (distilled water)	0.6903

Hemolysis rate = $(A - B/C - B) \times 100\% = 3.67\%$.

Figure 10 depicts the blood filtration process. Whole blood was filtered under gravity without the application of any external forces. Because the edges of the filter were enclosed and the blood flow was guided to the top of the filtration layers, as shown in Figures 2 and 3, the blood was forced to pass through all of the filtration layers before it flowed from the exit. During the process of WBC filtration, whole blood first met with the melt-blown materials with micrometer-grade pores and then flowed through the electrospun mat with nanograde pores. The bigger WBCs were first removed when they flowed through the melt-blown materials. When the blood flowed through the electrospun mat after it flowed through the melt-blown materials, the remaining WBCs were further filtered.

The numbers of blood cells before and after blood filtration are shown in Table IV. In Table IV, the number of RBCs, platelets, and time for filtration are also shown.

It was clear that filter B (made of melt-blown and electrospun mats) could remove more WBCs than filter A (made of melt-blown mats only). The residual number of WBCs from filter B was only up to 10^4 L^{-1}; this was hundreds of times smaller than those specified in international standards. The filtration efficiency of filter B (the number of WBCs after filtration/number of WBCs before filtration) was up to 99.99906%. Several kinds of viruses exist in WBCs, such as cytomegalovirus, Epstein–Barr virus, and human T-cell leukemia virus I/II, and they are usually transmitted with WBCs. Some of the virus can be detected by other medical methods, but cytomegalovirus is an important infectious agent for patients with low immunity, such as in patients with a history of bone marrow transplantation or organ transplantation and premature infants with low birth weight. The lower the amount of WBCs in transfusion blood is, the lower the risk is. For common patients, blood after filtration with filter A should be basically safe. However, filter B further decreases the WBC number, and their safety for the previous patients will be surely improved.

The filtration time of filter B was 65.5 min; this was much longer than the 2.5 min of filter A. The addition of electrospun

Table IV. Blood Filtration Efficiency Values of Filters A and B

		Filter A	Filter B
WBCs (L^{-1})	Before	$(5.1 \pm 1.1) \times 10^9$	$(5.1 \pm 1.1) \times 10^9$
	After	$(4.3 \pm 0.8) \times 10^5$	$(4.9 \pm 2.3) \times 10^4$
RBC recovery (%)		90.3 ± 0.9	87.4 ± 1.2
Filtration time (min)		2.5 ± 1.3	65.5 ± 6.2

mats increased the filtration resistance and caused a longer filtration time, as shown in Table IV. This could be explained by two aspects: (1) the pore diameter of the electrospun mats was much smaller, and (2) the wettability of the electrospun mat was poorer than that of the melt-blown mats with hydrophilic finishing. On the basis of the fact that no specific speed is demanded in blood filtration, such an increase in time is regarded as acceptable.

CONCLUSIONS

In this study, electrospun PBT nanofibrous mats were obtained with TFA and DCM as spinning solvents. The safety of using electrospun nanofibers for WBC filtration before blood transfusion was also studied. After that, the nanofibers were combined with PBT melt-blown nonwovens to form a new type of filter, the performances were evaluated and discussed by a comparison of the novel filter with a traditional WBC filter. SEM of the fiber morphology showed that the pore diameter and average fiber diameter greatly decreased; this is good for the functions of filtration and cell absorbency. The WBC filtration results further verify that the addition of electrospun PBT mats reduced the number of WBCs from 10^5 to 10^4 L^{-1}; this provides a much higher safety for transfusion, especially for patients with lower immunity. To further enhance the filtration efficiency, a future investigation will be conducted to improve the wettability of the electrospun PBT fibers.

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