Invited lecture/Review

Storage of Platelet-Rich Products

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Abstract:
Platelets are a natural source of signaling molecules, growth factors, cytokines and extracellular vesicles that modify the pericellular microenvironment in favor of tissue healing and regeneration. Autologous platelet-rich products, such as platelet-rich plasma are used in many fields of clinical medicine to benefit from these effects. Platelet-rich products are ideally used in more than a single application, but repetitive harvesting of concentrated platelets is time-consuming and impractical for patients and clinicians. In order to maximize the utility of priorly made products and create a supply of patients' own preparations, investigations of appropriate storage of platelet products have emerged. Recent explorations of hypothermic preservation, such as cryopreservation or freeze-drying have shown positive results, providing an efficient possibility of platelet-rich product storage retaining their growth factor, cytokine and chemokine activity.

Keywords: Platelet-rich plasma, Cryopreservation, Freeze-drying, Platelet storage, Cytokines, Growth factors, Healing

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1. Introduction.

Blood is an easily obtainable, abundant and dynamic diagnostic and therapeutic material. Clinical application of a blood fraction containing a higher concentration of platelets than whole blood, termed platelet-rich plasma (PRP), has been explored in many medical fields on various tissues to enhance healing by supplying growth factors, cytokines and other bioactive molecules (Andia et al., 2013). More recently, new diagnostic and therapeutic possibilities have emerged by the discovery of other small particles in body liquids (including plasma) termed extracellular vesicles (EVs). These have shown essential roles in transporting membrane proteins, cytosolic proteins and RNA (Tao et al., 2017). In order to emphasize this part of plasma contents, the term platelet and extracellular vesicle-rich plasma (PVRP) has been coined (Vozel et al., 2017; Vozel et al., 2021).

Despite recent optimized procedures of platelet-rich products repetitive production of these products by blood withdrawal and centrifugation is time-consuming and impractical for regular clinical practice (Steiner et al., 2022). In this aspect, several storage options of concentrated platelet-rich products have been investigated. The intention is to preserve ready-made platelet-rich products in a manner without losing their contents over time to the extent of diminishing the beneficial effect, and keep it available to be re-used in tissues, where repeated product applications are beneficial (Andia et al., 2020).

In recent years, several studies have been performed to evaluate the effect of different storage methods on platelet products. The most common measured parameters of storage feasibility are platelet degranulation, the release of functional proteins and their effect on vasculature, cell-growth and inflammation. Most studies focus on demonstrating the equivalence of preserved products to fresh products by comparing different points of platelet-rich product characteristics, such as the concentration levels of growth factors and cytokines relevant in healing mechanisms and the maintenance of their biological activity assessed by in vitro and/or in vivo functional assays (Andia et al., 2020). Other aspects of platelets have additionally been observed; the morphology of platelet membranes by electron microscopy, growth factor biomarker pattern and their kinetics, as well as their response to agonists, observed by flow cytometry and other modalities (Pan et al., 2016). Most notably involved in tissue repair, platelet-derived growth factor (PDGF), transforming growth factor (TGF), epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF) are commonly investigated in assessing the effect of storage on the function of plasma preparations (Andia et al.,2020; Brogna et al.,2020).

2. Essentials of platelet conservation.

The storage of platelet preparations was the subject of interest already when the sole purpose of concentrating platelets was transfusion. Until 1970s, cold storage at 4°C was the standard preservation technique for platelet concentrates, supported by the facts that the decreased metabolic rate preserves the product's blood clotting abilities and provides optimal bacterial growth inhibition (Becker et al., 1973). It was later described that platelets at room temperature show better in vivo survival after transfusion than preserved in cold environment (Becker et al., 1973; Murphy et al., 1971). Murphy et al. (1969) described the storage of platelets in the form of platelet-rich plasma and proposed to change the convention of cold storage to preserving products at 22 °C for maximum 4 days. Indeed, since Murphy's morphologic, metabolic and functional studies of platelets, platelet concentrates have been most commonly preserved on room temperatures (20-24 °C) in gas permeable bags with constant mixing. These measures prevent blood clotting and maintain the viability of platelet cells by promoting gas exchange. In this manner, the time of conservation has been shown to be limited to 5 to 7 days, most significantly due to bacterial contamination hazard and platelet viability deterioration (Waters et al., 2018).
During prolonged platelet storage a specific deterioration of function and viability of platelets occurs, referred to as the platelet storage lesion. The aim of efficient platelet storage is to keep them in a resting, non-activated state to retain their function. It has been found that with longer shelf-life at least a partial activation occurs (Seghatchian et al., 2001). This is observed as the release of alpha granules, altered glycoprotein expression and increased procoagulant activity with up-regulated glycolysis with elevated lactic acid concentration. The latter results in pH drop, deteriorating the viability of preparation (Rinder et al., 1991).

In cold or cryopreserved stored preparations, similar processes take place (Sandgren et al., 2006; Wood et al., 2016).

According to the current Blood Transfusion Centre of Slovenia guidelines, pathogen inactivated concentrated platelets are safe to use up to 7 days at 22 ± 2 °C [17]. Novel preservation techniques to such as timetril sulfoxyd, trechalosis or dry freezing have simplified platelet storage and notably prolonged the utility of these preparations. Indeed, in hypothermic conditions, fresh frozen plasma can be stored from 3 months (in conditions of -18°C) to 3 years (in conditions of -25°C) (Adams et al., 2015).

Four basic cold storage options exist for platelets as for other cells and tissues; hypothermic storage, cryopreservation (deep freezing), vitrification and freeze-drying (lyophilization). **Table 1** portrays the basic elements of different preservation methods (Cryopreservation and Freeze-Drying Protocols, 2022). Vitrification is the process of rapid cooling of the liquid medium in the absence of ice crystal formation.

Table 1. Different preservation methods for cells and tissues. Based on "Cryopreservation and Freeze-drying Protocols" (Adams, 2015).

	Hypothermic storage	Cryopreservation	Vitrification	Freeze-drying
Process	isothermal	slow cooling	rapid cooling	slow cooling sublimation desorption
Storage temperature	-4 °C	below -15°C	below -150 °C	room temperature (22-24°C)
State	aqueous solution	in frozen/glassy state	in glassy state	in glassy state
Storage duration	short term	long term	long term	long term

3. Mini review of platelet-rich products preservation

3.1 Room temperature

Several studies have discussed the storage of platelet-rich products on room temperature. Bausset et al. (2012) investigated the effect of storage on room temperature on growth factors in autologous PRP. Based on the results, they recommended using the concentrates on room temperature within 3 hours after preparation, even though the concentrations of PDGF and VEGF were shown to be well-sustained up to 6 hours. Previously, Marx reported that PRP remains stable for up to 8 hours after preparation, but release 70% of their growth factors within 10 min after activation and almost 100% within an hour (Marx et al., 2001). Wilson et al. (2018) reported that TGF- α1 (transforming growth factor alpha) in PRP retains its activity up to 4 hours at room temperature. Several publications describe an even longer room temperature availability. Moore at al. (2017) measured PDGF over 8 days and concluded that platelet products intended for tissue regeneration could be stored for at least 5 days. Wen et al. (2018) similarly reported the preservation and even rise of growth factors after 7 days room temperature storage in leukocyte rich-PRP.

3.2 Cold storage

Most commonly used cold storage modalities for platelet-rich products are deep freezing and freeze-drying, which is essentially freezing followed by water sublimation and subsequent removal of water vapor. These have been proposed as consistent methods for fabrication of stabile products, ready for future use. Recently, DeMello et al. (2022) investigated frozen PRP at -20°C for 6 months on fifteen healthy adult canine patients and observed growth factors levels after freezing and long-term storage (6 months without a preservation agent). The results revealed that all measured growth factors were present in measurable quantities, surviving the long-term storage. Pan et al. (2016) previously examined the release of growth factors in freeze-dried samples and reported that freeze-dried PRP remained rich in growth factors after storage for at least 4 weeks at room temperature.

Hosnuter et al. (2017) presented a study of autologous PRP storage in an office environment at -20 °C, in the freezing compartment of a standard refrigerator without using a preservation agent or a special carrier container. EGF, VEGF, PDGF-AB, IGF-1 (Insulin-like growth factor 1), TGF-β, and P-Selectin levels were immediately analyzed in the control group. The growth factors and P-selectin levels were still present, but all growth factors were significantly decreased in the autologous PRP samples stored at −20 °C compared to the control group.
Similarly, cold storage of platelet-rich products has been investigated in many fields of PRP or PVRP use, such as wound care, alopecia, musculoskeletal applications, dentistry, ophthalmology and more, some of which are discussed below (Daban et al., 2010; Anitua et al., 2021; Wolkers et al., 2002; Kandil et al., 2020; Shiga et al., 2016; Li et al., 2017).

3.2.1 Wound care

Pietramaggiori et al. (2006) explored the effect of platelet storage in freeze-dried PRP on chronic wounds. In a single dose freeze-dried PRP application to chronic wound with or without bio-stabilization, they evaluated the healing effects and activity of growth factors (TGF, PDGF, VEGF, and EGF) in a diabetic mouse model. Freeze-dried and biochemically stabilized freeze-dried platelets displayed abilities to modulate wound healing, enhancing proliferation of cells and vessel growth in granulation tissue and decreasing wound contraction. The cold storage processing did not affect the intra/extracellular ratio of growth factors, especially when platelets were biochemically stabilized. Using fibroblast proliferation assay, it was additionally investigated whether the preparation of samples affected the ability of platelets to stimulate cell growth in vitro, showing all platelet samples to exert equivalent in vitro abilities to increase cell proliferation compared to the room temperature control. Biochemically stabilized freeze-dried samples have proven to be by 40% more available for the release of growth factors.

3.2.2 Musculoskeletal

The benefits of freeze-dried preparations are besides the prolonged viability of products, the possible storage in the refrigerator or at room temperature in its powder form which is optimal for mixing with other materials, such as the artificial bone. In a study by Koga et al. (2021) the researchers evaluated the lyophilized form of PRP stored at -20°C for 4 weeks and discovered that the preparation remained safe to use in bone engineering up to 4 weeks after freeze-dried storage, with no side effects reported. Roffi et al. (2014) reported that immediate and 7-day release of growth factors of frozen PRP was lower compared to the fresh preparation, but the cold stored preparation effectively preserved the ability to induce proliferation and extracellular matrix production, as shown in chondrocyte and synoviocyte culture. Da Silva et al. (2018) similarly assessed the stability of PDGF, VEGF, TGF and EGF comparing fresh PRP and freeze-dried-PRP. They observed adequate proliferative activity of freeze-dried PRP on human umbilical endothelial cells and fibroblasts.
Shiga et al. (2016) also investigated the ability of cold stored human PRP in carrying out the functions of fresh PRP in a rat model on bone union in lumbar fusion surgery. The researchers reported that freeze-dried PRP maintained baseline levels of growth factors during the entire 8-week duration of the study. They analyzed three types of storage; human PRP of stored on room temperature with shaking, frozen PRP stored at -80°C and freeze-dried PRP with no further treatments. Platelet activation rates were assessed via flow cytometry, platelet count and growth factor (PDGF, TGF-α, VEGF - vascular endothelial growth factor and EGF). These were assessed by Growth Factor Membrane Antibody Array immediately after preparation and after 2, 4 and 8 weeks of storage. The authors reported no difference in PDGF concentrations after 4 weeks of storage in -80°C, but they decreased thereafter.

The results showed significantly reduced platelet counts on room temperature after 2 weeks, while the count remained relatively constant in the frozen and freeze-dried samples after 8 weeks of preservation. In room temperature samples almost no growth factors were detected after 8 weeks, the first clear reduction of expression occurring after 2 weeks. In the frozen PRP samples a significant expression of growth factors was maintained at 4 weeks, but decreased by 8 weeks for TGF-α, VEGF 2 and EGF. Freeze-dried samples ex-

hibited only slightly reduced levels of growth factors compared to fresh PRP levels. According to the findings, the researchers suggested that freeze-drying is the most suitable technique for storing PRP to maintain its biologic activity, preserving both platelet count and growth factor levels. They concluded that PRP is best stored at -80°C for 1 month or in dry-freeze state for up to 6 months. Moreover, Li et al. (2017) used 3D-printed biomaterial scaffolds for bone tissue engineering coated with freeze-dried PRP and showed significantly greater osteogenic differentiation induction compared to traditionally prepared PRP.

3.2.3 Maxillofacial surgery

Koga et al. (2021) conducted a study with freeze-dried PRP stored at - 20°C for 1 month, which was rehydrated and applied in five patients for sinus surgery mixed with bone grafting materials. The results were assessed 4 weeks after the surgery of maxillary sinus floor augmentation showing that the vertical augmented height was maintained and that the preparation remained safe to use in bone engineering up to 4 weeks after freeze-dried storage, with no side effects reported.

3.2.4 Ophthalmology

Lopez-Garcia et al. (2016) observed samples of PRP in eye drop form after 1, 3, 6 and 9 months of storage at -20 °C. In the first month evaluations were made at day 0, 1,2, 3, and 4 weeks. They reported the concentrations of EGF, TGF- α1, PDGF and albumin remained stable over 4 weeks at 4°C in both fresh and defrosted samples. No statistically significant differences were observed between growth factor concentration and the effects on cell proliferation and differentiation of cultured cells in fresh samples and defrosted samples after 1,3,4 or 9 months at -20°C. Anitua et al. (2021) similarly analyzed the biological contents and activity of freeze-dried plasma in eye drops after their storage at 4°C and at room temperature for 3 months with respect to fresh samples. They concluded that lyophilized plasma rich in growth factors eye drops conserves their biological features even without the use of lyoprotectants for at least 3 months.

4. Conclusions

In order to avoid potentially painful repetitive blood withdrawals and prolonged in-office visits, storage of priorly prepared autologous blood products has been explored. The preservation of platelet-rich products at low temperatures has been shown as a safe and effective manner of storage, demonstrated by assessments of cytokine and growth factor concentration levels and maintenance of biological activity in various functional assays. Storing samples in the refrigerator or at room temperature in lyophilized form offers a comfortable and simple method of creating a stockpile of therapeutic fluid on disposal for multiple applications, allowing the maximal yield of these preparations on tissues. Larger clinical studies confirming these conclusions as well as the standardization of procedures are required to consider these methods to be used in standard clinical practice.

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