Optimising quality of platelet transfusions
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Summary

Platelet concentrates (PCs), which are essential for modern transfusion practice, have a number of limitations regarding quality, safety and logistics. In this thesis, various studies about the preparation, leukocyte reduction by filtration, as well as quality measurements during storage of PC are described. Leukocyte reduction of PC was studied by a modification of the preparation method of PC via the buffy coat (BC) and by using new leukocyte reduction filters for PC. Quality testing of PC during storage was extended by two parameters (glycocalcin and soluble P-selectin) of which the concentrations gradually increased in the supernatant of PC during storage and which could easily be measured by ELISA assays. These parameters correlated well with sensitive platelet activation parameters.

Chapter 1, a general introduction, describes the origin and function of platelets, the use of PC in transfusion practice, and the preparation methods of PC via BC and platelet-rich plasma (PRP) method, or via platelet pheresis. Furthermore, specific problems, such as platelet refractoriness, PC leukocyte-reduction by filtration and the risks of platelet transfusion, are reviewed.

In Chapter 2, a method for the preparation of PC with low leukocyte concentrations meeting the requirements of leukocyte-depleted PC is described. The effects of variations in volume, shape and the haematocrit (Ht) of the BC and centrifugation forces on BC, which may affect the quality of the subsequently prepared PC, were studied. It is concluded that when BCs are centrifuged in long cylindrical containers that create a longer separation column, the quality of the PCs is improved. Combined with optimal Ht and volume of BC, this leads to a tenfold lower leukocyte contamination compared to the conventional BC bag, i.e. in 0.3 ± 0.19 x 10^6 leukocytes per single donor PC of 64 ± 4.5 ml. Platelet recovery was not affected by this method.

Leukocyte-reduction in PC via filtration is described in Chapter 3. The filtration characteristics of CO₂ and H₂O radio frequent glow discharge (RFGD)-treated non-woven poly(ethylene terephthalate) NW-PET were determined with a miniaturised filtration set-up. The downscaled test system for PC filtrations was shown to work well for research purposes and enabled good differentiation between different filter materials. NW-PET material, which is commonly used for red-cell filtration, is not platelet-compatible. Hydrophilic groups formed during RFGD treatment on the surface of NW-PET create a surface that was expected to be more platelet-compatible. Due to the RFGD treatment with water vapour or CO₂, the filter surface was significantly more compatible for platelets than the untreated NW-PET reflected by a significantly higher platelet recovery and flow rate. With
water vapour RFGD-treated NW-PET, leukocyte reduction was also significantly higher compared to untreated NW-PET.

Because water vapour RFGD-treated NW-PET showed a higher platelet recovery than did CO₂ RFGD-treated NW-PET, the robustness of the first treatment was tested by filtration of different types of PC. For these experiments, described in Chapter 4, the miniaturised filtration set-up was used with 5 different types of PC, i.e. freshly prepared and overnight-stored single BC-PC, overnight-stored single PRP-PC, overnight-stored pooled BC-PC and freshly prepared pooled BC-PC. In all filtration experiments, commercially available Sepacell material was used as a control. This study showed that leukocyte depletion did not differ significantly between the different types of PC, and that platelet recovery from freshly prepared pooled BC-PC was significantly higher compared to the other four types of PC. Furthermore, the filtration results of water vapour RFGD-modified NW-PET, tested with freshly prepared pooled BC-PC, did not change after γ-sterilisation or rinsing with HCl and subsequent storage at 20 °C or 37 °C for a maximum of 26 weeks.

Another approach for surface modification of NW-PET, by wet chemical treatment, is described in Chapter 5. Different PEO-PPO-PEO block copolymers were coated on NW-PET. After testing the wettability and the stability of the coating (after rinsing with PBS or with albumin solution), the coated filter material was tested in the miniaturised filtration set-up with freshly pooled BC-PC. The results of these studies demonstrated that the miniaturised filtration set-up was an ideal method for testing a large number of filters in paired experiments with a small amount of PC. However, despite good wettability and stability upon rinsing with PBS or albumin of the test matrices, this did not result in good filtration characteristics with PC, probably due to instability induced by γ-sterilisation. Other block copolymers, with polysiloxanes as the hydrophobic backbone, which are platelet compatible under static conditions according to the literature, also failed to give good results in the filtration mini set-up, with the exception of PS4.

In Chapter 6, the test results of a new platelet storage container (UPX80) are described and compared with a polyolefin (PO) container. Both containers have increased gas exchange properties compared to first-generation PVC containers. However, the gas exchange capacity of the UPX80 is more than 43% higher compared to PO containers. We tested these containers with freshly prepared, pooled and filtered BC-PC. PCs were made and tested in plasma as well as in synthetic medium (gluconate-acetate-citrate, GAC) with a very low (<8%) plasma content. Platelet metabolic, physical and activation parameters were measured. It was found that increased gas exchange did not result in improved platelet survival or decreased platelet activation during PC storage. From this study, we conclude that although the increased gas exchange of UPX80 compared with PO results in
significant lower $pCO_2$ and higher $pH$ and $pO_2$ concentration values, these changes do not result in significantly lower platelet activation during storage.

**Chapter 7** describes the correlation between soluble P-selectin, which is set free in plasma during platelet deterioration at storage, and other platelet activation parameters. Soluble P-selectin correlated well with sensitive platelet activation parameters such as the morphology score, $\beta$-thromboglobulin concentration and the percentage of P-selectin-positive cells. During storage, the concentration of soluble P-selectin after various periods of storage (1, 3 and 6 days) increased significantly. Furthermore, soluble P-selectin concentrations in plasma samples taken directly after blood collection or just before preparation of PC differ significantly, indicating that already during storage of whole blood on butane-diol cooling plates (which takes about 17 hours) platelet activation takes place. This difference in activation degree was not found when the percentage of P-selectin-positive cells at $t = 0$ and $t = 17$ hours were compared, indicating that the soluble P-selectin assay is more sensitive. Moreover, P-selectin can easily be measured in stored supernatants with a convenient ELISA, whereas $\beta$-thromboglobulin was measured by a radio-immuno assay. Another advantage of the assay for soluble P-selectin is that samples can be stored at $-80 \, ^\circ C$ until testing, whereas fixed PC samples are required for measurement of the percentage of P-selectin-positive cells as well as for the morphology score. Both of these last two assays have to be performed within 1 to 2 days.

Besides P-selectin, another soluble marker -glycocalicin-, which correlates with platelet activation parameters, is described in **Chapter 8**. Glycocalicin, a proteolytic fragment of glycoprotein Ib, is shed from the platelet membrane during platelet ageing. Apart from its diagnostic significance in differentiating between various types of thrombocytopenia, it also appeared to be a sensitive parameter for platelet activation. Glycocalicin correlated well with other platelet activation markers. In contrast to soluble P-selectin and other sensitive platelet activation parameters, glycocalicin levels increase significantly between the 6th and 8th day of storage. The conveniences of the ELISA, which can be performed on stored plasma samples, and its high sensitivity especially for platelet activation during prolonged storage of PC, render glycocalicin a valuable platelet activation parameter. Additional experiments demonstrated an increasing GC concentration also during platelet deterioration due to limited gas exchange. However, storage of platelets at 4 °C did not show a marked increase in GC concentration, indicating that the so-called cold activation of platelets does not resemble other forms of platelet activation.
Concluding remarks

There is an increasing demand for high-quality platelet concentrates for transfusions. The quality of PCs depends on collection and subsequent storage of whole blood. Furthermore it depends on the PC preparation method, the degree of leukocyte reduction and the PC storage conditions. Although some limits concerning storage and preparation seem to be reached there still is a need for improvement of PC quality. Further standardisation and research on blood collection can be a next step.

For further research on improving PC quality, the way of collection of whole blood will be an interesting topic. Finding an optimal method of mixing whole blood with anti-coagulant during collection and further standardisation (uniformity throughout whole Europe) of the blood collection and processing can both have a great impact on general PC quality. With respect to optimal mixing, a method of direct mixing the right amount of anti-coagulant with whole blood during collection has to be developed. Nowadays, during collection of whole blood, the first drop of blood is collected in a surplus of anti-coagulant. Only the last few millilitres are collected in a proper concentration (when properly mixed) of anti-coagulant. This can be of great influence on platelet function and quality. Research on a new method of mixing the anti-coagulant in the right concentration already directly during collection would be challenging. Further standardisation (throughout Europe) will possibly reduce the variation in PC quality and is hopefully one of the next steps in improving PC quality for transfusion.