Functional In Vitro Studies Of Buffy Coat Pooled Platelets Cryopreserved In Dimethyl-Sulphoxide With a New System

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ABSTRACT

Introduction Cryopreservation for long term storage of platelets (PLTs) represents a clinical useful method for avoiding platelet shortage. Many studies have tried to define, *in vitro* and *in vivo*, the entity and weight of storage-related PLTs lesions with discordant results related to different methods.

We have performed an *in vitro* prospective study to evaluate PLTs count, viability and function of buffy coat derived pooled platelet concentrates (BC-PLTs) treated with dimethyl-sulphoxide (DMSO) and cryopreserved at -80°C with an innovative patented system not requiring laminal flow hoods and external manipulations.

Materials and methods Each BC-PLTs was obtained from 5 buffy coats and pooled according to standard procedures. The final PLTs concentrates were leukoreduced by filtration and transferred to a 650 mL cryopreservation kit (Promedical ®) which allowed mixing with DMSO 25% in a closed system and following removal of supernatant without further manipulations. BC-PLTs were washed prior freezing with removal of at least 84% supernatant solution, suspended in homologous plasma from 1 of the 5 donors to a final concentration of 200 mL and frozen at - 80°.Selection criteria to make BC-PLTs available for this study was pooled PLTs concentration > 1250 x 10^9/L and a blood units collection time duration shorter than 6

minutes. All the 245 donors were healthy volunteers and they did not take any medication affecting PLTs function. BC-PLTs were analyzed immediately pre-freezing (T0) and 3 months after cryopreservation (CRY BC-PLTs).

The following parameters were assayed: PLTs count (PC), mean platelet volume (MPV), pH, flow cytometry (FACS) expression of CD41a, CD42b, CD61a, CD62p, PAC-1, Annexin V PLTs surface antigens and thromboelastography (TEG). All samples were analyzed also after dilution (1:4) with homologous plasma to approximately 400 $x10^9$/L PLTs (data not shown) and for bacterial contamination (BC). CRY BC-PLTs samples were thawed in a bath at 37°C for 5 minutes and evaluated promptly. All the tests were performed according to current European recommendations. PLTs swirl was furthermore visually assessed. Results were expressed as mean +/- standard deviation (SD). Results obtained at T0 and after 3 months were compared by paired sample t-test. Differences were considered as significant at p values <0.05.

Results In vitro cell parameters were measured on 49 BC-PLTs and 15 CRY BC-PLTs as reported in Table1. PC was only slightly reduced in CRY BC-PLTs while MPV was significantly increased in CRY BC-PLTs. There were no differences between the 2 groups in CD41a, CD61a, CD62p and Annexin V expression while a significant reduction in CD 42b, PAC-1 for CRY BC-PLTs was observed. TEG parameters were all significantly reduced in CRY BC-PLTs samples without affecting hemostasis. PLTs swirl was observed in all samples and BC was absent.

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Table 1

In vitro assays of pre-freeze and cryopreserved platelets

Conclusions The preliminary results of our investigation confirm the potential of a new system to overcome limits to PLTs storage. In fact, this method guarantees sterility and avoids excessive manipulations thanks to a closed system. Our method avoids PLTs apoptosis, as shown by absent expression of Annexin V. The adequate hemostasis achieved at TEG in both groups supports the hypothesis that *in vitro* PLTs activation/deterioration doesn't necessarily mirror an impaired hemostatic *in vivo* function of CRY BC-PLTs. The next step of current study will be to evaluate the influence of the observed *in vitro* activation changes on PLTs recovery and survival *in vivo.*

Disclosures: De Francisci: *Promedical:* Consultancy. **Reina:** *PROMEDICAL:* Consultancy. **Allegro:** *Promedical:* Employment.