Optimising quality of platelet transfusions
Kostelijk, E.H.

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Chapter 3

Filtration of platelet concentrates using $\text{H}_2\text{O}$ or $\text{CO}_2$ glow discharge treated non-woven PET
Filtration of Platelet Concentrates Using H\textsubscript{2}O or CO\textsubscript{2} Glow Discharge Treated Non-Woven PET

A.J.A. Klomp\textsuperscript{1}, E.H. Kostelijk\textsuperscript{2}, G.H.M Engbers\textsuperscript{1}, J.G.A. Terlingen\textsuperscript{1}, D. de Korte\textsuperscript{2}, W.G. van Aken\textsuperscript{1,2} and J. Feijen\textsuperscript{1}.

\textsuperscript{1}Polymer Chemistry and Biomaterials, Department of Chemical Technology, University of Twente, Enschede, The Netherlands, \textsuperscript{2}Sanquin Blood Supply Foundation, Division CLB, Amsterdam, The Netherlands

Abstract

BACKGROUND AND OBJECTIVES: Leukocyte filters containing unmodified poly(ethylene terephthalate) non-wovens (NW-PET) are not suitable for the preparation of leukocyte-poor platelet concentrates (PCs), because of the high loss of platelets due to adhesion of platelets to the non-woven surface. Improved platelet recoveries may be obtained by surface modification of NW-PET by radio frequency glow discharge (RFGD). The improvement of the filtration characteristics of NW-PET after H\textsubscript{2}O and CO\textsubscript{2} RFGD treatment were evaluated.

MATERIALS AND METHODS: The filtration characteristics of RFGD-treated non-wovens were studied with a filtration test system for laboratory scale filters. Miniaturising the filter size allows paired studies of several filter materials with one unit of freshly prepared PC using the buffy coat method.

RESULTS: The RFGD-treated NW-PET showed improved filtration characteristics compared with NW-PET (63\%). Filters containing NW-PET-H\textsubscript{2}O showed significantly improved filtration characteristics (83\%) compared with NW-PET filters. Moreover, NW-PET-H\textsubscript{2}O filters gave a significantly higher platelet recovery than NW-PET-CO\textsubscript{2} filters (76\%) (p < 0.0005).

CONCLUSIONS: The results of this study show that H\textsubscript{2}O RFGD treatment of NW-PET is an excellent method to obtain platelet-compatible non-wovens that can be used in leukocyte filters for PC.
INTRODUCTION

It has been demonstrated that leukocyte filters with polyester non-wovens, which are used for the removal of leukocytes from red cell concentrates, are not suitable for the preparation of leukocyte-poor platelet concentrates (PCs), because of the high loss of platelets [93]. Recently, it was shown that H$_2$O and CO$_2$ radio frequency glow discharge (RFGD) treatment of non-woven poly(ethylene terephthalate) (NW-PET) yield materials (NW-PET-H$_2$O and NW-PET-CO$_2$, respectively) that showed less interactions with platelets than did NW-PET during filtration of PC [94]. Air RFGD-treated NW-PET also gave a reduction in the interaction with platelets as compared with NW-PET. However, after prolonged RFGD treatment, shrinkage of the materials took place, preventing their application in filters.

Only H$_2$O-RFGD treatment of NW-PET yielded materials that significantly improved the platelet recovery. Differences in platelet recoveries between NW-PET-H$_2$O and NW-PET-CO$_2$ were rather small and are probably caused by differences in the chemical composition of the surfaces. For instance, NW-PET-CO$_2$ contained a significantly higher surface concentration of carboxylic acid groups than did NW-PET-H$_2$O [95].

In an earlier study [94], NW-PET was treated under various RFGD conditions and the most promising materials NW-PET-H$_2$O and NW-PET-CO$_2$ with respect to platelet compatibility were selected for further evaluation. In this article, the filtration characteristics of NW-PET-H$_2$O and NW-PET-CO$_2$ were studied in detail with a PC filtration test system with a higher non-woven surface area to PC volume ratio as used before. This will also increase the filter capacity for leukocyte removal. To obtain a maximal difference in platelet recovery between the two test filters, the PC load per test filter has to be preferably as low as possible. However, the PC load per test filter should not be too low, because otherwise the percentage of the PC lost in the dead volume of the filter becomes too large, which negatively influences the overall platelet recovery. The PC load per test filter should not be too high either, because in that case the test filters can be overloaded with leukocytes, causing a large decrease in the leukocyte retention. For the modified test system, a maximal PC loss of 8% in the dead volume of the filter was accepted, together with a minimal fraction of retained leukocytes of 90%. Since water rinsing of the RFGD-treated non-wovens significantly changed the surface composition of both CO$_2$ and H$_2$O RFGD-treated non-woven, resulting in a decreased number of carboxylic acid groups at the surface of both non-wovens [95], the effect of water rinsing on the filtration characteristics of CO$_2$ and H$_2$O non-woven was also studied in detail. For comparison, a non-woven from a commercial leukocyte filter for PC was included throughout the whole study.
EXPERIMENTAL SECTION

Filter materials
Polylethene terephthalate non-woven (abbreviated with NW-PET) was obtained from NPBI International BV (Emmer-Compascuum, The Netherlands). The control material was obtained from the Sepacell PL10(II)A filter (Asahi Medical Co., Tokyo, Japan), which contains 33 layers non-woven divided into four sections. Going from the inlet side to the outlet side of the filter, the structure of the non-woven changes from coarse (section I) to fine (section IV). All sections contain 4 layers, except for section four, which contains 21 layers. Only fabrics from section IV (abbreviated as NW-PET-S,IV) were used as control material in the biological evaluation. The physical characteristics of the non-wovens were determined according to the methods described elsewhere [96], summarised in Table 3.1.

Table 3.1: Physical characteristics of NW-PET and NW-PET-S,IV (mean ± SD; n≥3)

<table>
<thead>
<tr>
<th>Non-woven</th>
<th>porosity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>BET surface area&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Average fibre diameter&lt;sup&gt;c&lt;/sup&gt;</th>
<th>MFP&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MFP (%)</td>
<td>(m&lt;sup&gt;2&lt;/sup&gt;/gram)</td>
<td>(µm)</td>
<td>(µm)</td>
</tr>
<tr>
<td>NW-PET</td>
<td>84 ± 1.2</td>
<td>0.74 ± 0.19</td>
<td>2.6 ± 0.2</td>
<td>8 ± 1.1</td>
</tr>
<tr>
<td>NW-PET-S,IV</td>
<td>88 ± 0.9</td>
<td>0.83 ± 0.15</td>
<td>1.7 ± 0.2</td>
<td>8 ± 1.1</td>
</tr>
</tbody>
</table>

<sup>a</sup>the porosity of the non-wovens was determined from the weight and volume of dry samples, in which the mass of air was neglected and using a specific mass of polyether of 1.38 g/cm<sup>3</sup>. <sup>b</sup>the BET surface area was determined by gas adsorption measurements on a Micromeritics ASAP2400; <sup>c</sup>the average fibre diameter was determined from the diameters of 200 to 300 fibres using Scanning Electron Microscopy (SEM); <sup>d</sup>the Mean Flow Pore size (MFP) of non-wovens was measured using a Coulter® Porometer (type I).

Chemicals
Porofil, Leukoplate (plaxan) and phosphate-buffered saline (PBS, pH = 7.4) were purchased from Coulter Electronics GMBH (Krefeld, Germany), Labo International (Maarssen, The Netherlands) and NPBI, respectively. Air (purity > 99.5%) and CO<sub>2</sub> (purity > 99.995%) were purchased from Hoekloos (Schiedam, The Netherlands). Water was deionised with a Milli-Q-plus system (Millipore, Molsheim, France). All other chemicals were purchased from Merck (Darmstadt, Germany).
**RFGD treatment of NW-PET**

RFGD treatments of NW-PET were performed in a tubular reactor with a length of 80 cm and an internal diameter of 6.5 cm equipped with three externally placed, capacitively coupled electrodes. The powered (hot) electrode was placed at the centre of the tubular reactor. On both sides of the powered electrode, a grounded (cold) electrode was placed at a distance of 13 cm from the powered electrode. The electrodes were connected to a 13.56 MHz radio frequency generator (ENI Power Systems, Rochester, New York, USA) through a matching network (ENI Power Systems) [97].

NW-PET (71.5 cm²) was mounted in a glass frame, exposing 63 cm² of the non-woven to the gas plasma. Two glass frames were loaded in the reactor, each placed in the middle between a grounded electrode and the powered electrode. After evacuating the reactor to a pressure below 0.01 mbar, a gas flow of 25 cm³/min for CO₂ and a gas flow of approximately 2.5 ± 0.5 cm³/min for H₂O was established. The pressure was raised to 0.32 mbar. The RFGD treatment was computer-controlled. After 5 minutes, a pulsatile glow discharge (30 pulses, 1 second on, 1 second off) was applied with an input power of 50 watt. After the pulsatile glow discharge treatment, the gas flow was maintained for two minutes and the reactor was brought to atmospheric pressure with air. The H₂O and CO₂ RFGD-treated NW-PET (abbreviated by NW-PET-H₂O and NW-PET-CO₂, respectively) were removed from the reactor and stored in storage containers at 4°C to prevent surface rearrangements, until further use [97].

**Rinsing of RFGD-treated non-woven with water**

NW-PET, NW-PET-H₂O and NW-PET-CO₂ were cut into discs with a diameter of 26 mm. The discs were washed in water (50 ml) on a Vibrex VX R flatbed shaker (1 cycle/s, Labortechnik, Staufen, Germany) at room temperature (RT). After one hour, the discs were rinsed once with fresh water (25 ml, for 30 seconds). All samples were dried overnight in vacuo. All water-washed samples are marked with the superscript: (*).

**Preparation of pooled BC-PC**

Units of whole blood (500 ml) in the Biopack Composflex triple systems (NPBI) were centrifuged (Hettich Roto Silenta RP, Dépex, De Bilt, The Netherlands) for 15 minutes at 4000 x g, break 3, 20°C. The units were separated into components (plasma and red cells) with the Optipress (Baxter Fenwall, Lessines Belgium) leaving a buffy coat (BC) of approximately 65 ml in the collection bag (500 ml). 5 BCs (65 ± 5 ml, Ht: 49 ± 3 %) were stored overnight on a horizontal flatbed shaker (1 cycle/s, Helmer labs inc., Noblesville, IN, USA) at 20 ± 2°C. Subsequently, the BCs and one unit of thawed, fresh frozen human plasma (318 ± 16 ml, with CPD of the same ABO blood group) were pooled at RT. The
pool of BCs and plasma was centrifuged for 4 minutes at 1,507 x g, break 3, 20°C. BC-PC was transferred into a 300-ml transfer bag (NPBI) by means of a plasma extractor (Baxter Fenwall) until the red cells were approximately 1 cm from the top seal. The pooled BC-PC was stored at 20 ± 2°C on a flat bed shaker (1 cycle/s, Helmer labs inc.) until the filtration experiments were carried out.

**Characterisation of pooled BC-PC**

Platelet numbers in BC-PC were determined with a Sysmex K-4500 (Charles Goffin Medical Systems, Tiel, The Netherlands). Leukocyte numbers were determined by light microscopy (Olympus, Tokyo, Japan) with a Nageotte haemocytometer (Superior, Bad Mergentheim, Germany). For the Nageotte haemocytometer measurement, 50 μl of BC-PC were diluted with 450 μl of Leukoplate. Thereafter, the samples were incubated for 10 minutes at 22 ± 2°C. After filling the Nageotte chamber, the Nageotte haemocytometer was placed for 20 minutes in a humidified atmosphere and leukocyte numbers were counted in one compartment of 50 μl at a magnification of 250.

**Filtration procedure**

Home made filter holders (University of Twente, The Netherlands) with a flow area of 5.3 cm² were used for the filtration of PC. The filter holders contained 6 discs (unless mentioned otherwise) of NW-PET, NW-PET-CO₂, NW-PET-H₂O or NW-PET-S,IV. Filtration experiments with PC were performed according to the method described by Klomp [96].

PC (50 ml, unless mentioned otherwise) was transferred into a 50-ml Combitip (Eppendorf, Hamburg, Germany). The filtrate was collected in a 50-ml sampling tube (Greiner Labortechnik, Alphen a/d Rijn, The Netherlands). The distance between the reservoir and the filter outlet was set at 29 cm and the flow rate was controlled by gravity. Starting from the moment that PC had stopped entering the filter, a leak-out time of 2 minutes was taken into account for all filters. The test filter was considered to be blocked when the flow rate of filtered PC dropped below 2 ml/min. Unless mentioned otherwise, the filtered PC was collected in three subsequent fractions of about 10, 15 and 25 ml.

The filters were evaluated with respect to the hold-up volume (dead volume) of the filter, the platelet recovery and the leukocyte retention. Volumes of BC-PCs were calculated by dividing the net weight of BC-PC sample by the specific density of plasma (1.026 g/cm³). For calculating the leukocyte retention, the platelet recovery and the surface concentration of adhered platelets, the fraction of recovered platelets and the fraction of retained leukocytes, the following formulas were used:
1. Leukocyte retention (%) = \(1 - \frac{L \times V}{L_0 \times V_0}\) x 100% = total number of leukocytes after filtration / total number of leukocytes before filtration

2. Platelet rec. (%) = \(\frac{T \times V}{T_0 \times V_0}\) x 100% = total number of platelets after filtration / total number of platelets before filtration

3. Surface conc. of adhered platelets = \(\frac{(T_0 - T) \times V}{A}\) = number of adhered platelets / total surface area

4. Fraction of recovered platelets = \(\frac{T}{T_0}\)

5. Fraction of recovered leukocytes = \(\{1 - \frac{L}{L_0}\}\)

\(L\) = 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; \(V\) = PC-volume after filtration; \(V_0\) = PC-volume before filtration; \(A\) = total filter surface area (cm\(^2\)).
Statistical evaluation

Unless indicated otherwise, all values are expressed as the mean ± standard deviation (SD). Statistical analyses were performed using the Student t-test (t-test, two tailed; paired or unpaired, as appropriate) or Wilcoxon's signed rank test (WSR-test, one-tailed) [98;99].

In case of the Student t-test, the differences between several non-wovens with respect to surface composition and the differences between several filters with respect to flow rate and platelet recovery were considered significant in case the p-value was lower than 0.05. The Student t-test was performed with the computer programme InStat 2.03 (GraphPad Software Inc., San Diego, California, USA).

In case of the WSR-test, the differences between several filters with respect to flow rate, platelet recovery and leukocyte retention were considered significant when the p-value of the overall zero-hypothesis was lower than 0.10. In accordance with the Bonferroni method, the p-value has been corrected for multiple comparisons. When comparing differences between two filters, only the data of paired filtration experiments were taken into account. When the difference between two filters was found significant, both the p-value and the number of paired filtration experiments are given. The WSR-test was performed with a computer programme (SPSS/for HP-UX, Chicago, USA).

RESULTS

Improvement of test system

To improve the sensitivity of the earlier developed test system [96], the effect of the filter surface area on the filtration characteristics was studied by comparing filters with 2, 4 and 6 non-woven layers. The non-woven surface area per filter was in between 873 and 2620 cm$^2$ for NW-PET and NW-PET-H$_2$O, and in between 772 and 2316 cm$^2$ for NW-PET-S$_4$IV.

The data in figure 3.1 show that the mean flow rate of PC was lower with NW-PET than with NW-PET-H$_2$O or NW-PET-S$_4$IV. Furthermore, the mean flow rate decreased with increasing surface area of non-woven for all materials.

The leukocyte retention increased with increasing surface area of non-woven in the filter. However, the increase in leukocyte removal was not linear. The difference in the leukocyte retention between NW-PET-H$_2$O and NW-PET-S$_4$IV was small (2-6%) and decreased with increasing surface area of non-woven in the filter. The leukocyte retention was above 92% for all filters with six non-woven layers.

The platelet recovery decreased with increasing surface area of non-woven. For filters with two layers of non-woven the difference in platelet recovery between NW-PET and surface- treated non-wovens was about 5%. When using filters with six layers of non-woven, the difference in platelet recovery between NW-PET and
NW-PET-H2O increased to about 43%, while the difference between NW-PET-H2O or NW-PET-S,IV remained about 2%.

![Diagram](image_url)

**Fig 3.1** - Mean flow rate, platelet recovery and leukocyte retention of NW-PET, NW-PET-H2O and NW-PET-S,IV as a function of the surface area of non-woven. The filters were evaluated with a pooled BC-PC (1.2 x 10^9 platelets/ml and 6.0 x 10^4 leukocytes/ml, n=2).

The number of platelets adhering to the non-woven surface can be estimated by subtracting the total number of recovered platelets and the total number of platelets in the dead volume from the total number of platelets in the initial platelet concentrate. The platelets in the dead volume were considered not to adhere to the non-woven surface.

The data in figure 3.1 show an increasing surface concentration of adhered platelets with an increasing surface area of NW-PET. For both NW-PET-H2O and NW-PET-S,IV a small decrease of the surface concentration of adhered platelets with increasing surface area of the filter is observed, while for NW-PET a large
increase of surface concentration of adhered platelets with increasing filter surface area is noted (figure 3.2).

![Graph showing surface concentration of adhered platelets for NW-PET, NW-PET-H2O and NW-PET-S,IV as a function of the non-woven surface area of the filter. The filters were evaluated with a pooled BC-PC (1.2 x 10^9 platelets/ml and 6.0 x 10^4 leukocytes/ml, n=2).](image)

**Fig. 3.2** - Surface concentration of adhered platelets for NW-PET, NW-PET-H2O and NW-PET-S,IV as a function of the non-woven surface area of the filter. The filters were evaluated with a pooled BC-PC (1.2 x 10^9 platelets/ml and 6.0 x 10^4 leukocytes/ml, n=2).
Fig 3.3 - The fraction of recovered platelets (figure A) and the fraction of retained leukocytes (figure B) for NW-PET-H$_2$O as a function of the filtered PC-volume (total volume is 150 ml). The filters were evaluated with pooled BC-PC (1.3 ± 0.25 x 10$^9$ platelets/ml, n=4) with different leukocyte concentrations.
To determine the optimal BC-PC load per filter, filters with six layers NW-PET-H$_2$O and NW-PET-S,IV were used for filtration of 150 ml pooled BC-PC (figures 3.3 and 3.4). The results of this experiment show that the fraction of recovered platelets of both filters levelled off to about 97% with increasing filtered PC volume. However, after filtration of about 50 ml PC, the fraction of retained leukocytes for both filters decreased substantially.

Based on these results, filters with 6 layers of non-wovens and a BC-PC volume of 50 ml were used for further testing of the filtration characteristics of PET non-wovens. With the modified test system, the filtration characteristics of NW-PET, NW-PET-CO$_2$, NW-PET-H$_2$O and NW-PET-S,IV were extensively tested.

*Flow characteristics for filters with PET non-woven*

Table 3.2 shows the results of the filtration characteristics of NW-PET, NW-PET-CO$_2$, NW-PET-H$_2$O and NW-PET-S,IV. Blocking of NW-PET and NW-PET-CO$_2$ filters occurred in 24% and 5%, respectively, of the cases. None of the NW-PET-H$_2$O and NW-PET-S,IV filters were blocked. The results of the blocked filters were left out for calculation of the overall filtration data of the filters.

The mean flow rate for NW-PET (9 ml/min) was significantly lower than for NW-PET-CO$_2$ (12 ml/min, WSR-test, $p = 0.0002$, $n = 17$), NW-PET-H$_2$O (15 ml/min, WSR-test, $p < 0.0001$, $n = 23$) and NW-PET-S,IV (21 ml/min, WSR-test, $p < 0.0001$, $n = 17$). The mean flow rate for NW-PET-S,IV was significantly higher than for NW-PET-CO$_2$ (WSR-test, $p = 0.0079$, $n = 13$) and NW-PET-H$_2$O (WSR-test, $p = 0.0002$, $n = 29$). The mean flow rate for NW-PET-H$_2$O did not significantly differ from the mean flow rate for NW-PET-CO$_2$ (WSR-test, $p = 0.0294$, $n = 18$).
Fig. 3.4 – The fraction of recovered platelets (figure A) and the fraction of retained leukocytes (figure B) for NW-PET-H$_2$O as a function of the filtered PC-volume (total volume is 150 ml). The filters were evaluated with a pooled BC-PC (1.3 ± 0.25 x 10$^9$ platelets/ml and 25.0 ± 17.3 x 10$^4$ leukocytes/ml, n = 4).

**Table 3.2: Number of blocked filters, the mean flow rate, loss of BC-PC in the dead volume of the filter and the platelet recovery of NW-PET, NW-PET-CO$_2$, NW-PET-H$_2$O and NW-PET-S,IV.** The filtration experiments were performed using pooled BC-PC containing approximately 1.1 ± 0.16 x 10$^9$ platelets/ml

<table>
<thead>
<tr>
<th>Filter</th>
<th>number of experiments</th>
<th>number of blocked filters</th>
<th>mean flow rate (ml/min)</th>
<th>loss of BC-PC (%)</th>
<th>platelet$^1$ recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NW-PET</td>
<td>34</td>
<td>8</td>
<td>9 ± 3.8</td>
<td>8 ± 2.1</td>
<td>63 ± 7.9</td>
</tr>
<tr>
<td>NW-PET-CO$_2$</td>
<td>20</td>
<td>1</td>
<td>12 ± 4.8</td>
<td>6 ± 1.2</td>
<td>76 ± 10.2</td>
</tr>
<tr>
<td>NW-PET-H$_2$O</td>
<td>38</td>
<td>0</td>
<td>15 ± 4.0</td>
<td>5 ± 1.5</td>
<td>83 ± 5.4</td>
</tr>
<tr>
<td>NW-PET-S,IV</td>
<td>32</td>
<td>0</td>
<td>21 ± 6.6</td>
<td>5 ± 2.1</td>
<td>88 ± 5.4</td>
</tr>
</tbody>
</table>

$^1$Platelet recovery (%) = (T x V / (T$_o$ x V$_o$)) x 100 %.
Platelet recovery for filters with PET non-woven

No significant difference in the percentage of BC-PC loss (5-8%) in the dead volume of the filter was found between any of the filters.

The platelet recovery for NW-PET (63%) and NW-PET-CO₂ (76%) was significantly lower than for NW-PET-H₂O (83%, WSR-test, p < 0.0001 (n = 23) and p = 0.0002 (n = 18), respectively) and NW-PET-S,IV (88%, WSR-test, p < 0.0001 (n = 18) and p = 0.0005 (n = 13), respectively). The platelet recovery for NW-PET-CO₂ was significantly higher than for NW-PET (WSR-test, p < 0.0001, n = 17). In addition, the platelet recovery for NW-PET-H₂O was significantly lower than for NW-PET-S,IV (WSR-test, p < 0.0001, n = 30).

Figure 3.5 shows the platelet recovery for the different non-wovens as a function of the platelet concentration before filtration. The platelet recovery for NW-PET-H₂O and NW-PET-S,IV was not influenced by the platelet concentration of BC-PC before filtration. However, the platelet recovery for NW-PET slightly decreased when the platelet concentration of BC-PC decreased.

![Graphs showing platelet recovery as a function of platelet concentration (x10⁹ per ml) before filtration.]

Fig. 3.5 - The platelet recovery for NW-PET, NW-PET-CO₂, NW-PET-H₂O and NW-PET-S,IV as a function of the platelet concentration before filtration. The non-wovens were evaluated with a pooled BC-PC.
The fraction of recovered platelets (T/To) and the surface concentration of adhered platelets were determined as a function of the collected BC-PC volume. The results of the experiments are shown in figure 3.6.

Figure 3.6A shows that the fraction of recovered platelets for NW-PET-S,IV increased with increasing BC-PC load from 82% after 10 ml of BC-PC to 92% after 47 ml of BC-PC. After filtration of 10 ml of BC-PC, the fraction of recovered platelets for NW-PET-H_2O was about 17% lower than for NW-PET-S,IV, but after 47 ml, the difference between NW-PET-H_2O and NW-PET-S,IV with respect to the fraction of recovered platelets had decreased to about 4%.

With increasing volume of filtered PC, the surface concentration of adhered platelets increased for all non-wovens. For NW-PET this increase was larger than for the surface-treated non-wovens. The difference in surface concentration of adhered platelets between NW-PET and NW-PET-CO_2 was small after filtration of about 10 ml BC-PC, but became larger after filtration of more BC-PC.

Fig. 3.6 - The fraction of recovered platelets (figure A, T/To) and the concentration of platelets per surface area non-woven (figure B) as a function of the collected PC volume. The non-wovens were evaluated using a pooled BC-PC (1.1 ± 0.16 x 10^9 platelets/ml, n ≥ 8, ± SD).
After collecting 47 ml PC, the surface concentration of adhered platelets for NW-PET-H$_2$O (2.4 x10$^6$ platelets/cm$^2$) was lower than for NW-PET (6.7 x10$^6$ platelets/cm$^2$) and NW-PET-CO$_2$ (4.4 x10$^6$ platelets/cm$^2$). Moreover, the surface concentration of adhered platelets for NW-PET-H$_2$O was lower than for NW-PET-S,IV (3.2 x10$^6$ platelets/cm$^2$).

**Leukocyte retention for filters with PET non-woven**

The leukocyte concentration in the pooled BC-PC was about 16.5 ± 11.92 x 10$^4$ leukocytes per ml (n = 18). The filters removed between 86.2 and 95% of the leukocytes (table 3.3). Using NW-PET-H$_2$O (93%) significantly more leukocytes were removed than with NW-PET (88%, WSR-test, p = 0.0004, n = 13). The leukocyte retention for NW-PET-S,IV (95%) was significantly higher than for NW-PET (WSR-test, p = 0.0001, n = 10) and NW-PET-H$_2$O (WSR-test, p = 0.013, n = 15). Other differences in leukocyte retention were not significant.

**Table 3.3: Leukocyte concentration in pooled BC-PC before and after filtration and the leukocyte retention for NW-PET, NW-PET-CO$_2$, NW-PET-H$_2$O and NW-PET-S,IV**

<table>
<thead>
<tr>
<th>Filter</th>
<th>number of experiments</th>
<th>number of blocked filters</th>
<th>leukocyte concentration before filtration (x10$^4$ ml$^{-1}$)</th>
<th>leukocyte concentration after filtration (x10$^4$ ml$^{-1}$)</th>
<th>leukocyte$^1$ retention (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NW-PET</td>
<td>18</td>
<td>5</td>
<td>16.5 ± 11.92</td>
<td>2.2 ± 2.33</td>
<td>88 ± 7.2</td>
</tr>
<tr>
<td>NW-PET-CO$_2$</td>
<td>10</td>
<td>1</td>
<td>13.8 ± 13.49</td>
<td>2.6 ± 3.04</td>
<td>86 ± 9.8</td>
</tr>
<tr>
<td>NW-PET-H$_2$O</td>
<td>18</td>
<td>0</td>
<td>16.5 ± 11.92</td>
<td>1.5 ± 1.97</td>
<td>93 ± 5.4</td>
</tr>
<tr>
<td>NW-PET-S,IV</td>
<td>15</td>
<td>0</td>
<td>17.0 ± 12.56</td>
<td>1.2 ± 1.68</td>
<td>95 ± 5.1</td>
</tr>
</tbody>
</table>

$^1$Leukocyte retention (%) = (1 - total number of leukocytes after filtration/total number of leukocytes before filtration) x 100.
Fig. 3.7 - The leukocyte concentration in BC-PC after filtration as a function of the leukocyte concentration in PC before filtration. The filters NW-PET, NW-PET-CO$_2$, NW-PET-H$_2$O and NW-PET-S,I,V were evaluated using a pooled BC-PC.

Figure 3.7 shows the leukocyte concentration of BC-PC after filtration using NW-PET, NW-PET-CO$_2$, NW-PET-H$_2$O and NW-PET-S,I,V as a function of the leukocyte concentration before filtration. When filtering BC-PCs with less than 20 x10$^4$ leukocytes/ml, both NW-PET, NW-PET-CO$_2$ and NW-PET-H$_2$O gave a residual leukocyte concentration of < 1.5 x 10$^4$ leukocytes per ml, while NW-PET-S,I,V gave a residual leukocyte concentration of < 0.5 x 10$^4$ leukocytes per ml. However, when filtering BC-PCs with more than 20 x10$^4$ leukocytes/ml, the leukocyte concentration of all filters increased rapidly.

**Stability of RFGD-treated non-wovens: the effect of rinsing**

The effect of water rinsing and subsequent overnight drying *in vacuo* of NW-PET, NW-PET-CO$_2$ and NW-PET-H$_2$O on the filtration characteristics was investigated. The filtration data are shown in table 3.4.

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Table 3.4: Number of blocked filters, the mean flow rate, loss of BC-PC in dead volume of the filter, and the platelet recovery for NW-PET, NW-PET-CO₂, and NW-PET-H₂O, before and after rinsing with water. The index (w) indicates that the non-wovens were rinsed with water and subsequently dried to the filtration experiments. The filters were evaluated pairwise using pooled BC-PC \( (1.1 \pm 0.16 \times 10^9 \text{ platelets/ml}) \)

<table>
<thead>
<tr>
<th>Filter</th>
<th>number of experiment s</th>
<th>number of blocked filters</th>
<th>mean flow rate (ml/min)</th>
<th>loss of BC-PC (%)</th>
<th>platelet recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NW-PET</td>
<td>6</td>
<td>0</td>
<td>8 ± 0.8</td>
<td>7 ± 1.6</td>
<td>60 ± 7.2</td>
</tr>
<tr>
<td>NW-PET(^w)</td>
<td>6</td>
<td>0</td>
<td>9 ± 1.9</td>
<td>8 ± 1.2</td>
<td>63 ± 4.6</td>
</tr>
<tr>
<td>NW-PET-CO₂</td>
<td>6</td>
<td>0</td>
<td>11 ± 2.7</td>
<td>6 ± 1.8</td>
<td>75 ± 8.3</td>
</tr>
<tr>
<td>NW-PET-CO₂(^w)</td>
<td>6</td>
<td>0</td>
<td>12 ± 2.3</td>
<td>7 ± 1.9</td>
<td>72 ± 11.3</td>
</tr>
<tr>
<td>NW-PET-H₂O</td>
<td>6</td>
<td>0</td>
<td>14 ± 2.6</td>
<td>6 ± 1.9</td>
<td>79 ± 6.0</td>
</tr>
<tr>
<td>NW-PET-H₂O(^w)</td>
<td>6</td>
<td>0</td>
<td>17 ± 3.8</td>
<td>6 ± 1.9</td>
<td>81 ± 3.7</td>
</tr>
</tbody>
</table>

\(^w\)washed with water and subsequently dried overnight in vacuo prior to filtration

Table 3.4 shows that water rinsing and drying did not significantly change the mean flow rate of BC-PC during filtration. The mean flow rate for NW-PET\(^w\) (9 ml/min) was significantly lower than for NW-PET-CO₂\(^w\) (13 ml/min, t-test, \( p = 0.0052 \)) and NW-PET-H₂O\(^w\) (17 ml/min, t-test, \( p = 0.0049 \)). Moreover, the mean flow rate for NW-PET-CO₂\(^w\) was lower (not significantly) than for NW-PET-H₂O\(^w\).

The loss of PC in the dead volume of the filters for water-rinsed non-wovens was the same as for non-water-rinsed non-wovens (6-8%). Water rinsing and overnight drying in vacuo of non-wovens prior to filtration did not significantly change the platelet recovery of the filters. The platelet recoveries for NW-PET-H₂O\(^w\) (81%) and NW-PET-CO₂\(^w\) were significantly higher than the platelet recovery for NW-PET\(^w\) (63%, t-test, \( p < 0.0001 \) and \( p = 0.0214 \), respectively). Moreover, the platelet recovery for NW-PET-H₂O\(^w\) was higher (not significant) than for NW-PET-CO₂\(^w\) (72%, t-test, \( p = 0.0655 \)).

The results in figure 3.8 show that during filtration of the first 10 ml of BC-PC more platelets were recovered by NW-PET-H₂O\(^w\) than by NW-PET\(^w\) and NW-PET-CO₂\(^w\). Thereafter, NW-PET-CO₂\(^w\) recovered more platelets than NW-PET\(^w\).
After collecting 47 of ml BC-PC, the surface concentration of adhered platelets for NW-PET-H$_2$O$^W$ ($2.3 \times 10^6$ platelets/cm$^2$) was lower than for NW-PET$^W$ ($6.1 \times 10^6$ platelets/cm$^2$) and NW-PET-CO$_2$W$^W$ ($4.0 \times 10^6$ platelets/cm$^2$).

![Graph A](image1)

**Fig. 3.8** - The fraction of recovered platelets (A, $T/T_0$) and the surface concentration of adhered platelets (B) as a function of the collected PC volume. Prior to filtration, the non-wovens were rinsed with water and subsequently dried overnight in vacuo. The filtration experiments were performed with pooled BC-PC containing $1.0 \pm 0.15 \times 10^9$ platelets/ml ($n \geq 6$, ± SD).

**DISCUSSION**

The described test system (see also Klomp et al [96]) was optimised to enable a detailed evaluation of the filtration characteristics of surface modified non-wovens. In the optimised test system the ratio of non-woven surface area and BC-PC volume was increased with respect to the system used earlier [96]. The filtration experiments were performed with six layers of non-woven per filter and a BC-PC load of 50 ml, which corresponds to 9.4 ml BC-PC per cm$^2$ flow area. When using more than 50 ml BC-PC, the capacity for leukocyte removal of NW-PET-H$_2$O and NW-PET-S,IV filters was too low.
With the use of the new test system settings, a maximal loss of BC-PC in the dead volume of the filter of 6% and a minimal fraction of retained leukocytes of 93% were obtained. Platelet recovery varied between 39% for NW-PET and 84% for NW-PET-S,IV (figures 3.1 and 3.3). Compared to the 10% difference in platelet recovery between NW-PET and NW-PET-S,IV for the test system reported by Klomp [96], the new system enables a better differentiation between the characteristics of different filter materials.

Also with this new setting it was shown that RFGD treatment of NW-PET with water vapour or carbon dioxide led to a significant improvement of the filtration characteristics. Besides a significantly higher mean flow rate, H_{2}O RFGD treatment of NW-PET resulted in non-wovens giving a significantly higher leukocyte retention and platelet recovery as well. CO_{2} RFGD treatment of NW-PET significantly improved the mean flow rate and platelet recovery only. Moreover, the improvement of the platelet recovery by a CO_{2} RFGD treatment of NW-PET was significantly less than the improvement of the platelet recovery by an H_{2}O RFGD treatment of NW-PET (tables 3.2 and 3.3).

The highest loss of platelets occurred at the beginning of the filtration. All filters showed a relatively high percentage of retained platelets in the first fraction of about 10 ml of filtered BC-PC (figure 3.4). Elias-Elias [100] also observed the same effect for the commercial leukocyte filter Pall PL100.

Remarkable is the difference between H_{2}O and CO_{2} RFGD treatment of NW-PET with respect to platelet adhesion. The surface concentration of adhered platelets for NW-PET-CO_{2} during the first stage of the filtration was comparable to that of NW-PET. Only during the following stages of the filtration of BC-PC, NW-PET-CO_{2} exhibited a better platelet compatibility than NW-PET. NW-PET-H_{2}O showed a better compatibility throughout the whole filtration procedure.

This difference in platelet compatibility can be explained by differences in surface chemistry between NW-PET-CO_{2} and NW-PET-H_{2}O. RFGD treatment of NW-PET with water vapour leads to a lower surface concentration of oxygen-containing functional groups than does CO_{2} RFGD treatment [95]. This difference in degree of surface oxidation is most likely caused by differences in oxygen concentration available for oxidation during RFGD treatment and results in a large difference in surface concentration of carboxylic acid groups between NW-PET-H_{2}O and NW-PET-CO_{2}.

Probably, NW-PET-CO_{2} contains active sites that (indirectly) stimulate platelet adhesion. During filtration of BC-PC, proteins and platelets cover the active sites at NW-PET-CO_{2}. Thereafter, further adherence of platelets is diminished. Most likely, NW-PET-H_{2}O contains a lower surface concentration of active sites than NW-PET-CO_{2}. Consequently, NW-PET-H_{2}O shows directly from the start of the filtration of BC-PC resistance to platelet adhesion.

In spite of the 1.4 times higher surface concentration of adhered platelets measured for NW-PET-S,IV, a significantly higher platelet recovery was obtained with this material than with NW-PET-H_{2}O. The surface concentration of adhered
platelets remained constant when increasing the surface area of NW-PET-SJV (figure 3.2), but decreased slightly with increasing surface area of NW-PET-H$_2$O. Most likely, platelets are preferably removed in the first layers of filters with NW-PET-H$_2$O. This was also observed for filters with two layers of NW-PET-H$_2$O of which the top layer showed more adhered platelets than the bottom layer [94]. Probably, the resistance to platelet adhesion is mediated by plasma protein adsorption.

NW-PET-SJV showed a significantly higher leukocyte retention than NW-PET. This can be explained by differences in the physical characteristics of the filter, such as porosity, fibre diameter distribution and pore size distribution [96]. The leukocyte retention of NW-PET was improved by H$_2$O RFGD treatment. This might be caused by the improved accessibility of the non-woven for leukocytes (less platelets adhere to the non-woven surface) or by improved leukocyte adhesion (granulocytes).

Finally, rinsing of NW-PET and RFGD-treated non-wovens with water and drying in vacuo prior to the filtration of BC-PC did not change the filtration characteristics. Moreover, the remarkable difference in platelet adhesiveness between NW-PET-CO$_2$ and NW-PET-H$_2$O remained present (figure 3.8).

CONCLUSIONS

It is concluded that the test system developed can be used to better discriminate between surface-treated non-wovens with respect to their interaction with platelets. H$_2$O RFGD-treated NW-PET showed a better platelet recovery during filtration of BC-PC than CO$_2$ RFGD-treated NW-PET. Although rinsing with water did have an effect on the chemical composition of the surfaces, the filtration characteristics of these materials for BC-PC did not differ with respect to non-washed RFGD-treated non-wovens.

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