Platelet Storage – Current limitations and future solutions

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ABSTRACT

Platelets are small anucleate cell fragments found in the blood, which originate from large cells known as megakaryocytes. Platelets are widely used in transfusion medicine for therapeutic purposes. Platelet transfusions are beneficial for patients who are experiencing thrombocytopenic conditions or heavy bleeding and demands for platelet concentrates (PCs) are constantly on the rise. Current regulations that govern how PC units are prepared require that these be stored at a temperature of 22°C±2°C, under constant agitation for 5 days. This short shelf-life is due to the high susceptibility of this product to bacterial contaminations and platelet storage lesions (PSL). Many publications demonstrate how storing PCs at lower temperatures may help increase the shelf-life of this blood product. Prolonging the shelf-life will consequently decrease unnecessary wastage of valuable donations and the overall costs associated with PC production. Furthermore, cold stored platelets decrease both the risk of bacterial contamination and the occurrence of PSL.

Keywords: Platelet Concentrates, cold storage, prolonged shelf-life, quality, safety

1. INTRODUCTION

Platelet transfusion is considered an essential part of therapeutics and is beneficial for patients who are either suffering from a thrombocytopenic condition or experiencing heavy bleeding, which could be either due to trauma or surgery(1). Platelet concentrates (PCs) act by preventing further haemorrhage in affected individuals. Due to its wide range of essential purposes, PCs are constantly requested in high demands. In fact, in Europe and in the United States of America, approximately 5 million PCs are administered annually(2).

2. CURRENT STORAGE AND LIMITATIONS

As per the European Directorate for the Quality of Medicines and HealthCare guidelines, the general storage conditions currently implemented by blood banks for PCs is a temperature of 22℃±2℃, with constant agitation inside sterile gas-permeable bags and surfaces. To date, depending on national regulations, such conditions provide a maximum shelf-life of approximately 5 days. This may be extended up to 7 days if sterility testing is performed(3). These storage conditions, however, present some limitations, namely the high risk of pathogen contamination, as well as the occurrence of platelet storage lesions (PSL). PCs stored at a temperature of 22℃±2℃ are highly susceptible to microbial contamination, since the optimum temperature for most bacterial species to proliferate is at around 20℃(4). Bacterial contamination may lead to the introduction of life-threatening infections, which lead to sepsis, thus causing harm to patients(5). PSL is a condition triggered by the processing of PCs and may occur at any point in time between collection and transfusion, whereby platelets undergo an alteration in their morphology, functionality, and metabolic mechanism(6). In the event of such lesions, transfused platelets may not deliver an adequate therapeutic effect *in vivo*(7). In view of this, no standard testing parameters are being considered(8).

Such aforementioned limitations minimize the PC's shelf-life, and a short shelf-life will result in increased unnecessary wastage of expired PC units, decreased availability of PCs and an increased sense of pressure to keep up with high demands. Several publications illustrated the extent of such issues. It had been reported that the mean annual discard percentage amongst seventeen different clinics, located in several different countries around the world,

was 13%(9). Another publication revealed that the discard percentage in Australia alone was around 20%(10). Moreover, the production of such units is costly, which makes wastage a major financial loss.

3. COLD STORAGE – A POSSIBLE SOLUTION

Over the years, several different methods have been implemented in the hope of reducing PC wastage and enhancing product availability. An alternative approach, first introduced in the medical field back in the 1960s, was storing PCs at a temperature of 4±2℃. During this period, it was common practice to store all PCs in the cold, however, such a method was abandoned in the 1970s, due to newly emerged findings. Such findings suggested that although cold-stored platelets resulted to be superior to room temperature stored platelets through *in vitro* evaluations, these resulted to be inferior through *in vivo* evaluations(11). Hence, platelets were once again stored at the conventional 22±2℃ and this method of storage has been in place ever since, up until recently. Currently, the possibility of utilising cold-storage is being explored once again in several research studies and clinical trials.

Cold-stored PCs are slowly regaining popularity due to their ability to reduce the two main issues which limit their shelf-life, that is, bacterial contamination and PSL. Several studies are currently testing and demonstrating the various benefits of cold-stored platelets. Analyses are being performed to assess both the *in vitro* and the *in vivo* qualities of the cold-stored PCs. The wide range of *in vitro* and *in vivo* analyses, conducted in several different studies, on cold-stored platelets provide key findings regarding the impact that low temperatures have on platelets. The various outcomes derived were evaluated and interpreted accordingly in order to determine the underlying principles involved, and to establish whether platelet functions were preserved, diminished, or enhanced when compared to traditional storage. These findings, hence, allowed for the discovery of both the benefits and drawbacks of cold storage.

3.1 *The evidence so far*

The several different assessments performed have provided a wide range of significant information regarding the *in vitro* qualities of cold-stored platelets(12). It was found that upon lowering the temperature, metabolic processes within platelets were significantly slowed down(1,7,13,14). The slowdown of metabolism consequently lead to a reduced consumption of glucose and decreased production of lactate acid(15,16). In fact, through *in vitro* analyses, it was found that pH levels in cold stored PCs were relatively maintained, when compared to room temperature-stored PCs. Low pH levels led to the development of alterations in the platelets, and thus, loss of functionality and viability(17). This implies, therefore, that platelet integrity was better preserved at colder temperatures. It was estimated that the phenotype of cold-stored platelets was maintained for up to 10-14 days(14,18). Moreover, a temperature decrease, also led to a decline in microbial metabolism (19). This consequently reduced the rate of proliferation, and hence, the risk of the occurrence of bacterial contamination in the PCs. This has been demonstrated through several different studies(2,13). After approximately 5 days of storage, platelet density and platelet count in room temperature-stored PCs were found to be lower than those in cold-stored PCs(13,14). Such a finding further demonstrates how lower temperatures are able to better safeguard the *in vitro* qualities and parameters of the platelets. Additional *in vitro* analyses showed that mitochondrial function was significantly maintained in cold-stored platelets(2,15,18). Studies demonstrated how ATP generation by the mitochondria plays an essential role in platelet integrity, function, and viability(20). Mitochondrial viability could be calculated by the measurement of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) generation following stimulation by an agonist. It was found that levels of ROS and RNS in cold-stored platelets were greater than those in room temperature-stored platelets. This implies that cold-stored platelets would be more capable of becoming activated in the presence of an agonist. In fact, *in vitro* aggregation tests demonstrated that upon stimulation

with agonists, such as thrombin, ADP, collagen, alpha adrenergic and epinephrine, cold-stored platelets exhibited improved aggregation(17,21). In addition to this, the clots formed by cold-stored platelets upon aggregation, were found to be approximately 2.5 times stiffer than clots formed by platelets stored at room temperature and morphological analysis revealed that clots were denser and possessed greater crosslinks. Studies showed that this could have occurred due to the presence of factor XIII, which adhered to platelets upon exposure to lower temperatures(22). The increase in the amount of crosslinks could be explained through the presence of a greater amount of branching in clots produced by the platelets(23). Through macroscopic examinations of the units, it was also evident that unlike in room temperature-stored platelets, swirling - a simple test performed to evaluate morphology with the naked eye, was not seen in cold-stored platelets(15). This occurred since at a temperature below 15℃, platelets lose their conventional morphology, and shift from a discoid into a spherical shape(17,24). Furthermore evidence shows that at a colder temperature the platelet membrane is irreversibly damaged, subsequently causing phagocytosis of the platelets post transfusion(25).

In vivo assessments were also essential in providing information regarding how the platelets function once they are transfused. It was found that thrombin generation was increased when using cold-stored platelets(7). This enhanced thrombin generation, hence, led to a rapid activation process. In fact, it was found by several different studies that clots from cold-stored platelets formed significantly faster(2,7,13), consequently reducing bleeding times in actively bleeding patients by up to 40%(18). Therefore, cold-stored platelets showed an improved hemostatic response. However, on the other hand, it was found that transfused cold-stored platelets possessed a relatively reduced recovery rate. In fact, a study conducted in the early 1970s demonstrated that after just one hour post-transfusion, only 45% of the transfused platelets were recovered(26). It was estimated that the lifespan of cold-stored platelets was approximately a quarter of that of room temperature-stored platelets(13), with the half-life of room temperature-stored platelets being 3.8 days and that of cold-stored platelets being approximately 1.8 days(27). Such a short lifespan stems from the fact that the clearance rate of such platelets from the circulation was highly accelerated. A study highlights the fact that under low temperatures, platelets undergo modifications such as actin rearrangement, glycan clustering and lipid raft aggregation, which may influence the platelets' lifespan in the recipient's circulation(17). Furthermore, a study conducted in Germany demonstrated that cold stored platelets express a greater amount of apoptotic markers than room temperature-stored platelets, thus, making them more susceptible to an elevated clearance rate(28).

After evaluating the aforementioned significant findings, it was evident that cold-stored platelets possess a variety of both advantages and disadvantages. As can be visualized in Table 1, clinical trials demonstrated that although cold-stored platelets may present with some drawbacks such as a high clearance rate and a short lifespan *in vivo*, they also possess a wide range of benefits, which seem to outweigh the disadvantages acknowledged.

Advantages	
PC pH levels are maintained due to a slowdown in metabolism	(17)
The risk of bacterial contamination is reduced at lower temperatures	(2,13,15)
Mitochondrial function in cold-stored platelets is maintained	(20)
Platelets are more capable to become activated in the presence of an agonist	(17,21)
Stiffer clots are formed upon activation	(22)
Cold-stored platelets show an improved haemostatic response	(18)
A high clearance rate minimizes the risk of thrombosis	(7)

Prevents platelet functional defects and reduced effectiveness, which normally occur at room temperature	(6)
Disadvantages	
Short lifespan due to a high clearance rate *in vivo* following transfusion, hence requiring multiple transfusions	(13,26–28)
Low temperatures may cause alterations in platelet morphology	(17,24)
Swirling not seen in cold stored platelets	(15)

Table 1: Relative merits of cold-stored platelets

3.2. *Additional Considerations*

Acquiring knowledge regarding the different properties of both room temperature and cold-stored platelets allows for the proper management of PCs. The possibility of cold-stored platelet utilisation is being reconsidered due to its attractive elements and benefits being currently highlighted through several different clinical trials. Clinical trials, however, are also pointing out the drawbacks of the cold-storage method. The main drawback, identified by the majority of the studies, seems to be the platelets' short lifespan upon transfusion. This can be both beneficial as well as a limitation. A short lifespan will reduce the risk of late thrombosis following transfusion(18). Thrombosis is a potentially fatal condition characterized by the formation of clots within the vessels, which ultimately block the blood flow(29). Since platelets, which form an essential component in the formation of clots are reduced after some time, the risk of thrombosis will be reduced. On the other hand, a short lifespan *in vivo* will lead to a constant relapse in platelet levels, and hence, there will be a need for multiple transfusions to be administered in order to maintain an optimal therapeutic range. Multiple transfusions are not recommended since this may lead to a process known as sensitization. Sensitization occurs when an allergen, such as platelets within the transfused PC, triggers an immune response in the body, initiating an inflammatory reaction against the transfused product(30). The individuals that are sensitised will become hypersensitive and upon the subsequent transfusion, the allergen is recognized by the body, and a stronger immune response takes place. This will consequently cause the patient to present with allergy-like symptoms, which may even progress to anaphylaxis. The higher the number of transfusions, the greater the risk of sensitization(31), thus, multiple transfusion should be avoided.

4. CONCLUSION

Patients requiring PC transfusions may be categorized into two main groups: chronic thrombocytopenic patients, such as cancer patients, who are unable to produce enough platelets, and patients who are suffering from acute bleeding either due to a traumatic injury, childbirth, or surgery(32), but are otherwise able to produce their own platelets. In the case of acutely bleeding patients, a rapid hemostatic response would be required. As aforementioned, cold-stored platelets are able to provide such response with the formation of strong clots. This is ideal for acutely bleeding patients since a fast reaction would be needed to slow down the blood loss. On the other hand, in the case of chronic thrombocytopenic patients, a longer platelet lifespan would be required, since individuals would be dependent on the action of the platelets being transfused. In such cases, once the transfused platelets are cleared, subsequent transfusions would be needed. Unlike chronic thrombocytopenic patients, acutely bleeding patients will not require platelets with long lifespans since once the transfused platelets are cleared, the body would be able to produce its own platelets to maintain hemostasis. Due to such different requirements, it is being widely suggested that cold-stored PC transfusions are used for acutely bleeding patients, whilst room temperature-stored platelets are utilised for chronic thrombocytopenic patients(10). This will allow product management to be always dependent on the needs of the individual receiving the transfusion. Furthermore, studies are being conducted and

results are showing that adequate *in vitro* qualities are still maintained when PCs are stored at room temperature for the initial four days and then transferred to cold storage for the remaining sixteen days(33). Therefore, a system may be introduced whereby expired room temperature PCs, which are not used for chronic thrombocytopenic patients are transferred to lower temperatures and used for acutely bleeding patients, instead of being discarded after just five days.

COMPETING INTERESTS

Authors have declared that no competing interests exist

AUTHORS' CONTRIBUTIONS

All Authors contributed equality to the script, and all have read and approved the final manuscript.

CONSENT

Not applicable

ETHICAL APPROVAL

Not applicable

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