Transfusion and Apheresis Science 41 (2009) 97–104

Contents lists available at ScienceDirect

Transfusion and Apheresis Science

journal homepage: www.elsevier.com/locate/transci

Preparation, storage and quality control of platelet concentrates

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A B S T R A C T

Patients with thrombocytopaenia need transfusions of platelet concentrates to prevent or stop bleeding. A platelet transfusion should provide platelets with good functionality. The quality of platelet concentrates (PCs) is affected by the preparation method and the storage conditions including duration of storage, type of storage container, and storage solution (plasma or an additive solution). Different *in vivo* and *in vitro* techniques can be used to analyse PCs. Platelets can be collected by apheresis technique, and from whole blood using either the buffy-coat or the platelet-rich plasma method. PCs can be gamma irradiated to prevent occurrence of graft-versus-host disease in the recipient. Pathogen inactivation procedures have been developed to prevent transmission of bacteraemia.

1. Introduction

Any disruption of the vessel wall needs to be repaired. The repair system is called haemostasis, which gives rise to a blood clot that seals the damage (Fig. 1). It is important that the haemostatic system is functional; a dysfunctional system can lead to either bleeding or thrombosis. Platelets play an important role in the haemostatic process by sealing damaged blood vessels, forming a platelet plug and prevent blood loss. Once the damage to the vessel wall has been covered, the platelets retract the coagulum, to allow the blood to flow freely in the vessel.

The successful treatment of malignant haematological diseases is dependent on transfusion of blood components as these patients have a lack of functional blood cells that may be caused by their disease or chemotherapy treatment. Platelets are transfused to patients who are severely thrombocytopaenic or to patients with platelet dysfunction to prevent bleeding or induce haemostasis. To ensure good haemostatic function in the recipient it is important that the functionality of the platelets used for transfusion is well preserved.

2. Preparation and storage of platelet concentrates

Platelets are stored at room temperature with constant agitation, which is necessary for the maintenance of platelet viability [1–3]. Platelet concentrates (PCs) have until recently been stored for a maximum of five days due to the risk of bacterial growth. The introduction of bacterial detection systems and bacteria inactivation procedures has made it possible to extend the storage period up to 7 days provided the platelet function is well maintained. AuBuchon et al. [4] and Dumont et al. [5] found a reduction in recovery and survival after autologous transfusion of platelets stored for 7 days compared with platelets stored for 5 days. Dumont et al., however, concluded that the magnitude of the changes would not result in the diminution of transfusion efficacy in a clinical situation.

Many factors influence the quality of platelets during storage. These include the preparation method of the platelets [6], the plastic material of the storage bag [1] and the ability of bags to exchange gas across its surface [7]. Other important factors that effect the quality are the storage temperature [6,8,9], the type of anticoagulant used, the platelet concentration in the bag and the agitation [6].

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doi:10.1016/j.transci.2009.07.001

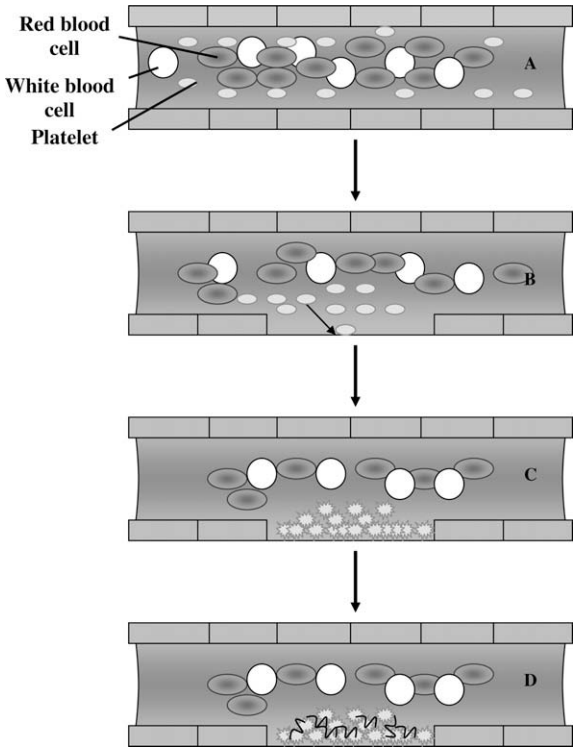

Fig. 1. The formation of a platelet plug at site of vascular injury. The laminar flow of the blood cells in an intact vessel with the platelets circulating close to the endothelium of the vessel wall (A). The platelets recognise vessel wall damage and adhere to the damaged site (B). Platelets undergo shape change, secretion and aggregate, forming a platelet plug (C). The platelet plug is stabilised by a fibrin network formed in the coagulation cascade (D).

2.1. Preparation methods

PCs can be prepared by the apheresis method (AP) or from whole blood using the buffy-coat (BC) or platelet-rich plasma (PRP) method. Platelets derived from whole blood are produced by the PRP method in the USA, whereas the BC method is used in Europe [10].

In the AP procedure, the blood from the donor is processed with a cell separator with an in-line centrifuge for platelet collection. The platelets are transferred to a collection bag, whereas the other blood cells and most of the plasma are returned to the donor. Various cell separators are available for platelet collection and have different collection principles [11]. It has previously been reported that cell separators induce various degrees of platelet activation [12,13].

The PRP and BC methods require pooling of platelets from several donors (usually 4–6) to obtain a platelet dose equivalent to that which can be obtained from a single donor by AP collection [10,14,15]. Since only one donor is required to produce a transfusion unit by AP technique the risk for immunisation is reduced as the recipient in this case is exposed to less antigen stimulation [16].

Using the PRP method to prepare PCs, whole blood is centrifuged by soft spin to prepare PRP followed by a high-speed centrifugation to obtain a platelet pellet. Most of the plasma is removed, and the platelets are stored in a reduced volume of remaining plasma. Alternatively, the platelets are re-suspended in a synthetic medium [17,18].

With the BC processing method whole blood is centrifuged at high-speed to prepare a BC. The BCs are pooled (often with a storage medium) and centrifuged to a platelet-rich supernatant that is transferred to the storage container [18].

2.2. Storage containers

Changes in pH in the PCs has been shown to greatly effect platelet viability [1,6,7]. To maintain pH during *in vitro* storage of platelets at 22 °C, containers with sufficient permeability for oxygen (O_2) and carbon dioxide (CO_2) are required [7,19]. First-generation storage bags made of polyvinyl chloride (PVC) plasticised with di(2-ethylhexyl) phthalate had poor oxygen permeability and therefore only allowed storage up to 3 days. Second-generation storage bags made of polyolefin [20] and PVC plasticised with tri(2-ethylhexyl) trimellitate [21] had increased gas permeability and allowed the storage period to be extended to 5 days. Storage containers made of PVC plasticised with butyryl-tri-*n*-hexyl-citrate have been developed. They have shown good gaseous permeability and result in good *in vivo* viability of platelets following transfusion [22,23].

2.3. Platelet additive solutions

The main energy source for platelets is the hydrolysis of adenosine triphosphate (ATP) and platelets must generate ATP continuously to meet their energy needs [24]. Platelets can generate ATP through two different metabolic pathways as shown in Fig. 2. One pathway requires oxygen, the tricarboxylic acid (TCA) cycle whereas the other, glycolysis, does not require oxygen. It has been suggested that the TCA cycle accounts for 85% of the ATP [19,25,26]. In the TCA cycle the substrate is free fatty acids in plasma [27] or acetate (if added to a synthetic medium). The end product is CO_2 [26]. Glucose metabolism may generate 15% of the ATP [26]. During glycolysis, glucose is converted to lactate and a free hydrogen ion [26]. The hydrogen ion can be buffered

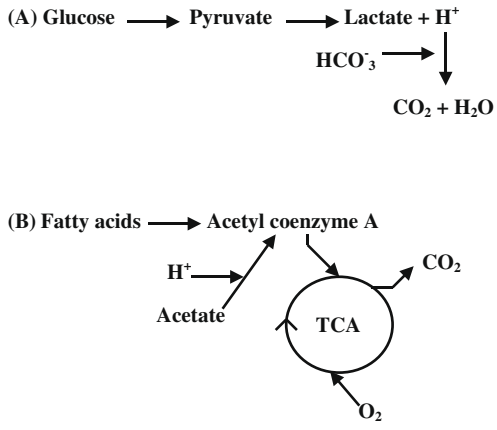

Fig. 2. Platelet metabolism. The glycolysis (A) and the tricarboxylic acid (TCA) cycle (B).

by the bicarbonate in the plasma and converted to CO_2 and H_2O [17,26]. The buffer capacity of the medium particularly bicarbonate will therefore also effect the pH level [28–30]. However, plasma only contains enough bicarbonate to buffer a lactate increase to about 28 mmol/L. Above that level, the pH will fall rapidly, which might result in a loss in platelet viability [17]. The decrease in pH however, is not entirely attributed to lactate formation. The amount of CO_2 also contributes considerably to the pH because CO_2 is a volatile acid [31]. It is therefore of great importance with gas permeable storage bags to keep the glycolysis to a minimum and allow the CO_2 that has formed to exit the PC through the walls of the container.

The use of a synthetic medium makes it is possible to include components in the storage environment that support good platelet function. It might also reduce the adverse transfusion reactions as most of these are caused by compounds present in plasma [32]. It was proposed that acetate should be included in the storage medium as an oxidisable fuel because 70–100% of the ATP might be produced aerobically [25]. The presence of acetate in the platelet additive solution (PAS) was shown to reduce lactate production [29,33]. Based on these findings, the additive solution PAS-II (T-Sol) was designed [34]. It is possible to store platelets for up to 7 days in a medium containing 70% PAS-II and 30% plasma [34]. PAS-III (InterSol), an additive solution that is used for pathogen inactivation procedures, has a similar composition to PAS-II, with the addition of phosphate [35]. The presence of phosphate improves the levels of adenine nucleotides [36]. Phosphate also has two counteracting effects on the metabolism. Phosphate acts as a stimulator of glycolysis but also as a buffer to prevent a decrease in pH caused by lactate production [36]. Composol and PAS-IIIM (SSP+) are two new storage solutions. PAS-IIIM is a modified PAS-III with the addition of potassium and magnesium and has been shown to improve the *in vitro* quality of platelets [37]. Composol has a similar composition to PAS-IIIM but lacks phosphate, Table 1. Platelets in Composol have been shown to have improved *in vitro* properties compared to platelets in PAS-II [38].

The composition of different additive solutions is shown in Table 1.

2.4. Prevention of transfusion-associated graft-versus-host disease

Leukocytes in the PCs can cause transfusion-associated graft-versus-host disease (TA-GVHD). TA-GVHD is mediated by the engraftment of allogenic T-lymphocytes [41]. The risk of developing TA-GVHD depends on the viability and number of contaminating lymphocytes, the susceptibility of the recipient's immune system to their engraftment and the degree of HLA disparity between donor and recipient. The risks of TA-GVHD is highest in recipients with immunodeficiency [42,43]. TA-GVHD is associated with a more than 90% mortality rate [41], and there is no effective therapy so far.

Leukocyte filtration is not effective enough to produce the level of lymphocyte removal required to prevent TA-GVHD [42]. PCs are gamma irradiated prior to transfusion to immunosuppressed patients to inhibit the proliferation of remaining lymphocytes and thus reduces the risk of TA-GVHD [41]. Luban et al. showed that the frequency of proliferating T-cells in the PCs decreased with irradiation dose. No growth was detected when a dose of 25 Gy was used [43]. Conflicting results have been reported with respect to whether gamma irradiation is harmful to the *in vitro* and *in vivo* properties of platelets [44–48]. An irradiation dose of 25 Gy has been recommended, and can be performed at any time during the storage period [42].

2.5. Prevention of transfusion-transmitted bacteraemia/sepsis

Bacterial infections transmitted by blood transfusion are associated with the rapid onset of sepsis and account for >10% of transfusion-associated fatalities [49]. PCs have been shown to be associated with greater risk of transfusion-transmitted bacteraemia than red blood cells. Since PCs are stored at room temperature, the introduction of a small number of contaminating bacteria during component preparation can result in high numbers during storage at this temperature [50].

To prevent transmission of bacteraemia, several methods have been developed to screen for bacteria-contaminating PCs. These include analysis of glucose levels, oxygen tension, detection by solid-phase laser cytometry, dielectrophoresis and automated bacterial culture [51].

Another option to prevent transmission of bacteraemia is through a pathogen inactivation procedure. The Intercept system uses amotosalen HCl (S-59) for pathogen inactivation. The Intercept process inactivates contaminating bacteria, viruses, and protozoa [35,50,52,53]. It also enables storage for longer duration than 5 days [35]. The Intercept system inactivates leukocytes and thereby eliminates the need for gamma irradiation to prevent TA-GVHD [54,55]. When added to the blood component, the amotosalen

Table 1
Composition of different platelet storage solutions.

	Plasma-lyte-A [39]	PAS-I [34]	PAS-II [34]	PAS-III [37]	PAS-IIIM [37]	Composol [40]
NaCl	90	70	115.5	77	69	90
KCl	5	10			5	5
$MgCl_2$	3				1.5	1.5
Na_3 citrate		30	10	10	10	11
Na acetate	27		30	30	30	27
Na phosphate		5		26	26	
Na gluconate	23					23
Mannitol		30				

Note. All values are expressed as mmol/L.
Platelet additive solution (PAS).

crosses the membrane or cell wall of the pathogen. Amotosalen reversibly intercalates into helical regions of DNA and RNA. Upon illumination with ultravilolet light (320–400 nm), amotosalen form covalent adducts and cross-links with nucleic acid, preventing replication [56,57]. A transfusion unit is suspended in 35% plasma and 65% PAS-III. The mixture, containing amotosalen HCl, is exposed to ultraviolet light to inactivate pathogens. The levels of residual amotosalen and free photoproducts are then reduced by compound adsorption device (CAD) treatment [57]. Varied *in vitro* results have been reported with respect to weather Intercept treatment is harmful to platelet function [58–60]. Several studies show that transfusion of Intercept treated PCs reduces *in vivo* recovery and survival [61], reduces corrected count increments (CCI) and shortens transfusion intervals compared with platelets in plasma or PAS-II [53,62–65]. Despite these drawbacks, Intercept platelets seem to be able to provide adequate haemostasis [63–65].

Another inactivation procedure developed called Mirasol PRT uses Riboflavin for inactivation of pathogens. Riboflavin intercalates between DNA and following treatment with ultraviolet light (265–370 nm) oxidises guanosine. This results in single strand breaks of nucleic acids and formation of covalent adducts [66]. Perez-Pujol et al. showed that Mirasol treated platelets had similar *in vitro* properties as non-treated control platelets [67]. This is in contrast to other studies, which have shown reduced *in vitro* properties of Mirasol treated platelets [68,69]. Treatment with Mirasol has also shown to reduce *in vivo* viability [68].

3. Quality testing of platelet concentrates

The quality of platelets during storage can be evaluated by determining the recovery and survival of the transfused platelets in thrombocytopaenic patients [70]. Calculation of corrected count increment (CCI) corrects for the patient's blood volume and the number of platelets in the PC when evaluating the post-transfusion platelet count increment. A 1 h post-transfusion CCI of 10–20,000 is considered a good response while a CCI of less than 7500 is a poor response and indicates transfusion failure [71]. An alternative approach is to measure the recovery and survival of fresh or stored radiolabelled platelets (^{51}Cr) in healthy volunteers [70]. However, as these *in vivo* studies are expensive and complex to perform, several studies use *in vitro* tests to assess *in vivo* viability, as discussed below.

3.1. Bleeding time

The Ivy bleeding time measurement can be performed if the patient receiving a platelet transfusion is relatively stable. The bleeding time has been shown to be inversely proportional to the platelet concentration in the range of 100–10×10^9 platelets/L [72]. It compares the shortening of the bleeding time with the increase in platelet concentration achieved. The bleeding time is affected by a number of factors not necessarily related to the platelet function. It is also complex to perform, resulting in operator variability. Because the thrombocytopaenic patients are often leukopaenic, there is risk of infection caused by the skin incision [17,73].

3.2. Blood gases

To evaluate the PC quality, pH, pCO_2 and pO_2 can be measured in the PCs. The pH has been shown to greatly affect the quality of the PCs. pH decrease during storage of PCs at 22 °C [1,31] which is due to glycolysis [7]. pH values of 6.4–7.4 do not correlate to *in vivo* viability [74]. A pH below 6.0 is associated with loss of viability [1,6] as is a pH greater than 7.4–7.6 [7]. pO_2 and pCO_2 reflect the permeability of the container. Increased pCO_2 leads to a pH decrease, which could be prevented by CO_2 egress from the storage container.

3.3. Metabolic parameters

The concentration of glucose and lactate in the PCs are commonly used as quality parameters. A decrease in glucose and an increase in lactate concentration during storage have been reported in several studies [4,58,75,76]. Depletion of glucose and increase in lactate concentration to more than 28 mmol/L can result in low ATP levels and a rapid pH reduction which might result in a loss of platelet viability [17,18,77]. Measurement of cytoplasmic leakage of lactate dehydrogenase (LDH) can also be used as a quality parameter and reflects platelet membrane damage. LDH has been shown to correlate to platelet survival ($r = -0.64$; [78]).

3.4. Swirling

Discoid platelets exposed to a light source reflect light and thereby producing the "swirling" phenomenon. Swirling is routinely used to evaluate the quality of PCs. Swirling is determined by examining a PC against a light source while gently rotating the container or gently squeezing the PC [79]. The presence of swirling is informative because it is highly effective in predicting a pH value in an adequate range. The presence of swirling was associated in 94% of cases with a pH value in the range of 6.7–7.5 [79]. This pH range is associated with adequate *in vivo* survival [1,7].

3.5. Hypotonic shock response (HSR)

Addition of a hypotonic solution to platelets results in initial swelling followed by a gradual decline as the platelets resume their baseline size. This can be measured spectrophotometrically at 610 nm [80]. Osmotic swelling of a cell increases cytosolic calcium and the release of ATP from the cell. This initiates several signalling processes in the cell. These responses lead to eventual K^+ and Cl^- ion and water egress from the cell and correction in cell size [81]. The response to hypotonic stress has been shown to be positively correlated with platelet viability [80,82].

3.6. Platelet activation

Platelets express a number of markers on their surface involved in haemostasis. Platelets become activated during

the preparation and storage of PCs. The activation can be measured as change in the surface expression of markers using flow cytometry. Both activation state and the reactivity of the platelets can be determined using flow cytometry [83]. Antibodies directed against activation-dependent epitopes are used to evaluate activation. Activation markers of interest that can be studied in such a way include P-selectin (CD62P), by the use of anti P-selectin, and the active conformation of GPIIb/IIIa, by the use of PAC-1 or indirectly by the use of anti-fibrinogen [83–85] as well as GPIb expression. *In vitro* platelet reactivity towards various agonists such as ADP, collagen, thrombin receptor activating peptide (TRAP) and thrombin can be studied [83]. The platelets in a sample can be detected based on their forward scatter (FS) and side scatter properties (SS) as shown in Fig. 3. FS is related to the cell size and SS to the cytoplasmic complexity/granularity of the cell. Once the platelets have been identified they can be analysed with respect of the property of interest (i.e. the activation epitope). The fluorescent signal can be expressed as the percentage of positive cells (relative to an irrelevant (isotype) control antibody) or as the mean fluorescence intensity (MFI) of the entire platelet population as shown in Fig. 3 [83].

Increased P-selectin expression during storage has been reported by several authors [12,86–89], whereas GPIb has been shown to decrease during storage [88,90]. The response towards different agonists have been shown to be decreased during storage [48,58,91]. It is, however, unclear whether the level of *in vitro* platelet activation in stored PCs correlates with *in vivo* survival and haemostatic function of platelets after transfusion [92]. Rinder et al. demonstrated that platelets characterised by increased *in vitro* activity are rapidly cleared from the circulation *in vivo* [93]. P-selectin and GPIb were shown to be involved in regulating post-transfusion platelet clearance by mediating adhesive interactions of platelets with counter receptors on macrophages and endothelia cells [94,95]. Significant negative correlations between P-selectin exposure and post-transfusion platelet recovery and survival have been reported [5,78,93]. However, in a recent study no such correlation could be detected [96].

3.7. Aggregometry

PRP is used for measurement of aggregation. PRP is stirred in a cuvette at 37 °C between a light source and a detector. Aggregation is initiated using an agonist such as collagen, thrombin or ADP. When platelets aggregate, the light transmission increases and is recorded as the rate of aggregation and maximum response. By comparing responses to different agonists platelet defects can be identified [97]. Aggregation measurements on platelets from PCs do not appear to be able to predict platelet recovery as platelets stored at 4 °C have better ability to aggregate than those stored at 22 °C [98] despite low recovery and survival.

3.8. Coagulation analysis

Free oscillation rheometry (FOR) and thromboelastography can be used to evaluate coagulation properties of whole blood and platelet-rich plasma [99,100]. FOR and thromboelastography can be used for analysis of coagulation as

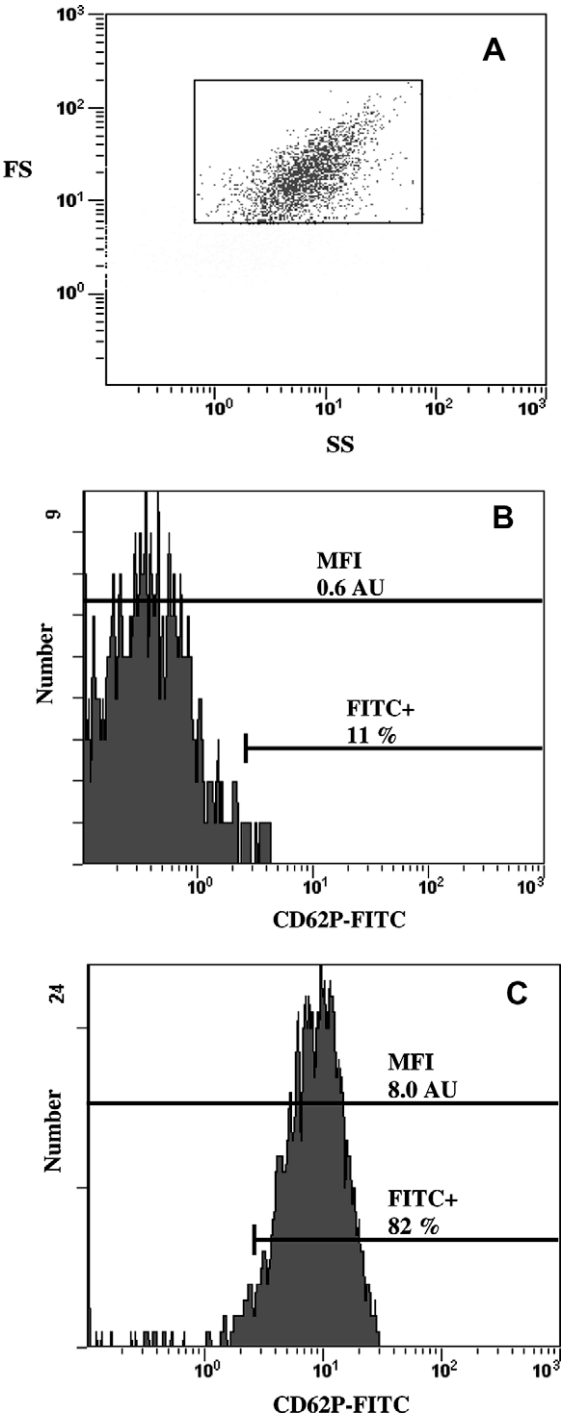

Fig. 3. Flow cytometry plots. The detection of the platelet population based on their forward scatter (FS) vs. side scatter (SS) properties (A). The spontaneous binding of fluorescein isothiocyanate (FITC) conjugated CD62P to platelets (B). The binding of CD62P-FITC to platelets following stimulation with thrombin receptor activating peptide (TRAP), TRAP-6 (C). The binding can be expressed as percentage of positive platelets (platelets within the FITC + region) or mean fluorescence intensity (MFI in arbitrary units, AU) of the entire platelet population.

measured by clotting time and changes in clot elasticity. The clot elasticity provides information about the fibrin network in the coagulum and the platelets' ability to support the formation of a clot and to retract the coagulum.

FOR analysis is performed using the ReoRox® 4 instrument (Medirox, Nyköping, Sweden). The sample is added to a cylindrical sample cup, which is set into free oscillation. The FOR analysis gives a curve from which the clotting time and the maximum elasticity (G'max) can be determined [99]. There are two commercially available thromboelastographs, TEG® 5000 (Haemoscope Corporation, Niles, IL, USA) and ROTEM® (Pentapharm GmbH, Munich, Germany). The TEG® 5000 consists of an oscillating sample cup. A pin is suspended in the blood sample by a torsion wire and is monitored for motion [101,102]. The TEG® analysis also gives a curve from which the reaction time (*R*) and the maximum amplitude (MA) can be obtained [102]. The ROTEM®, in contrast, has a fixed sample cup with a pin suspended in the blood sample. The pin oscillates and the movement is registered [103]. The ROTEM® analysis give rise to a curve from which the clotting time (CT) and the maximum clot firmness (MCF) can be determined [100].

The clotting time for the rheometer corresponds to *R* for TEG® and CT for ROTEM®. The G'max for the rheomter corresponds to the MA for TEG® and MCF for ROTEM®.

Some studies have reported a small increase in maximum clot retraction of platelets in concentrates with storage concomitant with a prolongation in time for the platelets to attain full clot retraction [48,91,104]. Other studies have not detected any change in clot retraction over storage [105,106].

4. Concluding remarks

The purpose of a platelet transfusion is to supply platelets with good haemostatic properties to patients with thrombocytopaenia. It is therefore very important to control the quality of platelet concentrates. The quality of platelet concentrates is affected by the preparation methods and storage conditions. The quality of PCs can be evaluated by measuring platelet viability following transfusion. Such measurements are expensive and complicated to perform and *in vitro* methods are often used to evaluate PC quality as surrogate tests to *in vivo* viability. The value of the commonly used *in vitro* tests in predicting *in vivo* viability is not clear and few of them directly measure the platelets haemostatic function. In the 1970's the hypotonic shock response was shown to correlate to *in vivo* viability [80,82]. However, in a recent study no such correlation was found [96]. pH values below 6.0 or above 7.4 have been shown to result in low viability [1,6,7], but pH in the range of 6.4–7.4 has been shown not to correlate with *in vivo* viability [74]. The relevance of increased platelet activation on *in vivo* viability is not clear. Some studies have shown a negative correlation between P-selectin expression and *in vivo* viability [5,12,78,93] but some report only a low correlation [12,93]. Furthermore, a recent study found no correlation between P-selectin expression and *in vivo* viability [96]. Thromboelastography and rheometry are new methods that can be used to evaluate the haemostatic properties of platelets in concentrates [48,91,106]. Their ability to be to predict the survival and the post-transfusion haemostatic efficacy of platelet concentrates needs to be further studied.

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