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TRANSFUSION
CLINIQUE ET BIOLOGIQUE

Transfusion Clinique et Biologique 25 (2018) 269–275

Update article

# General overview of blood products in vitro quality: Processing and storage lesions

## *Revue générale sur la qualité in vitro des produits sanguins labiles : lésions dues à la fabrication et à la conservation*

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Available online 31 August 2018

**Abstract**

Blood products are issued from blood collection. Collected blood is immediately mixed with anticoagulant solutions that immediately induce chemical and/or biochemical modifications. Collected blood is then transformed into different blood products according to various steps of fabrication. All these steps induce either reversible or irreversible "preparation-related" lesions that combine with "storage-related" lesions. This short paper aims to provide an overview of the alterations that are induced by the "non-physiological" processes used to prepare blood products that are used in clinical practice.


*Keywords:* Plasma; Platelet; Processing; Red blood cell; Storage; Transfusion

**Résumé**

Les produits sanguins labiles sont préparés à partir de don de sang. Celui-ci est collecté et immédiatement additionné de solutions anticoagulantes qui induisent les premières modifications chimiques et biologiques du sang prélevé. Une cascade de « lésions » est alors induite par l'ensemble des processus de fabrication des produits sanguins qui s'additionnent aux lésions de stockage qui s'accumulent durant la conservation de ceux-ci. Cette revue présente certaines modifications chimiques, biochimiques ou morphologiques qui résultent des conditions non physiologiques qui sont utilisées en routine pour « fabriquer » et « conserver » les produits sanguins labiles utilisés en pratique clinique.


*Mots clés :* Globules rouges ; Plaquettes ; Plasma ; Préparation ; Stockage ; Transfusion

## 1. Introduction

The ex vivo treatment of blood for transfusion is not trivial and the obtained labile blood products are impacted at all steps of the processes, from the donor to the patient. Obviously, the best product will be a fresh one without any preparation fulfilling all the requirements in terms of quality and safety. Biological qualification of all blood donations needs time implying that blood has to be stored until results are obtained. Furthermore, the blood needs to be transformed and separated in its different components because of logistical issues and blood cells particularities. Therefore, several strategies have been developed to store as appropriately as possible the blood components and to secure transfusion. It includes the addition of anticoagulants, centrifugations, filtrations and the separation of blood components, which are placed

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https://doi.org/10.1016/j.tracli.2018.08.162
1246-7820/

in various additive solutions. Finally, blood products may be treated using pathogen inactivation technologies or novel storage strategies to improve the quality of the blood products.

In the last two decades, an effort was put on the characterization of blood products using omic sciences in addition of continuous development in microscopy and cytometry analyses. The results hence obtained have deeply explored the content of blood cells and blood bags, and have greatly improved our understanding of ex vivo aging [1–4]. Beyond the acquired knowledge, they open the question of the qualification of blood products and the markers that should be used for the product specifications.

The present review will therefore present recent data on the effect of processing and storage on red blood cell concentrates (RCCs), platelet concentrates (PCs) and plasma. The focus is on in vitro data and the clinical data will not be specifically reviewed here.

## 2. Red blood cells

Red blood cell (RBC) lesions start when the blood is withdrawn from a donor. Then each following step to the patient will contribute to the storage lesions [5,6]. The ex vivo behavior of RBCs is influenced by the donor, the processing, the addition of an additive solution, and the cold storage in a permeable plastic. All of these steps change the metabolism, the protein content and function, and the morphology in a cascade of events [7–9]. Even if the donors' characteristics will not be treated here, donors have also an impact on the storage of RCCs [10–15].

The analyses of metabolites (where the achievement has exploded with the introduction of metabolomics during the last decade) [4] have shown a decrease in glycolysis and oxidative pentose phosphate pathway (PPP) rate, a decrease in energy metabolites, rewiring between the non-oxidative PPP and the glycolysis and accumulation of purine end products at the end of the storage. These rerouting in different metabolism pathways can be classified in three distinct zones as observed in statistical analyses of metabolomic data [16,17]. Hence, 8 extracellular metabolites able to discriminate the age of RCCs in three different phases were identified [18]. One of these purine end-product metabolites is the hypoxanthine that accumulates in RBCs as well as in the supernatant of RCCs. In case of transfusion of long-term stored RCCs, oxidation of circulating hypoxanthine by circulating xanthine oxidase will increase reactive oxygen species (ROS) that may initiate inflammation reactions [19]. Of interest, D'Alessandro and colleagues reported a negative correlation between the intracellular level of hypoxanthine and the post transfusion recovery in mouse and human (even though the data on human were less significant and required further investigations) [20]. In the same study, the authors also demonstrated that the level of hypoxanthine is decreased by hypoxia both in vivo (in volunteers exposed to high altitude) and in vitro (in RCCs stored at different levels of O2 saturation). All these researches confirmed the citrate metabolism (remnant of the TCA cycle), the influence of oxygen content and the influence of the composition of additive solutions. As for the use of additive solutions, where the composition and pH affect the metabolism, the action of diluting plasma triggers the excretion of urate during the first 7 to 10 days of storage that changes the antioxidant level of RCCs and that might affect in turn the RBC metabolism [10].

Because of the enzymatic regulation of several reactions in RBCs, protein functions are also impaired. For instance, phosphorylation activity, that requires ATP, is known to regulate glycolytic enzymes binding the band 3 [21,22]. Moreover, we have reported that the capacity of membrane protein phosphorylation is lost during the storage [23]. Within these regulations linked to band 3 complexes, the oxidation of GAPDH modulates the metabolism from glycolysis to PPP [24]. After 3-4 weeks of storage different types of oxidative damages are observed on proteins such as cysteine oxidation or protein carbonylation [25–29]. Protein complex reorganizations and migration were also detected such as the TALDO/SOD complex [30], the association of flottilin-2 to band 3 complexes [31], or the accumulation of peroxiredoxin II at the membrane [32]. On the contrary, another study on γ-irradiated or pathogen inactivated RCCs did not observe this accumulation of peroxiredoxin II [33].

At a higher cell level, morphology is also affected with a significant effect during the last two weeks of storage. The percentage of discocytes (or other reversible cell morphology) decreases and spherocytes are formed [9,34,35]. The formation of small RBCs were also recently reported after 28 days of storage [35]. All these modifications that reach more than 10% of the cell population decreases the transfusion efficacy because these types of RBCs are rapidly removed from the circulation once transfused (of note a surface loss of more than 18% is sufficient to trigger the elimination by the macrophages [36]). The cell deformability that is of primary importance to cross the capillaries is reduced after 3 weeks of storage [35] and spherocytes are known to have lower membrane fluctuations than discocytes [9]. All the cellular parameters are currently under investigation using different approaches of flow cytometry, fluorescence exclusion, quantitative phase microscopy or other microchannel-based analyses [9,35,37–40].

Finally, all these lesions end up with the formation of microvesicles [41] that accumulate in the supernatant [9,42,43]. These small particles can be generated by different ways (protein oxidation or phosphorylation like in thalassemic RBCs, or calcium stimuli) and contain a different proteome [27,44,45]. These microvesicles have procoagulant properties and might contribute to inflammation in some patients [46–49].

Different strategies have been employed or investigated to tackle the storage lesions or to reduce their formation such as rejuvenation procedures [50–52]. One of the solutions is the storage under anaerobic condition that improves energy metabolism and decreases the hemolysis [53,54]. Recent investigations also showed that this storage better preserves the mechanical properties of RBCs and decreases the number of plugging events in microchannels [55]. Moreover, a moderate reduction of oxygen content (below 20% of $O_2$ saturation on hemoglobin) increases the levels of both ATP and 2,3-DPG compared to standard storage [56].

## 3. Platelets

Platelet concentrates can be prepared in several manners. For instance, PCs can be either directly collected from a donor using an apheresis procedure or prepared by pooling buffy coats with an additive solution. Pooling of buffy coats can be done manually or with automated centrifuge separators. Several additive solutions can be used to replace part of the plasma content. Furthermore, PCs can be treated for pathogen reduction with the Intercept™ (Cerus, Concord, USA), Mirasol® (Terumo BCT, Lakewood, USA) or Theraflex (MacoPharma, Tourcoing, France) technologies in order to secure the product from bacterial contamination and/or emerging pathogens. Conventional platelets are stored at room temperature under agitation but, recently, we observe a regain of interest for the storage of platelets in cold conditions or for the cryoconservation [57]. The choice of the processing workflow is guided by the European and national guidelines and obviously by the production costs. Consequently, a large diversity of practices exists among the blood centers. Nevertheless, some tendencies appear to qualify the storage lesions and will be shortly reviewed here.

Similarly to RCCs, platelets are affected as soon as they are in an ex vivo environment and the extent of these lesions are both related to the donor characteristics and to way the blood component is processed and stored. The first easily observable process-related lesion occurs during the collection of apheresis PCs when aggregates are present in the products. Platelet activation during the procedure leads to the formation of aggregates and is highly dependent on the donor itself and on stress engendered by the procedure. These aggregates are reversible and will in general be dissolved within two hours under standard agitation. Further platelet lesions will then develop during the course of storage. So far, the quality of ex vivo platelets has been explored using visual inspection (presence of aggregates, swirling score), in vitro assays (aggregometry, flow cytometry, clinical chemistry) and more recently with metabolomics[4], transcriptomics [58,59] or proteomics [60].

Lesions in platelets stored under agitation at room temperature have been extensively reviewed [59,61–63]. Throughout the storage, platelets experience a functional decline highlighted by an increase of activation markers, morphological changes, mitochondrial dysfunction, loss of GPIbα and α-granule secretion, and a decrease of collagen and thrombin activated (COAT) platelets [61,64]. Release of immunomodulatory cytokine, chemokines and associated molecules known to be biological response modifiers has been reported in stored PCs [65]. In opposition to the linear decay of metabolism reported by in vitro functional assays, metabolomics approaches suggested the expression of discrete metabolic phenotypes during storage [66] and that metabolites present in stored PCs may be associated with platelet recoveries and survivals [67].

Pathogen reduction technologies tend to accelerate the apparition and extent of these lesions [63]. Intercept-treated platelets show metabolic changes, impaired mitochondrial function, accelerated passive activation, and altered agonist-induced platelet aggregation [68–74]. The risk of increased storage lesion rates following Intercept is higher for apheresis PC, especially when platelet contents are higher than $5 \times 10e11$ [75]. Whereas marginal global proteome alteration were reported following pathogen reduction treatments, Intercept seems to affect proteins involved in platelets activation an aggregation pathways [76]. Because these technologies rely on a photochemical action, reactive oxygen species are generated and consequently, a decrease of antioxidant power is observed in pathogen-reduced PCs [77]. Oxidative damages are also detected on peptides suggesting probable oxidation at the protein level [78]. In addition, using metabolomics we showed oxidative damages in stored Intercept-treated platelets compared to controls, in particular alteration of the purine and the glutathione metabolism and diminution of antioxidant defenses such as the conversion of urate to allantoin, only possible in humans under the action of reactive oxygen species [79–81].

Similarly, Mirasol-treated platelets stored at room temperature exhibit an increased expression of activation markers, higher lactate production and increased glucose and oxygen consumption, as well as lower ATP over storage time [69,82–96]. The ultraviolet light alone has been shown to contribute significantly to the lesions observed upon Riboflavin/UVB treatment [81]. The Mirasol treatment leads to hyper reactive platelets resulting in a reduction in the degranulation capacity upon stimulation [97]. Proteomics studies reveal that Mirasol impacts few proteins that are mostly related to actin polymerization, cytoskeleton organization and platelet shape change [76,98].

The Theraflex technology relies on a UVC illumination alone that has been shown to disrupt platelet surface disulphide bonds and activate the platelet integrin αIIbβ3 [99]. Efficient mixing of PCs during UVC treatment is essential to ensure homogeneous illumination of the blood components and improve bacterial inactivation. The enhanced agitation speed does not affect quality variables [100]. Activation markers and exposure of phosphatidyl serine as well as metabolic activity are increased in Theraflex-treated units. This treatment attenuates thrombus formation kinetics in vitro in microfluidic flow chambers, especially after storage [101].

A molecular model is emerging for explaining the quality decline of Intercept and Mirasol-treated platelet concentrates [63]. P38MAPK kinase is one of the central players in the signaling cascade that regulates the degranulation, mitochondria release, expression of surface glycoproteins, levels of mRNA expression, microvesicule release and development of apoptosis [63]. Further investigations are required to fully characterize the key role of this regulator in platelets. Moreover, further investigations are required to better understand the oxidative damages occurring in PCs treated for pathogen reduction [80]. The clinical efficacy of pathogen-inactivated platelets stored has been questioned as some reduction in post-transfusion recovery and survival in vivo was suggested [102]. A recent Cochrane systematic review including 12 completed trials with either the Intercept or Mirasol technologies reported that, in people with haematologic or oncologic disorders, there is high quality evidence that pathogen-reduced platelet transfusions increase the risk of platelet refractoriness and the platelet requirement. Moreover, they found moderate-quality evidence that pathogen-reduced platelet transfusions do not affect all-cause mortality, the risk of clinically significant or severe bleeding, or the risk of a serious adverse event [103]. Clinical data with the Theraflex technology are not yet available as the Phase III CAPTURE trial is ongoing [102,103].

Cold-stored platelets are conserved in refrigerator (2–6 °C) without agitation. This technique of conservation was abandoned in the 1970s because recirculation time of refrigerated platelets was dramatically reduced compared to platelets stored at room temperature. During storage, the Integrin GPIIbIIIa undergoes conformational changes to its activated form, an increase of expression of P-selectin and externalization of phosphtidyl serine as well as an irreversible morphological change are observed in refrigerated platelets [57]. Desialysation and clustering of the glycoprotein GPIbα lead to the exposure of N-acetylglucosamine (GlcNAc) and galactose. Recognition of exposed GlcNAc by the αMβ2 integrin on macrophages in the liver results in the rapid removal of platelets from circulation [104]. Galactose becomes exposed as storage progresses, which facilitates platelet clearance by hepatic Ashwell-Morell receptors [105]. However, refrigerated platelets present other advantages such as a reduced metabolic rate that allows the shelf life to be extended up to 21 days. Granule and cytokine release are decreased while microparticles are enhanced compared to platelets stored at room temperature. Bacterial growth is inhibited at cold storage therefore limiting the occurrence of transfusion-transmitted sepsis [57].

Cryopreserved platelets are stored at −80 °C after the addition of DMSO followed by prefreeze removal of supernatants. Of interest, cryopreserved platelets can be stored up to 2 years. Before use, platelets are thawed, resuspended in an adequate saline solution and rapidly transfused [106]. Cryopreserved platelets present a decrease of the glycoproteins GPIbα, GPVI and integrin αIIbβ and they are more activated and less responsive to basal stimulation than conventional platelets stored at room temperature [107,108]. Enhanced platelet degranulation has also been observed [107]. Nevertheless, cryopreserved platelets are haemostatically functional and are reported to be clinically efficient [109].

The pro-coagulant properties and extended shelf-life of cold-stored and cryopreserved platelets make them very attractive for a therapeutic use to stop bleeding and in military operational settings, for instance [110].

In a recent review, Waters et al. discussed the different platelet processing and storage strategies (i. e refrigerated and cryoconserved platelets) and how they may be combined, for example with pathogen reduction techniques, to alleviate the problems associated with conventional platelet storage. This novel concept may permit to improve the quality of the PCs and offer a panel of products that fits better the clinical needs [57].

## 4. Plasma

Different types of plasma products available worldwide depends on the collection, the time-to-freezing or the securisation procedure (quarantine, chemical or photochemical treatment), for instance. Fresh frozen plasma (FFP) are frozen plasma in less than 8 h post-donation and frozen plasma 24 (FP24) in less than 24 h. All these plasmas can be stored for several hours or days when thawed depending on requirements. Pathogen-reduced (PR) plasmas can be produced as S/D-plasma by using detergent and solvent extractions, followed by filtration. It is obtained after the pooling of more than thousand units of FP24. PR-FFP or PRI-FP24 are treated by a photochemical reaction using combination of riboflavin and UVB (Mirasol Pathogen Reduction Technology System, Terumo BCT); of methylene blue and visible light (Thereaflex MB-plasma (MB), Macopharma) or of amatosalen and UVA (Intercept Blood system, Cerus) before freezing. Methylene blue induces various protein lesions that notably affect fibrinogen and fibrin generation [111,112]. Lyophilization (Lyo-plasma) or freeze-drying procedures are also applied to produce a powder that is then reconstituted with sterile water. The Lyo-plasmas are derived from FFP, S/D-plasma or PR-FFP lyophilized by different techniques. The specifications for quality control are in general related to fibrinogen and factor VIII (that is a labile factor and thus the most sensitive one for quality control), amongst others parameters such as obviously the donor qualification, the volume, and the levels of cell contamination. In addition, several studies in the literature report data on factors and inhibitors of the coagulation, protein profiles, effect on the activated partial thromboplastine time (aPTT), the prothrombin time (PT) and the thrombin generation.

FFP is the fewer affected plasma compared to other preparations because of the short time-to-freezing. Factors are well preserved, in particular the factor VIII [113–115]. As for FP24 where the time-to-freezing is higher than 8 hours, factor VIII is decreased by around 20% whereas other factors are affected to a smaller extent [113,116]. Once thawed, the concentration of this factor is continuously decreasing quickly reaching the lower level of specification of 0.7 UI/mL [115,117]. The best results were obtained when the plasma was rapidly frozen [118].

The S/D treatment of plasma mainly affects the α2-antiplasmin and the total protein S with a decrease of 60 to 80% and of 20 to 38%, respectively [119]. Moreover, this type of plasma presents a pro-coagulant phenotype because of a reduced intact protein S level [119], as wells as an increased thrombin generation compared to FFP [119,120]. Of note, the level of α2-antiplasmin was improved by using the detergent Triton X-45 instead of Triton X-100. [121]

Another strategy to inactivate pathogens is the use of photochemical treatments. Mirasol technology well preserves the factors especially the sensitive one compared to other technologies with activities remaining around 96 to 100% compared to FFP [122]. MB induces a reduction of approximately 22–30% compared to FFP [114,115]. However, Osselaer et al. obtained slightly better results when using Intercept (20% of reduction in factor VIII). In this case, the plasma was frozen in less than 8 h post-donation, which explains the highest factor VIII level obtained. Indeed, this factor is already lower in FP24 than in FFP, therefore reducing the final level in PR-FP24 close to the threshold of 0.5 UI/mL [123]. This trend was also recently observed by Erickson et al. [124]. As for the other factors or inhibitors, they are also affected depending on the time-to-process where the best results were obtained with the freshest plasmas. Finally, a procoagulant phenotype was reported after Intercept treatment based on thrombin generation [120,123], like the S/D-plasma.

Last but not least, the lyophilized plasma provides alternative strategies to store plasma as powder especially in places where frozen storage solutions are not available [125]. These types of plasma are derived from FFP, S/D or PR-plasma. By consequences, their in vitro qualities reflect the in-coming product with low level of α2-antiplasmin for S/D-plasma [126], low level of factor VIII in PR-plasma [127], and a homogenous impact when using FFP [128]. The advantages of the lyophilized plasmas are the availability and the short time of reconstitution lower than 10 minutes [125].

In summary, the more the plasma is processed, the worst the impact. Nevertheless, all the available plasmas for transfusion (in general) respect the specifications and the different factors and inhibitors fall within the normal physiological ranges. Some of the preparations provide advantages such as levels of factors closest to the donor values, the inactivation of pathogens (the issues being mainly on emergent or unscreened pathogens) or the homogeneity in terms of volume and protein contents.

## 5. Conclusions

The knowledge on blood products and particularly on stored platelets and RBCs have been greatly improved in the last two decades. Even if there is an increasing interest to develop new quality parameters, it is still unclear what should be done in routine, because the risks related to both preparation-related and storage-related lesions effects in transfused patients are not. Nevertheless, the comparison to the physiological parameters can guide the decisions and advanced quality controls might be considered such as metabolite markers or cell dynamic (both RBCs) [55] and platelets [129].

## Disclosure of interest

The authors declare that they have no competing interest.

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