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Cytoskeletal reorganization after preparation of platelet concentrates, using the buffy coat method, and during their storage

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Abstract

Background and Objective. **The use of platelet transfusions has risen considerably in the last years. Changes occur in platelet biochemical and membrane properties during storage. We have analyzed the effect of platelet preparation and storage of platelet function through the evaluation of platelet cytoskeletal reorganization.**

Methods. **A blood sample was obtained from the donor and platelets were separated as standard platelet-rich plasma (PRP) (120 g, 20 min) (PRE sample). Aliquots were also collected immediately after preparation using buffy coat procedure of platelet concentrates (day 0) and after 1, 3 and 5 days of storage. Cytoskeleton composition in both low- and high-speed cytoskeletal fractions of detergent-lysed platelets was analyzed by gradient SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Presence of each contractile protein was quantified by densitometry.**

Results. **The method used to prepare platelet concentrates induced actin polymerization (actin increased to 163.5±4.8%, mean±SEM, n=8, p<0.001, considering actin values in PRE sample as 100%) with a concurrent increase in the association of actin-binding protein (ABP), myosin and α-actinin to the low-speed cytoskeletal fraction. During the first 24 hours of storage, cytoskeletal assembly was partially reversed (134.8±2.6% of actin, p<0.001) and actin polymerization increased gradually to 144.3±5.8% and 153.2±5.1% at days 3 and 5, respectively (p<0.001 for both days). ABP, myosin and α-actinin showed similar tendencies to those referred for actin. Conversely, during platelet preparation and storage, the contractile proteins associated with the high-speed cytoskeletal fraction decreased, due to reorganization of the contractile proteins to the low speed fraction.**

Interpretation and Conclusions. **The method used to prepare platelet concentrates (buffy coat procedure) induced cytoskeletal polymerization. This activating effect was partially reversed after 1 day of storage, although it increased progressively after 3 days of storage. The storage lesion may lead to defective cytoskeletal assembly in response to further stimulus. Analysis of cytoskeletal assembly is a sensitive method for detecting platelet activation caused by the concentrate preparation method and the storage conditions.**
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Key words: buffy coat, stored platelets, cytoskeleton, actin, platelet activation

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The main objective of platelet transfusions is to provide thrombocytopenic patients with a sufficient concentration of functionally active platelets. Platelets play an essential role in primary hemostasis. To promote hemostasis, transfused platelets should preserve adhesive and cohesive properties that allow them to interact with damaged vessels. The use of platelet transfusions has risen considerably in the last few years and indications for future requirements are continually increasing, linked particularly to new intensive therapeutic regimes associated with prolonged periods of bone marrow aplasia.[1,2]

In recent years the buffy coat method has become an alternative process for preparing platelet concentrates, particularly in Europe.[3,4] It has been suggested that this method causes less platelet activation and damage during platelet preparation.[5] During preparation and storage platelets are exposed to a variety of mechanical and chemical influences that may lead to their activation.[6-8] Besides signs of activation like disc-to-sphere transformation, extension of pseudopodes and loss of storage granules, platelets may display a swollen open canalicular system and changes in the structure of their α-granules. The resulting morphological, biochemical and functional alterations that occur in stored platelets have been defined under the term *storage lesion*.

Platelet activation involves shape change, increased levels of cytosolic Ca^{++} and polymerization of actin.[9] Platelet cytoskeleton contains two actin filament-based components: the cytoplasmic cytoskeleton, which fills the cytoplasm and mediates contractile events, and the membrane skeleton, which underlies the plasma membrane and regulates its shape and stability. The membrane skeleton plays a major role in the distribution of

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glycoproteins in the plasma membrane.[10,11] In the unstimulated platelet, only 30-40% of actin is polymerized into filaments. When platelets are activated, dramatic changes in the organization of the cytoskeleton take place. Pre-existing actin filaments become cross-linked.[12] There is an increased polymerization of actin monomers[13,14] onto filaments at the periphery of the platelets that fill the developing filopodia.[15,16] The network of filaments binds myosin,[17,18] and this interaction generates the tension required for the centralization of granules and the retraction of filopodia with their externally bound fibrin clots. Some actin filaments modulating proteins, such as profilin and gelsolin, control linear actin assembly in response to activation, while others such as actin-binding protein (ABP) and α-actinin establish the three dimensional architecture of the assembled filaments and connect them to the extracellular receptors.

The objective of the present study was to analyze the effect of the preparation method and storage conditions of platelet concentrates on the functional state of platelets, through evaluation of platelet cytoskeletal reorganization by gradient SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

Materials and Methods

Preparation of platelet concentrates

Blood was obtained from healthy blood bank donors and collected with citrate-phosphate-dextrose into a quadruple-bag with an integral PL-1240 platelet storage pack and a plasma container. Blood cells were sedimented by centrifugation at 2650g for 10 min. Platelet poor plasma (PPP) and the buffy coat were transferred to auxiliary bags by an automated process (Compomat®, NPBI, Amsterdam, The Netherlands). Platelets were recovered from the buffy coat, as a platelet-rich plasma (PRP) by a second centrifugation at 280g for 3 min and collected into the storage bag (PL-1240)(Baxter Fenwalt®, Valencia, Spain). Platelet concentrates (n=8) were stored with continuous agitation on a flat-bed agitator, at 22°C for a maximum of 5 days.

Blood sampling and platelet washing

A sample of blood was obtained from each donor (n=8) and platelets were separated as standard platelet rich plasma (PRP) (120g, 20 min) (PRE sample). Aliquots were also collected immediately after platelet concentrates preparation and after 1, 3 and 5 days of storage. Platelets from PRP and aliquots from different days of storage were washed three times with equal volumes of citrate-citric acid-dextrose (93 mM sodium citrate, 7 mM citric acid and 140 mM dextrose), pH 6.5, containing 5 mM adenosine and 3 mM theophyline. The final pellet was resuspended in a Hanks' balanced salt solution (136.8 mM NaCl, 5.3 mM KCl, 0.6 mM Na_2HPO_4, 0.4 mM KH_2PO_4, 0.2 mM $NaH_2PO_4 2H_2 0$) and incubated for 20 minutes at 37°C.

Obtaining cytoskeletal proteins

Platelet cytoskeletons were obtained according to the procedure described by Jennings *et al.*[19] with minor modifications.[20] Platelet suspensions were adjusted to 1.2×10^9 plts/L. Samples were treated with an equal volume of a lysis buffer containing 2% Triton X-100, 100 mM Tris-HCl, 10 mM ethylene glycol bis (β-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA) and 4 mM ethylenediaminetetraacetic acid (EDTA) (pH 7.4), 2 mM phenylmethylsulfonyl fluoride (PMSF), 1 μg/mL leupeptin and 1 mM benzamidine. Triton-insoluble residues, corresponding to the low-speed cytoskeletal fraction, were isolated by centrifugation at 12,000g for 5 min at 4°C in a microfuge. Supernatants were collected and centrifuged at 100,000g for 3h using polycarbonate tubes in a Beckman Ultracentrifuge (using a Ti 70 rotor) to obtain residues corresponding to the high-speed cytoskeletal fraction. Both low- and high-speed cytoskeleton fractions were washed twice with a washing buffer without Triton X-100 at 4°C, then solubilized with washing buffer containing 2% sodium dodecylsulfate (SDS) and heated at 100° C for 5 min. Samples were frozen at –40°C until a 7-12% gradient SDS-polyacrylamide gel electrophoresis (SDS-PAGE)[21] was performed.

Densitometric analysis of cytoskeletal proteins

Proteins in profiles from both low- and high-speed cytoskeleton fraction were stained with Coomassie brillant blue R250, and densitometrically quantified.[22,23] In our study, stained protein bands were densitometrically analyzed using digital video technology provided by a computerized image analyzer running specific software (Lecphor; Biocom 200, Les Ulis Cedex, France).[24] Bands were manually selected on the monitor screen of the system. The software automatically scanned the selected lane for protein bands, analyzed the color density of each protein band and integrated areas beneath densitometric peaks. To facilitate comparisons between gels, the quantification of protein bands was performed under standard conditions. In cytoskeletal proteins, the area of each protein peak in the lane containing cytoskeletons extracted from PRE sample was calculated and considered one hundred percent. The association of the main cytoskeletal proteins after preparation and during storage was determined and expressed as a relative value with respect to proteins in PRE profiles.

Statistics

Data from 8 different experiments were expressed as mean±SEM and paired *t*-test was applied for statistical analysis considering protein values in PRE profiles as references. A p level < 0.05 was considered statistically significant.

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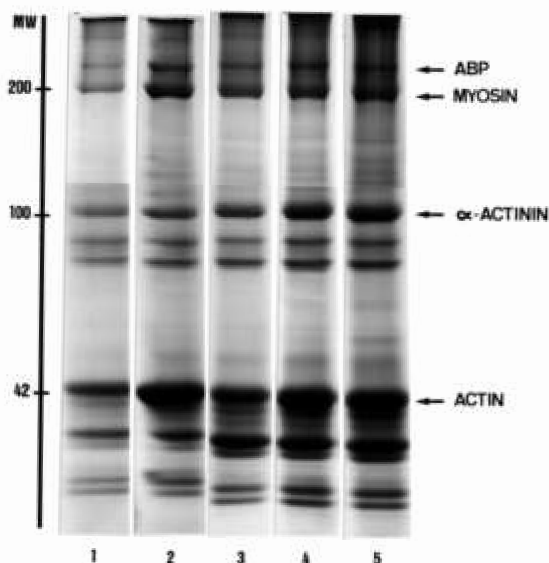

Figure 1. Effect of the preparation procedure and storage conditions on cytoskeletal reorganization. Coomassie blue stained SDS-PAGE profiles corresponding to the low-speed cytoskeletal fraction of platelets before (lane 1), after preparation (lane 2) and at 1 (lane 3), 3 (lane 4) and 5 (lane 5) days of storage. Preparation of platelet concentrates induced an incorporation of contractile proteins to the low-speed fraction. It was partially reversed after 1 day of storage and increased gradually after 3 and 5 days.

Results

Analysis of proteins associated with the low-speed cytoskeletal fraction

Figure 1 shows SDS-PAGE profiles corresponding to the low-speed cytoskeletal fraction from aliquots of the blood unit (PRE sample: lane 1), after platelet concentrates preparation (day 0: lane 2) and after 1, 3 and 5 days of storage (lanes 3, 4 and 5, respectively). Profiles correspond to an equal number of platelets.

Actin-binding protein (ABP), myosin, α-actinin and actin were the major cytoskeletal proteins recovered at the low-speed cytoskeletal fraction. Incorporation of these proteins was densitometrically analyzed using digital video technology. Values of the different cytoskeletal proteins in PRE profiles were determined and expressed as one-hundred percent. Incorporation of the contractile proteins to this fraction in samples obtained after preparation and during storage of platelet concentrates were expressed as relative values (Figure 2).

Preparation of platelet concentrates (by buffy coat procedure) induced a statistically significant increase in the incorporation of actin to the low-speed cytoskeletal fraction (increase of 63.5±4.8%; mean±SEM, n=8, $p<0.001$). After the first 24 hours of storage, cytoskeletal polymerization was partially reversed (actin increased of 34.8±2.6%, over its corresponding value in PRE profiles). During storage, the actin filaments formation increased gradually 44.3±5.8% and 53.2±5.1% at days 3 and 5, respectively. Increases in actin polymerization during the whole period of storage were statistically significant ($p<0.001$).

Figure 2 shows bar diagrams representing increments in ABP, myosin and α-actinin recovered in the low-speed cytoskeletal fraction during the study performed. Incorporation patterns for the major contractile proteins, ABP, myosin and α-actinin, showed similar tendencies to those referred for actin. Differences among data in profiles from days 0, 1, 3 and 5 versus PRE profiles reached statistical significance for the proteins studied (**$p<0.001$ and *$p<0.05$, as shown in Figure 2). Thus, results indicate that the obtainment method (day 0) and long periods of storage induced polymerization of the cytoskeleton which may reflect platelet activation.

Proteins associated with the high-speed cytoskeletal fraction

Profiles shown in Figure 3 correspond to proteins associated with the high-speed cytoskeletal fraction of samples before (lane 1), after preparation of platelet concentrates (lane 2) and during 1, 3 and 5 days of storage (lanes 3, 4 and 5, respectively). As in Figure 1, profiles correspond to equal number of platelets. After preparation of platelet concentrates (lane 2) there was a decrease in the density of the proteins associated with this fraction. This decrease was related to the increased amount of cytoskeletal proteins observed in the corresponding profiles from the low-speed cytoskeletal fraction (Figure 1, lane 2). This observation was also extensible to profiles from days 3 (lane 4) and 5 (lane 5).

Densitometric evaluation of the actin present in the high-speed cytoskeletal fraction was performed. Actin in profiles from a PRE sample was considered as 100% and values in profiles from samples 0, 1, 3 and 5 were expressed as relative values with respect to PRE sample.

Results indicated that the presence of actin in the high-speed fraction decreased in profiles from samples 0 (decrease of 23±2%). After one day of storage the cytoskeletal organization had a tendency to recover the unstimulated state (decrease of actin of 4.2±1.2%). During 3- and 5-day storage, the presence of actin in the high-speed cytoskeletal fraction decreased 14.1±2.3% and 17.2±5.7%, respectively.

During platelet preparation and storage, the contractile proteins associated with this fraction decreased due to cytoskeletal assembly proteins reduced to the low-speed cytoskeletal fraction.

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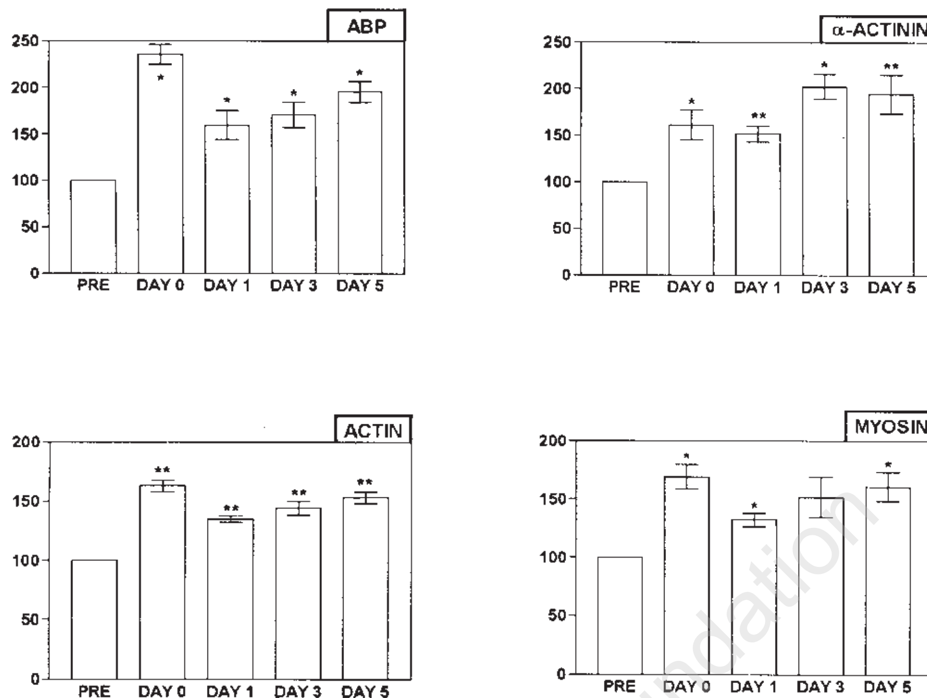

Figure 2. Bar diagrams represent the major cytoskeletal proteins (actin-binding protein, α-actinin, actin, myosin) recovered at the low-speed cytoskeletal fraction. Values for the different cytoskeletal proteins in PRE profiles were determined and expressed as 100%. Incorporation of the contractile proteins to this fraction in samples after preparation of platelet concentrates (day 0) and during storage for 1, 3 and 5 days were expressed as relative values (mean±SEM). Experiments were performed in 8 different platelet concentrates (n=8). **p<0.001 and *p<0.05; ABP = actin-binding protein.

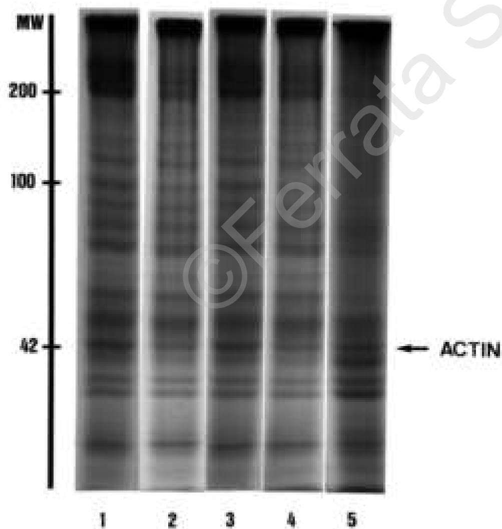

Figure 3. Coomassie blue-stained SDS-PAGE profiles corresponding to the high-speed cytoskeletal fraction of platelets before (lane 1), after preparation (lane 2), and at 1 (lane 3), 3 (lane 4) and 5 (lane 5) days of storage. After preparation of platelet concentrates and at days 3 and 5 there was a decrease in the density of proteins associated with this fraction, due to reorganization of contractile proteins to the low-speed cytoskeletal fraction.

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Discussion

This study was designed in an attempt to analyze the effect of the preparation procedure and storage, under blood bank conditions, on platelet function, through evaluation of platelet cytoskeletal organization. The method used to prepare platelet concentrates induced polymerization of contractile proteins. Storage for up to 3 days resulted in an increased cytoskeletal assembly. According to our data, analysis of cytoskeletal reorganization was a sensitive method to detect platelet activation due to the method used to prepare platelet concentrates and to the storage conditions.

Platelet concentrates are currently stored at 22°C for up to 5 days under continuous agitation.[25] The storage period is limited to 5 days since longer storage is known to increase the risk of bacterial contamination. During storage the continuos agitation is considered necessary[5] for adequate gas diffusion through permeable plastic bags. Maintenance of a satisfactory pH level, between 6.4 and 7.4, improves postransfusional viability. However, agitation has some deleterious effects on stored platelets causing activation and structural damage. Considerable morphologic, biochemical and functional changes of platelets during storage have been documented. Studies using an electron-microscope revealed a loss of organelles and progressive

destruction of cell membranes.[26] These alterations probably lead to functional impairment of stored platelets.

Results obtained from our studies confirmed that storage of platelet concentrates, under standard blood bank conditions, at 22°C with continuous agitation to 5 days, induces a storage lesion which can be detected by electrophoretic analysis of platelet cytoskeleton. Unstimulated platelets rearrange their cytoskeletal components after activation. Platelets possess two well differentiated cytoskeletons, the membrane and the cytoplasmic skeletons.[10,12,27] Actin is the major constituent of both of them. The molecular assembly of actin into a filament network and the organization of other structural proteins of the cytoskeleton are of critical importance for platelet shape change and internal contraction.[16,28] During these events, the contractile proteins that constitute both membrane and cytoplasmic cytoskeletons rearrange themselves through poly and depolymerization processes.

Our studies demonstrate that the platelet preparation method induced cytoskeletal assembly with actin polymerization. This observation would reflect that during preparation of concentrates, platelets are exposed to centrifugation and, therefore, to shear stress which results in a certain degree of activation. After 1 day of storage, the activation caused by the platelet concentrates preparation method was partially reversed, as shown by a decreased incorporation of contractile proteins to the cytoskeleton, suggesting that these platelets may be more active functionally than after immediate isolation. Storage periods longer than 3 days induced cytoskeletal polymerization. In relation to changes in actin polymerization, our results would be in agreement with other authors' work in which the DNAse inhibition assay[9] was applied to detect changes in globular actin. The cytoskeletal and structural changes observed may imply functional alterations in stored platelets.

During storage of platelet concentrates there are various changes including secretion and loss of the granules content. This reaction would lead to modifications in the expression of glycoproteins at the platelet surface. Flow cytometric methods have been applied to assess changes in glycoprotein expression. While these methods are accurate for detecting changes in activation-dependent antigens, their value on assessing modifications of major glycoproteins (especially GPIb) is more limited. Most studies on the expression of surface glycoproteins have found that GPIb remains relatively constant or decreases slightly, while GPIIb/IIIa increases during platelet storage.[29,30] However, these changes are moderate and no relationship has been found with the adhesive and cohesive properties of platelets, as demonstrated by perfusion studies: while platelet adhesion increases or remains equal, platelet aggregation decreases dramatically.[31] Moreover, shape changes, fragmentation of platelets and cellular interaction of platelets with leukocytes may impair the detection of activation markers.[2] Considering these facts, the analysis of cytoskeletal changes may be a more sensitive method to follow the platelet state during storage.

After 5 days of storage, analysis of the high-speed cytoskeletal fraction showed diffuse protein profiles. In fact, it has been previously found that with increasing storage time, a Ca^{++}-dependent proteolysis of actin leads to functional defects in the membrane skeleton.[32] This fact would result in an impaired stabilization of the lipid bilayer with fragmentation of the plasma membrane, as has been shown by electron microscopy.[26]

Cytoskeletal assembly plays a critical role in binding and localizing signaling molecules. Since repeated activation of platelets may result in alternate cycles of poly- depolymerization of cytoskeletal proteins causing platelet refractoriness, signal transduction processes may be impaired in stored platelets. This fact would lead to a insufficient platelet response. Further studies need to be performed to elucidate this question.

Contributions and Acknowledgments

EE was the principal investigator; she carried out the biochemical measurements and statistical analysis. MD-R was responsible for handling and interpretation of data and direct supervision. EE and MD-R wrote the paper and, with GE, were responsible for the design of the study. GE was involved in the conception of the study and the correction of the manuscript. ML and RM contributed to the execution of the study by providing the platelet concentrates. AO was responsible for the funding and facilities, making possible the accomplishment of this work.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

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References

1. Lee VS, Tarassenko L, Belihouse J. Platelet transfusion therapy: Platelet concentrates preparation and storage. J Lab Clin Med 1988; 111:371-83.
2. Klinger MHF. The storage lesion of platelet: ultra-

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structural and functional aspects. Ann Hematol 1996; 73:103-12.
3. Murphy S, Heaton WA, Rebulla P. Platelet production in the Old World and the New. Transfusion 1996; 36:751-4.
4. Pietersz RNI, Reesink HW, Huijgens PC, van Oers MHJ. Preparation of leukocyte-poor platelet concentrates from buffy coats. IV. Clinical evaluation. Vox Sang 1988; 55:129-32.
5. Keegan T, Keaton A, Holme S, Owen M, Nelson E, Carmen R. Paired comparison of platelet concentrates prepared from platelet-rich plasma and buffy coat using a new technique with ^{111}In and ^{51}Cr. Transfusion 1992; 32:113-20.
6. Bode AP. Platelet activation may explain the storage lesion in platelet concentrates. Blood Cells 1990; 16:109-26.
7. Rinder HM, Snyder EL. Activation of platelet concentrate during preparation and storage. Blood Cells 1992; 18:445-56.
8. Rinder HM, Murphy M, Mitchell JG, Stocks J, Ault KA, Hillman RS. Progressive platelet activation with storage: evidence for shortened survival of activated platelets after transfusion. Transfusion 1991; 31: 409-14.
9. Feinberg H, Sarin MM, Batka EA, Porter CR, Miripol JE. Steward M. Platelet storage: changes in cytosolic Ca^{++} actin polymerization an shape. Blood 1988; 72: 766-9.
10. Fox JEB, Boyles JK, Steffen PK, Anderson LK. Identification of a membrane skeleton in platelets. J Cell Biol 1988; 106:1525-38.
11. Bertagnoli ME, Beckerle MC. Evidence for the selective association of a subpopublation of GP IIb/IIIa with the actin cytoskeletons of thrombin-activated platelets. J Cell Biol 1993; 121:1329-42.
12. Fox JEB, Boyles JK, Reynolds CC, Phillips DR. Actin filament content and organization in unstimulated platelets. J Cell Biol 1984; 98:1985-91.
13. Jennings LK, Fox JEB, Edwards HH, Phillips DR. Changes in the cytoskeletal structure of human platelets following thrombin activation. J Biol Chem 1981; 256:6927-32.
14. Casella JF, Flanagan MD, Lin S. Cytochalasin D inhibits actin polymerization and induces depolymerization of actin filaments formed during platelet shape change. Nature 1981; 293:302-5.
15. White JG. Arrangements of actin filaments in the cytoskeleton of human platelets. Am J Pathol 1984; 117:207-17.
16. Hartwig JH. Mechanism of actin rearrangements mediating platelet activation. J Cell Biol 1992; 188:1421-42.
17. Fox JEB, Phillips DR. Role of phosphorylation in mediating the association of myosin with the cytoskeletal structures of human platelets. J Biol Chem 1982; 257:4120-6.
18. Stark F, Golla RE, Nachmias VT. Formation and contraction of a microfilamentous shell in saponin-permeabilized platelets. J Cell Biol 1991; 112:903-13.
19. Jennings LK, Fox JEB, Edwards HH, Phillips DR. Changes in the cytoskeletal structure of human platelets following thrombin activation. J Biol Chem 1981; 256:6927-32.
20. Kometani M, Sato T, Fuji T. Platelet cytoskeletal components involved in shape change and secretion. Thromb Res 1986; 41:801-9.
21. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage. Nature 1970; 227:680-5.
22. Kometani M, Sato T, Fuji T. Platelet cytoskeletal components involved in shape change and secretion. Thromb Res 1986; 41:801-9.
23. Phillips DR, Jennings LK, Edwards HH. Identification of membrane proteins mediating the interaction of human platelets. J Cell Biol 1980; 86:77-86.
24. Escolar G, Diaz-Ricart M, Garrido M, et al. A variant of Glanzmann's thrombasthenia which fails to express a GPllb-IIIa related epitope that is recognized by a specific monoclonal antibody (C17). Br J Haematol 1992; 81:545-51.
25. Holme S, Vaidja K, Murphy S. Platelet storage at 22°C: Effect of type of agitation on morphology, viability and function *in vitro*. Blood 1978; 52:425-35.
26. White JG. Ultrastructural changes in stored platelets. Blood Cells 1992; 18:461-75.
27. Escolar G, Krumwiede M, White JG. Organization of the actin cytoskeleton of resting and activated platelets in suspension. Am J Pathol 1986; 123:86-94.
28. Gerrad JM, Schollmeyer JV, White JG. The role of contractile proteins in the function of the platelet surface membrane. Amsterdam:Elsevier/North-Holland Biochemical Press, 1981. p. 217-51.
29. Michelson AD, Adelman B, Barnard MR, Carroll E, Handin Rl. Platelet storage results in a redistribution of glycoprotein lb molecules; evidence for a large intraplatelet pool of glycoprotein lb. J Clin Invest 1988; 81:1734-40.
30. Fijnheer R, Modderman PW, Veldman H, et al. Detection of platelet activation with monoclonal antibodies and flow cytometry: changes during platelet storage. Transfusion 1990; 30:20-5.
31. Mazzara R, Escolar G, Garrido M, Pereira A, Castillo R, Ordinas A. Evaluation of the transfusion effectiveness of various platelet concentrates by means of an *in vitro* perfusion technique. Transfusion 1991; 31:308-12.
32. Snyder EL, Horne WC, Napychank P, Heinemann FS, Dunn B. Calcium-dependent proteolysis of actin during storage of platelet concentrates. Blood 1989; 73:1380-5.

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