Optimising quality of platelet transfusions

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Chapter 5

Chemical coatings for improving platelet compatibility of non-woven PET used for filtration of platelet concentrates
CHEMICAL COATINGS FOR IMPROVING PLATELET COMPATIBILITY OF NON-WOVEN PET USED FOR FILTRATION OF PLATELET CONCENTRATES


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Abstract

Non-woven PET (NW-PET) material is widely used for leukocyte removal from red blood cell concentrates. It is, however, not platelet-compatible. Because of good leukocyte removal properties and low-cost aspects, it is interesting to modify this material into platelet-compatible material for development of a leukocyte removal filter for platelet concentrates (PC).

Based on literature study, experimental coatings that were hydrophilic and protein-compatible were selected and used for coating of NW-PET material. After testing the wettability and stability of experimentally coated NW-PET, filter material was tested in triplo with three different PCs in a downscaled filtration set-up.

After coating of NW-PET with different polymers (block copolymers from poly[ethyleneoxide](PEO), poly[propyleneoxide](PPO) and polysiloxanes), the wettability increased. These coatings were not stable after gamma-sterilisation and during filtration. The downscaled filtration set-up is an excellent way of testing a maximum amount of filters with minimal amounts of PC. According to the filtration results with different PCs, only PEO-polydimethylsiloxane-PEO type 4 seemed to be platelet-compatible. This material might be used for further development of a leukocyte removal filter for PC. The predicted protein and platelet compatibility under static conditions according to the literature did not correlate with platelet compatibility under flow conditions.
INTRODUCTION

In leukocyte-depletion of blood or blood components by filtration, three mechanisms are involved: mechanical sieving direct adhesion and indirect adhesion [111]. Leukocytes can bind directly to the filter surface but also indirectly when leukocytes adhere to platelets that have already adhered to the filter material. Granulocytes are removed by all three mechanisms, monocytes are mainly removed by adhesion and mechanical sieving, while lymphocytes are removed by sieving only. Therefore, lymphocyte removal mainly depends on the pore size and granulocyte and monocyte removal mainly depends on chemical properties of the filter surface. Residual leukocytes in buffy-coat (BC)-derived PC are mainly lymphocytes [87; 112], thus removal of leukocytes from PC should mainly depend on a mechanical sieving mechanism. For platelet concentrate (PC) filtration, a filter that does not induce platelet adhesion is necessary. For this purpose, the filter surface can be modified to obtain a non-adhesive surface for platelets. The effect of a platelet-compatible surface on adhesion of granulocytes and monocytes is unknown but of minor importance because of the low concentration of these cells in PC.

For removal of leukocytes from red blood cell concentrates, non-woven poly(ethylene terephthalate) (PET) filter material is frequently used. However, this material is not platelet-compatible and has to be modified to render it suitable as a leukocyte-removal filter for PC. For this purpose, biocompatible polymers were selected from the literature and tested as coatings on the NW-PET material.

Several ways of surface modification to increase blood compatibility exist, which can be divided in five main groups [113]:
1. change of hydrophilicity or hydrophobicity of the surface
2. applying a negative charge on the surface
3. immobilisation of physiologically active compounds on the surface
4. masking of the surface with coatings
5. combinations of one of the above-mentioned methods

Ad 1. For maximum platelet compatibility, the adsorption of adhesive proteins needs to be minimised. Some adhesive proteins possess the Arg-Gly-Asp (RGD) sequence. This sequence is responsible for specific binding to the platelet integrin αIbβ3 (GpIib/IIa), which is present in closed conformation on non-activated platelets. The RGD sequence is present in fibrinogen, von Willebrand factor (vWF), fibronectin, vitronectin and thrombospondin. These proteins are called adhesive proteins, in contrast to albumin, which lacks the RGD binding site. These adhesive proteins bind to αIbβ3 on activated platelets when the open (active) conformation is present.

By increasing the hydrophilicity or hydrophobicity or both, protein adsorption on a (filter) surface can be minimised, resulting in less platelet interaction with the filter surface [67]. Hydrophilic surfaces also induce less
conformational changes of proteins, which, in turn leads to less platelet interaction. Despite the low protein adsorption on hydrophobic surfaces, these surfaces are not suitable for filtration due to low wettability capacity of the hydrophobic surface. A combination of hydrophobic and hydrophilic surfaces (domain structure) shows a low platelet interaction as well. Proteins keep their native conformation when adhered to this type of surface. The hydrophobic as well as the hydrophilic part of the protein can bind to a specific domain. In this way, proteins can cover the surface. However, the wettability of this surface type is not optimal and depends on the relative amount of hydrophilic domains compared to the hydrophobic domains.

In conclusion, hydrophilic surfaces induce the lowest protein/platelet interaction combined with the highest wettability capacity, rendering these surfaces the most preferable for development of platelet-compatible filter material.

Ad 2. Cell membranes of platelets and leukocytes have a negative charge under physiologic conditions. A positively charged surface will have a strong interaction with both cell types. A negatively charged surface will repulse both and thus cause a decreased platelet adhesion, which is preferable for platelet filters.

Ad 3. For immobilisation of physiologically active compounds at the surface, anti-platelet compounds such as prostaglandins, aspirines, apyrases and heparin can be used. These have an inhibiting effect on platelet deposition. Besides this platelet-deposition-inhibiting effect, heparin also has an inhibiting effect on the intrinsic pathway of the coagulation.

Ad 4. By covering the surface with body-compatible material, the surface can be masked and will not be recognised as non-corporal material. There are three different methods to accomplish this:
* simulation of physiologic surface of arteries and veins (endothelialisation)
* immobilisation of non-adhesive proteins to the surface
* simulating of the blood cell surface by applying phospholipids [114-116]

Endothelialisation is not suitable for filtration material due to storage instability, high costs, difficult sterilisation and processing. Immobilisation of non-adhesive proteins, such as albumin, to the surface is possible, but also creates storage problems and infection possibilities. However, simulating of the blood cell surface by applying phospholipids is a good possibility, but costly [114;117].

Ad 5. Combination of one of the above-mentioned methods. For example, surfaces can be covered by poly[ethylene-oxide] (PEO) chains with a terminal sulfate group. These can function as negatively charged tails. By applying a negative charge to the surface, the hydrophilicity of the surface will increase as well.
In our study we decided to make the surface of the NW-PET more hydrophilic. For this purpose, we used a number of block copolymers consisting of variable numbers of hydrophobic and hydrophilic blocks. The hydrophobic block of the copolymers binds via irreversible adsorption to the filter surface, whereas the hydrophilic tails (connected to the hydrophobic block) give the filter a hydrophilic surface character. We mainly used the hydrophilic polymer PEO, which is known for its platelet-compatible property [67]. As hydrophobic block, poly[propyleneoxide] (PPO) and polysiloxane polymers were tested. The hydrophobic polymers were bound to the PEO and tested in different structures. We used for example ABA and AB structures. In case of ABA structure, two tails (A) of PEO are hold together by the hydrophobic middle part (B), whereas the AB structure contained only one hydrophilic tail.

The stability of the coating depends on the interaction between the hydrophobic part and the NW-PET. It was expected that a long hydrophobic block would result in multiple interaction sites with PET and therefore in a stable coating. As surface-active compounds we used several block-copolymers, including PEO-PPO-PEO compounds, which were tested with (1) an increasing PPO content (more interaction with filter surface) with equal PEO content: type 1 < type 2 or (2) an increasing content of PEO (increasing hydrophilicity) with equal PPO content: type 3 < type 4 < type 5. Applying these block copolymers can lead to a homogeneous coating of 60-100Å [67;71]. Also AB structures (PS1 and PS2) were tested in which the hydrophobic part consisted of hydrophobic polysiloxanes (PS). Polysiloxanes were tested in the ABA block copolymer structures as well: PS3 and PS4 (PEO-poly(dimethylsiloxane)-PEO), DBE1-3 (dimethyl siloxane-ethyleneoxide (EO) block copolymers) and DBP1 (dimethyl siloxane-poly(propyleneoxide-ethyleneoxide) block copolymers) are all ABA block copolymers.

Before biological evaluation, coated materials were tested with respect to stability of the coating, hydrophilicity and wettability. An iodine complexation test was performed to test the amount of coating adhering to the filter surface.

The modified non-wovens were tested with platelet concentrates (PCs) in a downscaled filtration set-up. We focused on platelet compatibility. This was done by filtration experiments in which the platelets of the initial PC and in various fractions of the filtrate were analysed (platelet counts and morphological evaluations before and after filtration). With this approach, many leukocyte counts were omitted, allowing more filtration experiments to be performed in parallel on one day. In filtrates from filters with an acceptable platelet recovery (> 70%), leukocytes were counted as well.
MATERIALS AND METHODS

Preparation of PC

Platelet concentrates were made according to the single-BC method. Donations of (500 ± 50 ml) whole blood from the same ABO-Rh blood group were collected in 70-ml of citrate-phosphate-dextrose (CPD) in quadruple systems (Biopack Compoflex, NPBI, Emmer-Compascuüm, The Netherlands). After storage at 20 ± 2°C for 12-16 hours on butane-diol cooling plates [30], whole blood donations were processed to single BC-PC [87] by means of an automated device (Compomat G4, NPBI, Emmer-Compascuüm, The Netherlands) according to standard methods as described before [106]. After platelet counts had been made, BC-PC were stored overnight in a platelet incubator (22 ± 2°C) on a horizontal flatbed shaker (1 cycle/s) (Helmer lab Inc, Noblesville, IN, USA) until filtration on the next day. Before filtration, the BC-PC were pooled to suspensions of approximately 300 ml and with platelet counts of 0.8-1.2 x 10⁹/ml. For this purpose, a sterile connecting device was used (SCD 312, Haemonetics, Braintree, USA). One pool of PC was used for 6 downscaled filtration experiments. After pooling, the PC pool was split in two equal parts. One part was directly used for 3 filtrations while the other part was kept in the platelet incubator until use for the next 3 filtrations.

Cell counts

Platelet counting was performed on a Cell-Dyn whole blood counter (Sequoia-Turner Co, Mountain View, CA, USA). Prior to filtration, the leukocytes were counted electronically with a Coulter Multisizer II (Coulter Electronics, Mijdrecht, The Netherlands). After filtration, the leukocytes were counted by fluorescence light microscopy in a Nageotte bright-line counting chamber (Superior, Bad Mergentheim, FRG). For this measurement 100 μl filtered PC was added to 400 μl acridine orange solution (0.05 mg/ml in PBS). Leukocyte counting was only performed in starting point material and in filtrates when the platelet recovery was more than 70%.

Coating of filter material

Filter discs of 26 mm were cut from non-woven polyethylene terephthalate (PET) material and chemically coated by submerging the material in a 0.5% (w/v) surfactant solution in water for 15 minutes. Subsequently, unbound surfactant was removed by extensively rinsing the filter with water. The filters were dried in air on filtration paper.
Chemical coatings for improving platelet compatibility

Wettability test
To test the wettability, water drops were placed on the surface. Unmodified NW-PET did not absorb water at all. After coating, drops were absorbed directly. The scoring was qualitative: good, average and bad (+, ± and -). Unmodified NW-PET was judged as bad (-).

Iodine complexation test
Iodine forms complexes with ethers. The amount of ethers can be measured by depletion of iodine from an iodine solution. Because the stochiometric complexation of iodine with (adsorbed) ether compounds is unknown, only relative measurements were performed. The non-woven filter material (about 0.4 g) was submerged for 15 minutes in 10 ml I_2 solution (0.005 M) containing HCl (0.1 M (from 1M solution, Merck, Darmstadt, Germany)). The extinction before and after complexation was measured with a spectrophotometer at a wavelength of 500 nm. In this way, the depletion of iodine was calculated. The amount of I_2 was expressed as μmol I_2/g NW-PET material.

Stability tests for coatings
The stability of the coatings was tested by comparing wettability and iodine complexation of the materials before and after submerging in PBS. Coated NW-PET had been submerged in PBS or albumin solution in PBS (4 g/l) for 30 minutes and was subsequently rinsed three times with water and thereafter dried on filtration paper.

Gamma sterilisation of filter discs
Filter discs were gamma sterilised with 25 kGray at Gammaster (Ede, The Netherlands).

Coatings
Surface active compounds (PEO-PPO-PEO) (ICI, Middlesbrough, UK) were tested in homologous series with increasing PPO content combined with a comparable amount of PEO:
- type 1 < type 2
or increasing PEO contents combined with comparable PPO content:
- type 3 < type 4 < type 5.

We also tested various dimethylsiloxane block copolymers (PS1-4, DBE1-3 and DBP1) from Gelest (Tullytown, PA, USA). Coatings from Gelest were not tested for wettability and iodine complexation. Physical and chemical data from the manufacturer were used instead.
Preparation of filters

Per filtration, 6 coated discs with a diameter of 26 mm were used and put in a home-made filter holder. Sepacell filter material (PL10(II)A, Asahi, Medical CO., Tokyo, Japan) was used as control. For this purpose, 6 filter discs with a diameter of 26 mm were cut out of the non-coarse section (the 21 layers closest to the outlet) of the Sepacell filter.

Downscaled filtration

To allow performance of experiments with limited volumes of PC, a 6:1 downscaled filtration was performed that allowed 6 paired filtration experiments with one PC of about 300 ml. For each filtration experiment, 6 filter discs of the same type were put in a specially made Perspex filter holder (made at CLB), also described in chapter 3 and 4. For each filtration, 50 g of PC was poured in a 50-ml disposable Combitip (Eppendorf, Hamburg, Germany) that was connected to the filter holder with 15.5 cm of PVC tube. The distance between the upper edge of this tip and the filter discs inside the holder was 26 cm.

The time between entrance of the PC in the filter and the first visible drop in the outlet of the filter was called the wettability time. The filtration time was measured as the time between PC entering the filter holder and the first air entering the filter holder after the PC had gone through. The filtrate was collected in three parts, i.e. the first two parts of 5 ml and the remaining part.

Samples for platelet counting and morphology were taken from the pool before every filtration and after filtration from the first, second and remaining fraction of the filtrate and the pool of filtration fractions. Samples for leukocyte counts were taken from the pool and, if necessary, from the total filtrate.

Weights were converted into volume by using a specific gravity for PC (plasma) of 1.026 g/cm³. When the flow rate became lower than 2 ml/min for more than 60 s, or when the total filtration time exceeded 480 s, filtration was stopped and called blocked. The volume of the filtrate after 480 s flow time was used for calculations of the flow rate.
Chemical coatings for improving platelet compatibility

The leukocyte retention and platelet recovery were calculated as follows:

\[
\text{Leukocyte retention (\%)} = \left\{1 - \frac{\text{total number of leukocytes after filtration}}{\text{total number of leukocytes before filtration}}\right\} \times 100\% = \frac{L \times V}{L_0 \times V_0} \times 100\% 
\]

\[
\text{Platelet rec. (\%)} = \left\{\frac{\text{total number of platelets after filtration}}{\text{total number of platelets before filtration}}\right\} \times 100\% = \frac{T \times V}{T_0 \times V_0} \times 100\%
\]

\(L = \) leukocyte concentration in total filtered PC (sum of fractions); \(L_0 = \) leukocyte concentration in PC before filtration; \(T = \) platelet concentration in total filtered PC; \(T_0 = \) platelet concentration in PC before filtration; \(V = \) volume of the filtered PC; \(V_0 = \) volume of PC used for filtration

**Platelet morphology**

For morphological evaluation of the PC, 50 \(\mu l\) of PC was fixed with 250 \(\mu l\) of 0.5% glutaraldehyde in PBS and stored at 4\(^{\circ}\)C for future evaluation. Morphology was judged by a modification of the Kunicki score [107] with light microscopy (Leitz, Wetzlar, Germany) with oil immersion (1000x). The number of discoid cells per 100 cells was multiplied by 4, the number of filled dendrites by 2 and the number of spheres by 1. A score of 250-300 indicated a good PC quality.

**Statistical analysis**

Statistical comparisons and correlation coefficient calculations were carried out with the computer programme Instat 2.03 (GraphPad Software, San Diego, CA, USA) for two-tailed Student t-tests. p-values < 0.05 were considered significant.
RESULTS

Physical and chemical testing of filter materials

NW-PET coated with block copolymers from PEO-PPO-PEO 1-5 were evaluated by iodine complexation tests and wettability tests. For this evaluation materials were not subjected to gamma sterilisation. Only limited decreases for the iodine complexation were found upon rinsing of the coated filters (table 5.1), with no strong effects on wettability, leading to the conclusion that the coatings were stable.

Table 5.1: Coating of NW-PET with PEO-PPO-PEO compounds. Results of iodine complexation tests and wettability tests. After coating, the materials were rinsed with PBS or albumin solution (4 g/l) (see Materials and Methods).

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Rinsing solution</th>
<th>Iodine Complexation (μmol/g) (n=1-2)</th>
<th>Wettability (+, ±, -) (n=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non</td>
<td>-</td>
<td>5.1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>PBS</td>
<td>2.3</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td>5.1</td>
<td>-</td>
</tr>
<tr>
<td>PEO-PPO-PEO</td>
<td>-</td>
<td>16.0</td>
<td>+</td>
</tr>
<tr>
<td>Type 1</td>
<td>PBS</td>
<td>8.5</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td>9.0</td>
<td>+</td>
</tr>
<tr>
<td>PEO-PPO-PEO</td>
<td>-</td>
<td>18.7</td>
<td>+</td>
</tr>
<tr>
<td>Type 2</td>
<td>PBS</td>
<td>7.9</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td>10.9</td>
<td>+</td>
</tr>
<tr>
<td>PEO-PPO-PEO</td>
<td>-</td>
<td>39.0</td>
<td>+</td>
</tr>
<tr>
<td>Type 3</td>
<td>PBS</td>
<td>28.4</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td>30.8</td>
<td>+</td>
</tr>
<tr>
<td>PEO-PPO-PEO</td>
<td>-</td>
<td>17.5</td>
<td>+</td>
</tr>
<tr>
<td>Type 4</td>
<td>PBS</td>
<td>11.0</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td>13.2</td>
<td>+</td>
</tr>
<tr>
<td>PEO-PPO-PEO</td>
<td>-</td>
<td>19.0</td>
<td>+</td>
</tr>
<tr>
<td>Type 5</td>
<td>PBS</td>
<td>16.4</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td>14.9</td>
<td>+</td>
</tr>
</tbody>
</table>
Testing filter holder

**- Plasma filtrations**

Before using the filter holder with PC the flow properties were tested with plasma \( (n=3) \). The filter holder was filled either with 6 layers of uncoated NW-PET or PEO-PPO-PEO type-5-coated NW-PET. There was a significant difference \( (p = 0.0061) \) in wettability time between coated and untreated NW-PET filter material, whereas recovery and flow rate were similar (table 5.2).

**Table 5.2**: Testing filter holders with 6 layers of untreated or 6 layers of PEO-PPO-PEO type-5-coated NW-PET by means of plasma filtrations \( (n=3) \).

<table>
<thead>
<tr>
<th>Plasma filtrations</th>
<th>6 layers untreated</th>
<th>6 layers PEO-PPO-PEO type 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate (ml/min)</td>
<td>15.8±1.99</td>
<td>16.8±1.60</td>
</tr>
<tr>
<td>Wettability time (s)</td>
<td>12±2.9</td>
<td>3±0.6*</td>
</tr>
<tr>
<td>Product recovery (%)</td>
<td>96±0.2</td>
<td>96±0.2</td>
</tr>
</tbody>
</table>

*: significant difference \( (p < 0.01) \)

**-Filtrations with Sepacell material**

PEO-PPO-PEO type 5 was used as control in the first filtration experiments but due to some blockages we decided to use Sepacell material as control in further experiments, because of former good results with this filter type [chapter 4; 105]. For this purpose we performed filtration experiments with 6 layers of Sepacell material. Every PC was tested with Sepacell material to rule out possible filter blockages that were caused by the filtration set-up or by the PC itself. For Sepacell material, we found a platelet recovery of 92 ± 3.5%, flow rate 16.0 ± 4.38 ml/min and a wettability time of 2 ± 0.3 s \( (n=19) \). Morphology scores before and after filtration were not significantly different \( (319 ± 30) \).

**- Filtrations with uncoated NW-PET**

Uncoated NW-PET material \( (3, 6 \text{ and } 12 \text{ layers}) \) was used for filtrations with PC. Increasing numbers of layers caused increasing wettability times, increasing numbers of blockages and decreasing platelet recoveries. During filtration, when no blockage was seen, the amount of platelets increased in the subsequent filtration fractions. In case of blockage, a decreasing amount of platelets was counted in subsequent filtration fractions. However, for almost all filtrations with 6 or 12 layers of untreated material the overall platelet recovery was low (table 5.3).
Table 5.3: Platelet (P) recovery and flow rate of PC filtered with 3, 6 and 12 layers of untreated NW-PET material

<table>
<thead>
<tr>
<th>Number of filter layers</th>
<th>Wettability time (s)</th>
<th>P recovery (%)</th>
<th>Flow (ml/min)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>7 ± 2.2</td>
<td>81 ± 2</td>
<td>7.5 ± 1.7</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>14 ± 8.7</td>
<td>41 ± 32x</td>
<td>3.6 ± 2.5x</td>
<td>13x</td>
</tr>
<tr>
<td>12</td>
<td>45 ± 8.7</td>
<td>blockagexx</td>
<td>blockagexx</td>
<td>34</td>
</tr>
</tbody>
</table>

x: For 3 out of 16 filtrations it was impossible to make trustworthy calculations because the total filtration times were longer than 30 minutes. xx: total filtration time longer than 30 minutes (after these filtrations the experiments were restricted to 480 s, see Materials and Methods section).

**Filtrations with coated NW-PET**

- **Stability of coating after gamma-sterilisation**
  Non-wovens coated with PEO-PPO-PEO type 1-5 and subsequently gamma-sterilised, were tested in a filtration set-up with PC. Filtrations blocked and gave platelet recoveries lower than 20% (n = 3 for each coating type). After these tests, the stability of the gamma-sterilised coatings was tested. Upon rinsing with albumin or PBS, the iodine complexation measurements showed that no coating was left on gamma-sterilised NW-PET (data not shown). Therefore, it was decided to perform biological evaluations with PC in first instance with non-sterilised material.

- **Filtrations with PEO-PPO-PEO type 5 coated NW-PET**
  Six NW-PET discs coated with PEO-PPO-PEO type 5 were used as (non-sterilised) filter material and put in a filter holder. In general, bad filtration results were observed (n = 13) with large variability in platelet recovery, flow rate and platelet counts in the subsequent filtration fractions. In the subsequent filtration fractions, both increasing and decreasing amounts of platelets were seen. Flow rates were low (2.7 ± 0.76 ml/min) and platelet recoveries were around 30% (28 ± 16.5%). This was due to blockage: all filtration times were longer than 480 s and artificially stopped at that time. With this filtration time calculations were made. The wettability time was 5 ± 1.3 s. Due to filtration, the morphology score decreased (p < 0.01) from 305 ± 18 to 202 ± 119.

- **Filtrations with polysiloxane based coatings on NW-PET**
  Filtrations were performed with 6 layers of NW-PET coated with a group of 8 different polysiloxane-containing coatings. Filters were not gamma-sterilised. The filtration results showed a lot of variability. Because initial results with PS1 were hopeful, we performed more than 3 filtrations with this coating, but in additional
filtrations several blockages (5 in 12 filtrations) occurred. With PS1 coating, morphology scores before and after filtration did not differ significantly (324 ± 16 (before) and 278 ± 94 (after)). Filtration results of all polysiloxane coatings are summarised in table 5.4. In case of blockage, platelet recoveries were calculated after artificially ending the filtration at 480 s. For all coatings the wettability time was significantly reduced compared to untreated material (tables 5.3 and 5.4), with slightly higher values for DBP1. This coating is, due to the propyleneoxide content in the hydrophilic block, somewhat less hydrophilic than the other coatings. Only the results with respect to platelet recovery and flow rate for PS4 are promising, especially because no effects on platelet morphology were found.

Table 5.4: Platelet recovery (P rec.), flow rate, wettability (time of first drop) and morphology scores of PC filtrations with 6 layers of polysiloxane NW-PET material (n = 3 except when indicated differently).

<table>
<thead>
<tr>
<th>Siloxane coating</th>
<th>P rec. (%)</th>
<th>Flow rate (ml/min)</th>
<th>Wettability (s)</th>
<th>Morphology before</th>
<th>Morphology after</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS1 (n=12)</td>
<td>73 ± 19</td>
<td>7.1 ± 3.3</td>
<td>3 ± 0.5</td>
<td>324 ± 16</td>
<td>278 ± 94</td>
</tr>
<tr>
<td>PS2</td>
<td>30 ± 11.9</td>
<td>2.6 ± 0.52</td>
<td>3 ± 0.0</td>
<td>335 ± 9</td>
<td>335 ± 9</td>
</tr>
<tr>
<td>PS3</td>
<td>24 ± 3.2</td>
<td>2.3 ± 0.20</td>
<td>3 ± 0.0</td>
<td>335 ± 9</td>
<td>335 ± 9</td>
</tr>
<tr>
<td>PS4</td>
<td>82 ± 7.1</td>
<td>8.8 ± 1.61</td>
<td>3 ± 0.6</td>
<td>335 ± 9</td>
<td>335 ± 9</td>
</tr>
<tr>
<td>DBE1</td>
<td>43 ± 2.1</td>
<td>3.2 ± 0.18</td>
<td>3 ± 0.0</td>
<td>335 ± 9</td>
<td>335 ± 9</td>
</tr>
<tr>
<td>DBE2</td>
<td>27 ± 5.8</td>
<td>2.2 ± 0.29</td>
<td>3 ± 0.0</td>
<td>313 ± 24</td>
<td>255 (n=2)</td>
</tr>
<tr>
<td>DBE3</td>
<td>58 ± 20.2</td>
<td>4.4 ± 2.05</td>
<td>4 ± 0.0</td>
<td>313 ± 24</td>
<td>245 ± 18</td>
</tr>
<tr>
<td>DBP1</td>
<td>45 ± 9.1</td>
<td>3.4 ± 0.59</td>
<td>5 ± 0.6</td>
<td>313 ± 24</td>
<td>245 (n=2)</td>
</tr>
</tbody>
</table>

Several characteristics of the polysiloxane coatings, such as molecular weight and/or viscosity are shown in table 5.5. In general, viscosity is a good indication for molecular weight: the higher the viscosity, the higher the molecular weight (see data for DBE1-3 and DBP1). The percentage of non-siloxanes is an indication for the ratio between the hydrophobic backbone and the hydrophilic tails.
Table 5.5: Properties of tested polysiloxane coatings according to the manufacturer.

<table>
<thead>
<tr>
<th>Coating</th>
<th>% Non-siloxane</th>
<th>Viscosity (Nms(^{-2}))</th>
<th>Molecular weight</th>
<th>Water solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS-1</td>
<td>82</td>
<td>130</td>
<td>nda</td>
<td>+</td>
</tr>
<tr>
<td>PS-2</td>
<td>80</td>
<td>45</td>
<td>nda</td>
<td>+</td>
</tr>
<tr>
<td>PS-3</td>
<td>45-55</td>
<td>300-350</td>
<td>2200-2600</td>
<td>nda</td>
</tr>
<tr>
<td>PS-4</td>
<td>75-80</td>
<td>250-350</td>
<td>1000-1500</td>
<td>nda</td>
</tr>
<tr>
<td>DBE-1</td>
<td>75</td>
<td>20</td>
<td>600</td>
<td>+</td>
</tr>
<tr>
<td>DBE-2</td>
<td>80</td>
<td>40-50</td>
<td>1000</td>
<td>+</td>
</tr>
<tr>
<td>DBE-3</td>
<td>80-85</td>
<td>125</td>
<td>3600</td>
<td>+</td>
</tr>
<tr>
<td>DBP-1</td>
<td>65-70</td>
<td>1800</td>
<td>20,000</td>
<td>+</td>
</tr>
</tbody>
</table>

Prefix: PS = dimethylsiloxane-ethyleneoxide copolymer; D = dimethylsiloxane; B = block; EE = poly[ethyleneoxide]; P = propyleneoxide-ethyleneoxide copolymer; nda = no data available.

- Fractionated collection of filtrate

The filtrate was collected in 3 fractions of 2 times 5 ml and the remaining volume. In all 3 subsequent fractions, platelets were counted. Platelet concentrations in filtration fractions were expressed as a percentage of the platelet concentrations in the unfiltered PC (fig. 5.1). In all filtration experiments with coated filters there was an increase in platelet concentration between the first and second filtration fraction. However, between the second and third filtration fraction there was either an increase or decrease in platelet concentration, reflected in the large vertical error bars for the points related to the third fraction. A decrease in platelet concentration between the second and third fraction resulted in all cases in a blockage of the filter (volume of filtered PC << 50 ml), after variable filtered volumes, reflected in the large horizontal error bars. Some examples of change in platelet concentrations in subsequent filtration fractions are shown in figure 5.1, in which filtration characteristics of various coated NW-PET filters and control Sepacell filter material are shown. Unblocked filtrations led to a filtration volume of almost 50 ml (figure 5.1), whereas in case of blockage, this volume was never reached. Filtrations with untreated filter material gave variable results (table 5.3); this would mean rising and descending lines. A drawing of a line calculated from the average would not depict an average behaviour of this filter type and is therefore omitted.
Fig. 5.1 - Relative concentrations of platelets flowed through the filter (%) in the cumulative subsequent filtration fractions. During filtration, the platelet counts in the subsequent filtration fractions were related to that in the unfiltered PC (%). This was plotted against the cumulative volume of the subsequent filtration fractions (ml). Filtration characteristics of 4 filters are shown: Sepacell (n = 12)(+); NW-PET coated with PS-1 (n = 12)(△); DBP-1 (n = 3)(△) and PS-2 (n = 3)(x).

DISCUSSION

Prior to evaluation of the coated non-wovens, the test system was validated. Control experiments with our home-made filter holders were performed with plasma instead of PC, with uncoated and PEO-PPO-PEO type-5-coated NW-PET. Results of plasma filtrations with uncoated or coated NW-PET showed a good flow and a high product recovery with different wettability times (as expected), proving the system was suitable for our filtration experiments. Sepacell filter material was used as a control for filtrations with platelets, because it is known for its good platelet compatible properties. Experiments with Sepacell material showed that filtrations with PC in our test system are possible and give good filtration results (high flow and high platelet recovery). Filtrations with PC in the same system with untreated NW-PET resulted in low recoveries and/or blockage. Thus, using the downscaled filtration set-up with 6 layers of NW-PET,
Platelet compatibility properties of coated NW-PET can be investigated. Platelet compatibility is good when platelet recovery as well as flow rate are high, as shown by the results obtained with Sepacell material (> 90% recovery and flow > 10 ml/min).

Although the wettability and the iodine complexation test of PEO-PPO-PEO type 1-5 coating on NW-PET were promising, filtration results with PC were disappointing after gamma-sterilisation of the coated material. It might be possible that released coating material activates the platelets, resulting in blocked filters. Therefore, we tested PEO-PPO-PEO type 5 and 3 in additional control experiments in which PCs (n = 3) were incubated with these coatings for 10 minutes in concentrations higher than, equal to or lower than those maximally present during filtrations (calculated on the assumption that all coating was released in a limited volume). None of the concentrations showed any influence on platelet morphology or platelet aggregation response towards ADP (data not shown). Thus, platelet incompatibility of the coating itself is not playing a role in our experiments.

Because the coated NW-PET that were evaluated after gamma-sterilisation resulted in blocked filters, it was concluded that coatings were not stable upon gamma-radiation. This was confirmed by physical and chemical analysis of the coated material, because iodine complexation as well as wettability was much less after rinsing with PBS. Therefore, we decided to delete the gamma-sterilisation step from initial platelet compatibility tests of coated NW-PET.

According to the literature, PEO-PPO-PEO coating [67;71;113;118] should give a blood-compatible coating. However, the fibrinogen adsorption and platelet adhesion in the study of Amiji et al. [67] were tested under static conditions. We tested under flow-conditions and it is possible that due to local high shear stress, the conformation of glycoprotein-Ib (GpIb) on the platelet membrane and/or vWF changes [7]. This can induce binding of vWF to GpIb and binding of this complex (present on the platelet membrane) to available groups on the filter surface. PEO tails may not be suitable to prevent this binding by complete masking of the surface characteristics. Other unknown processes specific to the coating are possible as well. Filtrations with Sepacell material (also non-woven PET) did not show any blockage, indicating that in this filter the NW-PET is completely modified by the coating, allowing no adhesion of adhesive proteins.

Before filtration experiments, stability of the coating was tested by iodine complexation and wettability tests after rinsing the coated filter material with albumin solution or PBS. These tests gave good iodine complexation and wettability results, which both increased after coating compared to uncoated material (table 5.1). This indicates that the coating is still present on the filter surface after rinsing with albumin solution or PBS. However, this test does not seem to be representative for plasma-adhesive proteins which may specifically bind to the coating. Klomp [70] have described the importance of the amount (and conformation) of fibrinogen adsorption as an important factor for platelet incompatibility. This was not tested in our preliminary tests in which albumin was
used. It also might be possible that the hydrophobic part of the coating connected to PET is competing with plasma proteins and (partially) replaced by those. Another factor is the flow rate during filtration, something that was not tested during the albumin rinsing experiments, which were performed under almost static conditions.

The filtration results obtained with PS1 and PS2 were quite different, and table 5.5 shows that PS1 and PS2 differ most in viscosity (a molecular weight indication), whereas the percentage of non-siloxane (hydrophilicity indication) is comparable. The different filtration results of PS1 and 2 might be caused by a difference in stability of the coating, because in general a higher molecular weight and longer polymer chains result in a higher stability of the coating. For the DBE compounds, the best results were found with the highest molecular weight and the highest percentage of non-siloxanes.

The differences in recoveries between PS3 and 4, as well as the results obtained with DBE1-3 and DBP1 indicate that a high percentage of non-siloxane is important for biocompatibility of the coating. With respect to the differences between PS1 and PS4, both coatings with relatively high platelet recoveries and therefore potentially interesting, the ABA structure of PS4 compared to the AB structure of PS1 might explain the better results, because the hydrophilicity will be higher for the ABA structure. However, due to the limited number of tests with PS4, we have to be careful to launch PS4 as a promising candidate, as we learned from further experiments with PS1. Despite this precaution, PS4 is the only coating that seems worthwhile to investigate in more detail, especially with respect to stability, in more extensive tests.

CONCLUSION

The downscaled filtration system is suitable for testing new filter materials for leukocyte-depletion of platelet concentrates. Only coating PS4 might be a promising candidate for further investigations. The predictive value of wettability and iodine complexation tests is very limited and not suitable for judgements about possible platelet compatibility.

Also plasma and albumin compatibility shown in static test systems are not suitable to predict platelet compatibility in a filtration set-up. Therefore, more research has to be performed to find factors that are involved in specific platelet compatibility under these circumstances.

Acknowledgements

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