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Review

Platelet microparticles in cryopreserved platelets: Potential mediators of haemostasis

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ABSTRACT

Platelet concentrates can be cryopreserved in dimethylsulphoxide (DMSO) and stored at –80 °C, which increases the shelf-life from 5 days to up to 4 years. Cryopreserved platelets have been shown to contain a high number of microparticles that have *in vitro* haemostatic activity. Further, when transfused, cryopreserved platelets have been shown to be at least as haemostatically active, as liquid-stored platelets, if not more so. Given that microparticles are traditionally considered to be pro-coagulant, it is likely that their presence in the cryopreserved component contributes to this haemostatic effect. However, as microparticles are known to mediate many physiological and pathological processes, including in thrombosis and cancer development and progression, further work is warranted to fully understand the functional scope of the microparticles in cryopreserved platelets.

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1. Introduction

Platelets are 2–4 μm cell fragments produced by budding of the megakaryocyte cytoplasm. Platelets are essential in mediating haemostasis, but also play a role in modulating the immune response (reviewed in References 1 and 2). Platelet transfusions are administered to prevent or treat bleeding in patients with quantitative or qualitative defects in their platelets, and are most often required prophylactically for the treatment of haematological malignancies [3]. Additionally, platelet transfusions may be required to stop bleeding, as a result of severe trauma or surgery, due to loss or dilution of platelets and intravascular consumption of clotting factors [4].

Platelets for transfusion are stored at room temperature with constant agitation, and under these conditions the platelet shelf life is limited to between five and seven days,

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depending on the storage media and institutional jurisdictions. This limitation has been imposed as the storage of platelets at room temperature results in a heightened risk of bacterial growth and damaging changes to platelet structure and function [5,6]. This short shelf life makes the use of platelets in remote or military environments problematic, thereby failing to meet the expected standards in resuscitative care [7]. Considerable efforts have been directed towards the development of platelet alternatives and substitutes, in order to negate the challenges associated with fresh liquid preserved platelets. The cryopreservation of platelets offers an alternative to liquid-stored platelets with the ability to improve the shelf life, from 5 days to up to 4 years [8], and overcomes some of the logistical issues that surround fresh components [9].

2. Platelet cryopreservation

The cryopreservation of platelets involves the addition of between 5 and 6% dimethylsulphoxide (DMSO), followed by removal of the DMSO containing supernatant and freezing at –80 °C [10], as summarized in Fig. 1. DMSO is a cryoprotectant, which is added in order to minimize platelet damage by partially solubilizing the plasma membrane. This minimizes membrane disruption associated with the formation of ice crystals, which may puncture the plasma membrane and cause cell lysis [11]. However, DMSO can cause toxicity and adverse reactions such as headache, nausea and vasoconstriction have been reported when the dose of DMSO exceeds 1 g/kg of recipient body weight [11,12]. Therefore, the majority of DMSO is removed prior to freezing, and using our published method [13], the mean residual amount of DMSO is 1.4 ± 0.1 g per unit, which is well below the dose associated with toxicity. Removal of DMSO prior to freezing also simplifies post-thaw processing [10]. When required, the platelets are thawed in a 37 °C water bath and resuspended in either a unit of freshly thawed plasma [9,13] or a small volume of saline [14]. If not transfused immediately, they may be stored, without agitation, for 6 hours at room temperature. This shelf-life has been imposed primarily due to the risk of bacterial growth in the component due to the open manufacturing system employed for DMSO addition.

At present, cryopreserved platelets are not routinely transfused in a civilian setting, and the practice is primarily limited to military environments [11], where over 1000 units have been transfused during the last 10 years [9,15]. Further, cryopreserved platelets have been used clinically for over 30 years (reviewed in Reference 16). Despite being used in these clinical situations, only a single randomized clinical trial has been conducted. This study of patients undergoing cardiac surgery revealed that cryopreserved platelets were more capable of reducing blood loss and limited the need for more blood products, when compared to fresh liquid-preserved platelets [17]. The improved clinical effect occurs despite a reduction in platelet number following cryopreservation, when compared to the platelet count prior to freezing (*in vitro* recovery) [17]. A reduction in *in vivo* recovery has also been noted, although *in vivo* survival of cryopreserved platelets is well maintained [14,16].

The relative paucity of clinical data means that the mechanisms mediating the improved *in vivo* effects are not well understood. Cryopreserved platelets are known to have an altered surface receptor phenotype, with a loss of key functional glycoproteins and impaired *in vitro* aggregation responses [18–20], which are traditional *in vitro* markers

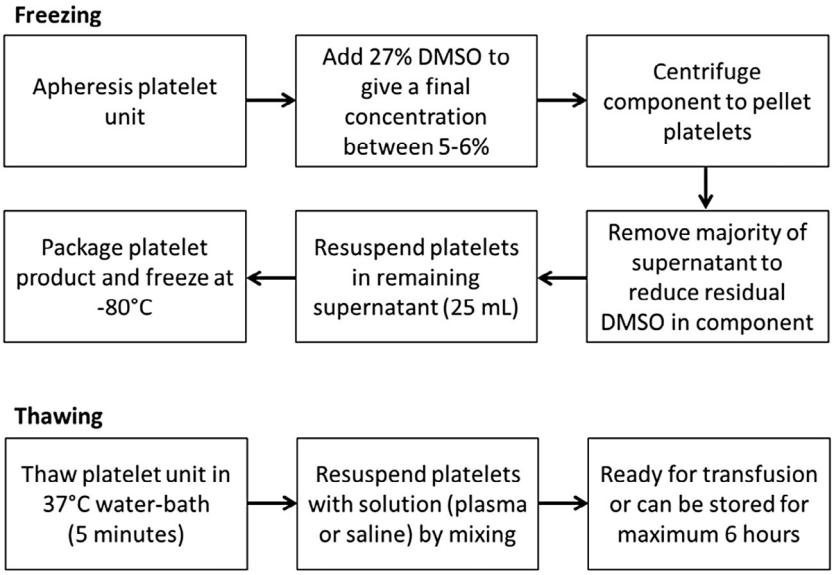

Fig. 1. Summary of the general procedure for cryopreservation and thawing of platelets. Dimethylsulphoxide (DMSO) is added to an apheresis platelet unit to give a final concentration between 5 and 6%. The platelets are pelleted by centrifugation and the supernatant containing DMSO and plasma is removed prior to freezing to reduce post-thaw manipulations and to minimize the DMSO toxicity when transfused. Platelets are then frozen and stored in a freezer at –80 °C. When required for transfusion, platelets are thawed at 37 °C until they reach a temperature of approximately 30 °C. Platelet units are then resuspended in a solution, usually either plasma or saline, and can be transfused immediately or stored at room temperature for a maximum of 6 hours.