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Production and preservation of platelet concentrates. Evaluation of elutration technigue and advanced preservation technologies.

Elias-Elias, Mona K.

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Document Version

Publisher's PDF, also known as Version of record

Publication date:

1991

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Elias-Elias, M. K. (1991). *Production and preservation of platelet concentrates. Evaluation of elutration technigue and advanced preservation technologies.* s.n.

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SUMMARY

This thesis includes various studies on improving the collection and preservation of the platelet product. The conventional differential centrifugation method of platelet isolation, which quantitatively is the common source of platelet supply, has several shortcomings. The technique depends solely on difference in specific gravity between the blood cell components. Therefore, cells with similar specific gravities (e.g. red cells and granulocytes, or platelets and lymphocytes) are difficult to separate. Attempts to increase the resolution of platelets and white cells by modification of the technique, occur at the expense of platelet yield. The manual preparative manipulations - including a two-step centrifugation, resuspension and agitation result in a combination of activation and damage processes. Platelets are exposed twice to high shear forces, created by the large diameter of the centrifuge. Platelets remain in the vicinity of red and white cells during processing, and mediators leaking from shear damaged platelets and erythrocytes secondarily activate other unaffected platelets. Packing of platelets during processing add to the initial activation and damage. Therefore, the development of relatively gentle methods of platelet isolation, or pharmacological strategies interfering with the processing damage and with platelet activation following contact with damaged cells appears a necessity.

One of the undesirable factors, compromising both the platelet quality *ex vivo* and the recipient response to platelet transfusions, are the leukocytes contaminating the platelet product. Therefore, leukocyte removal appears highly desirable as well.

Chapter 1 is a general introduction to this thesis. It describes the current methods of platelet concentrate production, and the improvements introduced to the techniques, which serve the purpose of this study. It also introduces the problem of alloimmunisation and the potential approaches to this problem in view of other works. Finally, it addresses the problem of the platelet storage lesion, its possible etiology and approaches to prevent or at least minimise it.

Chapter 2 and 3 describe the evolution of the elutriation technique. A preliminary introduction to the technique, dating back to 1985 when the machine was manually controlled serves as a background. The technique uses a single venous access. Another major advantage of centrifugal elutriation is the avoidance of cell-cell contact. Platelets are elutriated out of the buffy coat as soon as it is formed. No pelleting and no long contact with red and white cells. There is no exposure to osmotic effects of special media for density gradient separation as is the case with counterflow centrifugation. Platelets are centrifugated only once in the small diameter of the spinning rotor, during a relatively brief separation time and thus exposed to minimal shear stresses.

The semiautomated surge elutriation and the autosurge are compared. The technique became self adjustable to donor variables and additional safeguards measures were introduced. In our hands, no difference was observed as far as platelet yield and leukocyte contamination are concerned. The actual advantage is the ease of operation and the elimination of determining the donor's blood composition prior to the procedure.

Chapter 4 defines optimal conditions for collection and extended storage of elutriated platelets. Relatively little work has been done to evaluate

optimal storage conditions for the apheresis platelet concentrates and even less work has been done on elutriated platelets in particular. The ex vivo parameters of the elutriated platelets, relative to pooled multiple donor platelets with comparable parameters are described. The study reveals that the elutriated platelets perform better than pooled platelets when both products are stored during 7 days under the same conditions. The storage characteristics of elutriated platelets are further improved by modifying the surface area available for gas exchange. The pH could be kept around 7 during the entire storage period, platelet metabolism slowed, and platelet damage limited. The study confirms that leukocytes, being higher in pooled platelet concentrates (PC), adversely affect the platelet quality during storage. However, the processing effect by the two different techniques - elutriation versus differential centrifugation - is not excluded so far. In order to investigate this effect alone, leukocytes are removed from both products prior to storage.

In chapter 5, both leukocyte-depleted single and pooled platelet concentrates are stored for 7 days under the same conditions and the same parameters used in the previous study were determined. It appears from this study that, despite the leukocyte removal, elutriated platelets are still less activated and less damaged than pooled platelets. The recovery and haemostatic capacity of elutriated and filtered leukocytes are satisfactory.

Chapter 6 evaluates two versions of the new-generation leukocyte adsorption filter. The polyester Pall filter has a high affinity to leukocytes while it allows platelets to pass freely without adhering to the foreign filter material. The efficiency of the filter is determined by fraction analysis, from which it appears that filtration of 5 PC units - instead of 6 - insures a leukocyte depletion below the critical immunogenic threshold of 10 million leukocytes, assessed by flow cytometric measurements. The performance of the smaller version of the filter is more efficient. The filtered product fulfills the criteria of a 'leukocyte-free' single or multiple donor platelet concentrate. Leukocytes are absent in more than 92% of the units tested, irrespective of the PC type and the detected leukocytes never exceed 2 million cells in a pool of 6 units. Both filters keep platelet integrity and function intact.

Since platelets collected by the differential centrifugation method are more activated and more damaged than those collected by elutriation (chapter 4 & 5), an attempt has been undertaken in chapter 7 to protect these platelets from the processing damage and to minimise the activation-induced storage lesion. A stable prostacyclin analogue, Iloprost, is incubated with platelets before the hard spin. This appears to act favourably on platelets by virtue of its cytoprotective and platelet inhibitory effect. Platelet release and lysis are significantly reduced in Iloprost-treated platelets, and platelet ultrastructure is protected. The reversibility of inhibition has been assessed by synergistic aggregation response, which was restored by 80% on day 5 of storage. The concentration used in the study is not likely to produce side-effects after transfusion. The stability of this compound makes it a good candidate among the anti-platelet agents for stabilization of PC, since it can be added in the collection bag to interfere with the initial platelet damage and activation. Its sustained effect would also interfere with subsequent platelet activation following contact with damaged cells and which is accentuated by processing and storage.

FINAL CONSIDERATIONS

In view of the results described in this thesis we conclude that:

1. Elutriation is a relatively gentle technique for platelet isolation compared to the differential centrifugation technique.
2. Optimal storage conditions should be defined for each product exclusively. Because of the diversity in preparation techniques, product composition, container types and volumes, agitation type, anticoagulant used etc., it is likely that what may be true for random donor units may not be for single donor platelet concentrates.
3. The new generation leukocyte adherence filters offers the possibility to prepare leukocyte-depleted platelet concentrates, fulfilling the criteria required to prevent or minimise alloimmunization. Filtration by these filters, in addition combines the advantage of preservation of platelet integrity and maintaining the platelet yield without the necessity to use pharmacological inhibitors. If further clinical studies prove that the long-term cumulative effect of residual leukocytes on alloimmunisation is negligible, filtration would be an effective, cheap and simple approach, avoiding manipulation of the recipient immune system.
4. Platelets can be protected from the processing damage and the storage lesion could be minimised by using a stable prostacyclin analogue. This practical approach has potential benefits which justify clinical trials to be done to confirm the improved quality observed *ex vivo*.

PROSPECTIVE DIRECTIONS

An exciting, fast moving and promising field remains the use of plasma-free synthetic media for platelet storage. Besides the potential of saving plasma for fractionation and therapy, unnecessary plasma proteins and enzymes could be eliminated and transfusion reactions caused by incompatible plasma proteins could be avoided. The risk of blood associated infections could be reduced and inactivation of residual viruses facilitated. These media can support higher platelet counts per unit compared to plasma and, therefore, would be of value in storage of apheresis PC. The quality of platelets stored in some non-plasma medium is now significantly improved compared to conventionally stored platelets, and *in vivo* results are promising. The combination of platelet metabolic manipulation together with the use of additive solutions may provide opportunities for extended storage beyond 5 days, with complete plasma separation. Leukocyte depletion of platelets stored in plasma-free medium is worth being investigated.