Transfusion Medicine, 2001, **11**, 199–205

Improved platelet compatiblity of water vapour glow discharge treated non-woven poly(ethylene terephthalate) leukocyte-reduction filters for different types of platelet concentrates

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Received 31 August 2000; accepted for publication 23 November 2000

SUMMARY. Non-woven poly[ethylene terephthalate] (NW-PET) filter fabric, usually used for leucocyte removal of red cells, was modified by water vapour glow discharge (WVGD) treatment to improve platelet compatibility.

Modified filter material was evaluated with different kinds of platelet concentrates (PCs). In addition, modified filter materials were γ-sterilized and tested after different time intervals at different storage conditions.

Modification of the filter material resulted in an improved platelet recovery after filtration of PC from 57 to about 80%. No significant difference in platelet recovery was observed when filtering either freshly prepared (79 ± 3·5%, mean ± SD), overnight-stored single BC-PC (78 ± 3·3%), overnight-stored single PRP-PC (75 ± 8·8%) or overnight-stored pooled BC-PC (79 ± 8·9%). However, freshly prepared pooled BC-PC gave a significantly higher platelet recovery (84 ± 3·5%). Leukocyte depletion did not differ significantly between the different types of PC. γ-Sterilization and subsequent storage of the modified filter material for 5, 14 and 26 weeks at 20 °C or 37 °C had no significant influence on the filtration results of overnight-stored pooled BC-PC.

The results of the present study show that WVGD-treated NW-PET is platelet compatible and can be used for leucocyte removal from preferably BC-PC. It can be γ-sterilized and stored for at least 6 months prior to filtration without affecting the platelet recovery and leucocyte removal.

Keywords: leukocyte filtration, platelet concentrates.

Transfused platelet concentrates (PCs) containing leucocytes may cause adverse reactions such as HLA alloimmunization and (non)febrile transfusion reactions (Andreu *et al*., 1988). These reactions can be minimized by removal of leucocytes through filtration of PCs with leucocyte reduction filters (The Trial to Reduce Alloimmunization to Platelets Study Group, 1997; Claas *et al*., 1981; van Marwijk Kooy *et al*., 1991; Williamson *et al*., 1994). Common whole blood or red cell concentrate (RCC) filters are not suitable for PC, because platelets become activated, resulting in low platelet recoveries. Because of easy processing properties and cost aspects, the non-woven polyethylene therephthalate (NW-PET), frequently used for RCC filters, was selected for the development of a PC-filter. Water vapour glow discharge (WVGD) treatment of NW-PET has been shown to render the material more platelet-compatible (Klomp, 1998). During this process, hydrophilic groups like hydroxil, epoxide, keton/aldehyde, carboxylic acid and ester groups are formed at the surface of the NW-PET.

The modified material was tested in a down-scaled filtration set-up by measuring flow rate, platelet recovery and leucocyte reduction of small amounts of PC. Because the preparation of PC varies, resulting in different qualities (Fijnheer *et al*., 1990), the filtration characteristics may differ. Therefore, the WVGD-treated NW-PET filters were tested with several types of PC prepared via the platelet-rich plasma (PRP) method

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(Slichter & Harker, 1976) (after overnight storage of the PC), the single-buffy-coat (BC) method (Pietersz *et al*., 1985) (either freshly prepared or overnight-stored) and the pooled-BC method (Boomgaard *et al*., 1994b) (either freshly prepared or after overnight storage).

To test the effects of sterilization on the WVGD treatment, filter material was subjected to sterilization. Because steam autoclaving places a severe burden on the NW-PET and ethylene oxide is no longer used because of environmental concerns, it was decided to study the effect of 25 kGy gamma radiation treatment on modified NW-PET with respect to filtration characteristics with PC. The stability of the WVGD treatment and the γ-sterilization on the NW-PET were tested after both treatments and after storage at 20 and 37 °C for, at the maximum, 26 weeks. During storage of the modified NW-PET, the oxidized non-woven surface shows rearrangements resulting in a decrease of the wettability as result of loss of oxygen containing groups into the top layer of the sample. Therefore, rinsing the modified material with HCl and water tested the stability of the hydrophilic groups on the NW-PET formed by the WVGD modification. After rinsing, the filters were stored at 20 °C. All stored and treated filters were tested by filtration with overnight-stored pooled BC-PC.

MATERIALS AND METHODS

Blood collection and PC preparation

PCs were prepared from whole blood in three different ways, the PRP method (Slichter & Harker, 1976), the single BC method (Pietersz *et al*., 1985) and the pooled BC method (Boomgaard *et al*., 1994b).

A volume of 500 ± 50 mL of whole blood was collected in 70 mL of citrate–phosphate–dextrose (CPD) in PVC/DEHP quadruple systems (Biopack Compoflex, NPBI, Emmer-Compascuüm, The Netherlands). The pooled BC-PC were prepared in bottom-and-top bags (NPBI). After storage at 20 ± 2 °C for 12–16 h on butane-diol cooling plates (Pietersz *et al.*, 1989), whole blood donations were processed into PRP-PC, single BC-PC or pooled BC-PC.

PRP-PC

For PRP-PC, blood was first separated into red cells and PRP by centrifugation for 7 min at 1000*g*, brake 3, 20 °C (Hettich Roto Silenta R/P, Dépex, De Bilt, The Netherlands). PRP was pressed under reduced flow into an empty satellite bag by an automated device for component preparation, i.e. Compomat (G4) with Compomaster software (Loos, 1985) (NPBI). PRP was subsequently centrifuged for 6 min at 3000*g* at 20 °C. The Compomat was then used to remove plasma until about 70 mL of plasma was left. The platelet pellet with 70 mL of plasma was left undisturbed for 90 min at room temperature before manual resuspension. Before pooling, PRP-PC were stored overnight in 600-mL PVC/DEHP bags in a platelet incubator (22 ± 2 °C) on a horizontal flatbed shaker (1 cycle s^{-1}) (Helmer labs Inc, Noblesville, IN, USA). Prior to filtration, the platelet concentration in the PRP-PC was measured and pools of two PRP-PC were made by means of a sterile connecting device (SCD 312, Haemonetics, Braintree, MA, USA) so that an equal platelet concentration was reached in each pool. This pool was split again, and PCs originating from one pool were used for paired filtrations with WVGD-treated filter material and control material (Sepacell).

Single BC-PC

Blood for single BC-PC was processed to PC from single donations (Pietersz *et al*., 1985) by use of a Compomat according to standard methods as described before (Boomgaard *et al*., 1994a).

For each paired experiment, four single BC-PCs were pooled by means of a sterile connecting device (SCD 312, Haemonetics). This pool was split in four BC-PCs again and two BC-PCs were used directly for filtration, while the other two were stored overnight in a platelet incubator until filtration on the next day. This allowed pairing for different filter types and fresh vs. overnight PC storage. The whole procedure was repeated six times.

Pooled BC-PC

BCs of the same ABO and Rhesus blood group were prepared in bottom-and-top triple bags (NPBI). Blood was separated into plasma and red cells by centrifugation at 3990*g* for 15 min, brake 5, 18 °C. BCs were prepared by means of a Compomat G4 with Compomaster software. After preparation, BCs were left undisturbed for 1 h at room temperature. For pooling, four BCs and a plasma unit were connected via a sterile docking device, and plasma was added until a net weight of about 550 g. After homogenization, pools were centrifuged at 1252*g* for 4 min, brake 3, whereafter PCs were pressed into Thrombo pool bags, 1000 mL (Compoflex F501, NPBI). These PCs were directly used or stored overnight in a platelet incubator prior to filtration.

Cell counts

Platelet counting was performed on a Cell-Dyn whole blood counter (Sequoia-Turner Co, Mountain View, CA, USA). Prior to filtration, leucocytes were counted

electronically with a Coulter Multisizer II (Coulter Electronics, Mijdrecht, the Netherlands). After filtration, leucocytes were counted by fluorescence light microscopy in a Nageotte bright-line counting chamber (Superior, Bad Mergentheim, Germany). For this measurement, 100 μL of filtered PC was added to 400 μL of acridine orange solution (0·05 mg mL^{-1} in PBS). Sensitivity of this method was 0·5 leucocytes per μL (one cell counted in the counting chamber means 0·05 leucocytes per μL).

Water vapour glow discharge treatment of NW-PET

NW-PET filter material (obtained from NPBI) was treated with WVGD in a radio frequency glow discharge process (Klomp, 1998) during which water vapour is brought into a reactor in which the NW-PET is placed. A discharge process initiates reactive plasma formation of the water vapour, producing ions and radicals. This reactive plasma initiates formation of hydrophilic groups on the surface of the NW-PET (Klomp, 1998).

Preparation, sterilization, storage and rinsing of the filters

Filter discs were cut with a diameter of 26 mm from untreated and WVGD-treated NW-PET material. To remove water-soluble polymer surface groups, part of the gas-plasma-treated NW-PET material was rinsed with 50 mL, 10 mM HCl solution on a Vibrax VXR flatbed shaker (1 cycle s^{-1}, Labortechnik, Staufen, Germany) for 1 h at room temperature. Rinsing with water (25 mL for 30 s) and drying overnight at room temperature followed this treatment. Subsequently, all glow discharge-treated NW-PETs including the rinsed ones were γ-sterilized (25 kGy, Gammaster, Ede, the Netherlands). The gas-plasma-treated NW-PET discs (non-sterilized, γ-sterilized, and rinsed and γ-sterilized material) were stored at room temperature in closed containers. For accelerated ageing, γ-sterilized WVGD-treated NW-PET material was stored at 37 °C in closed containers.

Material from a platelet leucocyte depletion filter with good properties (Sepacell filter material (PL10(II)A, Asahi, Medical Co., Tokyo, Japan (Boomgaard *et al*., 1994b)) was used as control. This material was also NW-PET that was made platelet compatible with polymer coating (details unknown to us). For this purpose, six filter discs with a diameter of 26 mm were cut out of the non-coarse section (21 layers most close to the outlet) of the Sepacell filter.

Downscaled filtration

To allow performance of paired experiments with limited volumes of PC a 6 : 1 down-scaled filtration was performed. For each filtration experiment, six filter discs of the same type were put in a specially made Perspex filter holder (made at the University of Twente or at the CLB). For filtration, 50 g of PC were poured in an 50-mL disposable Combitip (Eppendorf, Hamburg, Germany) which was connected to the filterholder with 15·5-cm tubing (3 × 4·55 mm, NPBI). The distance between the upper edge of the Combitip and the filter discs inside the filter holder was 26 cm and 29 cm from the outlet.

The time between entrance of the PC in the filter and the first visible drop in the outlet of the filter was called the wetting 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 flowed through. Weights were converted into volume by applying specific gravity for PC (plasma) of 1·026 g cm^{-3}. When the flow rate became lower than 2 mL min^{-1} for more than 60 s or when the total filtration time exceeded 720 s, filtration was stopped. This filter was called blocked. The leucocyte retention and platelet recovery were calculated as follows:

$$\text{Leukocyte retention (\%)} = \{1 - [\text{total number of leucocytes after filtration}]/[\text{total number of leucocytes before filtration}]\} \times 100\%$$

$$= \{1 - [L \times V]/[L_0 \times V_0]\} \times 100\%$$

$$\text{Platelet recovery (\%)} = [\text{total number of platelets after filtration}]/[\text{total number of platelets before filtration}] \times 100\%$$

$$= \{[T \times V]/[T_0 \times V_0]\} \times 100\%$$

where L = leucocyte concentration in total filtered PC (counted in pooled fractions); L_0 = leucocyte 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 μL of PC was fixed with 250 μL of 0·5% glutardialdehyde in PBS and stored for future evaluation at 4 °C. Morphology was judged by a modification of the Kunicki score (Kunicki *et al*., 1975) evaluated by light microscopy (Leitz, Wetzlar, Germany) with oil immersion (1000×). The number of cellular discs per 100 cells was multiplied by 4, the number of

Table 1. Characteristics of differently prepared PC

PC type	*n*	Platelet concentration ($\times 10^9$ mL^{-1})	Leucocyte concentration ($\times 10^6$ mL^{-1})	Morphology score
single PRP-PC overnight	8	0·9 ± 0·38	0·24 ± 0·086	263 ± 35
single BC-PC overnight	6	1·0 ± 0·12	0·06 ± 0·029	335 ± 24
single BC-PC fresh	6	1·0 ± 0·09	0·07 ± 0·025	346 ± 17
pooled BC-PC overnight	12	1·0 ± 0·10	0·17 ± 0·127	249 ± 37
pooled BC-PC fresh	4	1·1 ± 0·33	0·25 ± 0·257	353 ± 35

PC type and number (*n*) prepared in different ways, with platelet and leucocyte concentrations and morphology scores before filtration. Every filtration with an overnight-stored single BC-PC was paired with a filtration with a fresh single BC-PC.

filled dendrites by 2 and the number of spheres by 1. A score higher than 250–300 indicated a good PC quality.

Statistical analysis

Statistical comparisons and correlation coefficient calculations were carried out with the computer programs Instat 2.03 (GraphPad Software, San Diego, CA, USA) for two-tailed Student's *t*-tests. The statistical program SPSS (SPSS7.5, SPSS Inc., Chicago, IL, USA) was used for multiple comparison analysis (ANOVA) in case of the filter storage study. P values $< 0{\cdot}05$ were considered significant.

Table 2. Filtration characteristics of differently prepared PC

PC type	*n*	P rec. (%)	L red. (%)	Flow rate (mL min^{-1})
Single PRP-PC overnight				
WVGD NW-PET	8	75 ± 8·8	94 ± 1·4	10 ± 3·7
Sepacell	8	87 ± 5·8	95 ± 1·3	12 ± 4·9
Single BC-PC overnight				
WVGD NW-PET	6	78 ± 3·3	95 ± 0·6	10 ± 1·2
Sepacell	6	87 ± 2·2	95 ± 1·0	12 ± 2·6
Single BC-PC fresh				
WVGD NW-PET	6	79 ± 3·5	93 ± 1·6	15 ± 4·8
Sepacell	6	90 ± 3·1	95 ± 1·6	15 ± 7·7
Pooled BC-PC overnight				
WVGD NW-PET	4	79 ± 8·9	99 ± 0·3	7 ± 3·0
Sepacell	2	83/91	99/98	11/17
Pooled BC-PC fresh				
WVGD NW-PET	4	84 ± 3·5	96 ± 2·4	15 ± 2·8
Sepacell	4	92 ± 4·4	96 ± 1·3	16 ± 4·1

Platelet recovery (P rec.; %) and leucocyte reduction (L red.; %) after filtration and flow rate (mL min^{-1}) during filtration measured with different PC types filtered with six layers of water vapour gas-plasma-treated NW-PET (WVGD NW-PET) and Sepacell material.

Table 3. Filtration characteristics after storage of WVGD-treated NW-PET filter material under different circumstances

Filter type Storage time (weeks)	P rec. (%) ($n = 4$)	Flow rate (mL min^{-1}) ($n = 4$)
H_2O PET		
5	79 ± 8·9	7 ± 3·0
14	85 ± 5·1	6 ± 2·0
26	54 ± 37·7	5 ± 3·8
H_2O PET, γ		
5	83 ± 5·2	7 ± 1·7
14	87 ± 3·4	7 ± 1·7
26	83 ± 4·2	6 ± 2·1
H_2O PET, γ, 37 °C		
5	82 ± 3·4	6 ± 0·9
14	84 ± 4·5	6 ± 2·2
26	83 ± 5·8	6 ± 3·2
H_2O PET, γ, HCl		
5	81 ± 6·1	7 ± 0·9
14	83 ± 4·8	6 ± 1·9
26	82 ± 1·9	6 ± 1·5

Platelet recoveries (P rec.; %) and flow rates (mL min^{-1}) of differently treated WVGD NW-PET material at different time points, filtered with overnight-stored pooled BC-PC. Modifications: γ-sterilization (γ), storage at 37 °C (37 °C) and rinsing with HCl (HCl).

RESULTS

Preparation of different types of PC

Table 1 shows quality characteristics of the different PCs. All PC had comparable platelet concentrations (1×10^9 mL^{-1}). The leucocyte concentration in overnight-stored single PRP-PC was significantly higher than in fresh and overnight-stored single BC-PC. PRP-PC and overnight-stored pooled BC-PC scored lower in morphology ($P < 0{\cdot}01$ and $P < 0{\cdot}001$) compared to single BC-PC, fresh and stored overnight, and pooled BC-PC, stored overnight.

Testing down-scaled set-up with control material

To monitor the down-scaled filtration set-up, besides NW-PET, for every PC an aliquot was filtered with control material (six layers of Sepacell) as well. The Sepacell filter material never blocked and gave platelet recoveries of 88 ± 5·5% (mean ± SD; $n = 34$; range 70–96%). In the down-scaled filtration set-up the performance was comparable to that obtained with full-scale PC (Boomgaard *et al*., 1994b). The average flow rate was 14·8 ± 5·20 mL min^{-1}, with a leucocyte reduction of 96 ± 2·2% ($n = 34$) and no effect on the morphology score.

Untreated NW-PET

Before using NW-PETs modified by WVGD treatment, these filters were tested without this modification. For this purpose, overnight-stored pooled-BC-PC were used. Including 3 out of 12 experiments in which the untreated filter was blocked, we found a platelet recovery of 57 ± 14% ($n = 12$).

Influence of different types of PC on filtration results tested with PET-H_2O

Platelet recovery did not differ significantly between freshly prepared or overnight-stored single BC-PC or overnight-stored PRP-PC. However, freshly prepared pooled BC-PC showed a significantly higher platelet recovery compared to the other types of PC (Table 2). Flow rates of freshly prepared pooled BC-PC were significantly higher ($P < 0{\cdot}05$) than those of single or pooled overnight-stored pooled BC-PC, and flow rates of single freshly prepared BC-PC were higher than those of overnight-stored pooled-BC-PC (Table 2). Leukocyte reduction and flow rate did not differ significantly per type of PC and between control (Sepacell material) and WVGD-treated NW-PET material (Table 2). Morphological scores before (Table 1) and after filtration did not differ significantly (scores after filtration not shown).

Influence of storage, γ-sterilization and rinsing with HCl on WVGD-treated filters

WVGD-treated NW-PET filters were tested after storage of the filters for 5, 14 and 26 weeks with overnight-stored pooled BC-PC. After γ-sterilization, filters were stored at room temperature or at 37 °C. The stability of WVGD treatment was tested after rinsing these filters with HCl before γ-sterilization and storage. Filter material that had been stored at room temperature and not γ-sterilized was used for control experiments. After 5, 14 and 26 weeks of storage, no difference in overall platelet recovery was measured after filtration. Storage temperature, γ-sterilization or rinsing with HCl did not influence the results (Table 3). After 26 weeks, platelet recoveries of filtrations with the non-sterilized material, stored at 20 °C, gave a high standard deviation (SD), caused by two out of four filtrations that blocked (total filtration time longer than 720 s). Due to this high SD and the low number of tests, a significant difference with other filter types was not demonstrable.

There was no influence of the treatments and storage

conditions on the flow rates of PC through the filters (Table 3). Moreover, flow rates of PC through filters with Sepacell control material and WVGD-treated NW-PET did not differ (data not shown). However, glow discharge-treated NW-PET material stored at 20 °C, without γ-sterilization, showed variation in flow rate due to blockage of two filters out of four. The γ-sterilized NW-PET showed a more reproducible flow rate and gave no blockage. Leukocyte reduction did not differ significantly for different treatments of the WVGD-treated filters (99·0–99·9%). Morphological scores before and after each filtration were not significantly different (248 ± 37·5 and 250 ± 37·1, respectively).

DISCUSSION

Morphological scores of platelets are used to assess the quality, notably the degree of platelet activation of PC. The unexpected differences in morphological scores between the differently prepared PC could not completely be explained by the preparation method or handling of the PC. In the case of PRP-PC, the low morphology score correlates with a higher activation rate of this type of PC (Fijnheer *et al*., 1990), due to the preparation method in which platelets are pelleted against the plastic container. In contrast to the PRP-PC, we have no explanation for the unexpectedly low morphology score of the overnight-stored pooled BC-PC. However, we observed this effect in other (unpublished) studies as well, in which the morphology score improved within the first 3 days of storage. According to morphological score, overnight-stored pooled BC-PC and PRP-PC are the most activated PC. However, in comparison with single BC-PC, which were not activated, these do not give a lower platelet recovery after filtration. So, the degree of platelet recovery after filtration cannot be explained by the degree of activation of the PC, because in activated and in non-activated PC, platelet recoveries after filtration are similar.

After storage of the treated filters for 26 weeks at room temperature or 37 °C, a statistically significant decline in filter performance is not demonstrable. However, the filtration results from non-sterilized filters vary more than the filtration results from γ-sterilized filters, including blockage of some non-sterilized filters. This might be caused by a stabilizing effect of γ-sterilization on the surface characteristics of WVGD-treated NW-PET. An explanation could be a possible cross-linking effect of the WVGD-treated NW-PET by γ-sterilization. Good filtration results were obtained with WVGD-treated NW-PET stored at 37 °C for 26 weeks, indicating that this modification of the material might be stable for almost 2 years at room temperature (storage at 37 °C is used for accelerated ageing: 26 weeks storage at 37 °C is approximately similar to 2 years storage at room temperature (Gennaro, 1985; Wells, 1988)).

In all filtration experiments, the control material (Sepacell) shows a significantly higher platelet recovery than did WVGD-treated NW-PET (differences between 5 and 16%). This can be due to the total filter surface of this material, which is smaller than the NW-PET. Experiments by Klomp (1998) showed that 1·4 times more platelets adhere to the Sepacell surface compared to the NW-PET (2·4 vs. $3{\cdot}2 \times 10^6$ platelets cm^{-2}). This means that if equal filter surfaces (of NW-PET and Sepacell filter material) are compared with each other instead of equal layers, the difference might be minimal. The leucocyte reduction for WVGD-treated NW-PET filters and the control (Sepacell) material does not differ significantly.

We conclude that WVGD treatment of NW-PET modifies the surface of the NW-PET, which changes the chemical composition of the surface structure of this material, resulting in a material that is more platelet-compatible. If used as a filter material, this results in significantly improved filtration characteristics, an increased flow rate, increased leucocyte retention and increased platelet recovery (Klomp, 1998). Subsequent γ-sterilization seems to be essential to stabilize WVGD treatment-induced modifications.

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