Platelet concentrates (PC) prepared from buffycoats in a quadruple bag CPD-SAG M system proved to have a good survival in vitro after storage for 72 h at 22°C (Vos Sang 1985, 49: 81-85). Storage of these PC for 7 days at 22°C on a linear platelet recirculator, was studied using 6 different types of bag made of polyvinylchloride (PVC) with either DEHP and/or TOTM as plasticizer. From Biotest the 76 standard PVC (n=1/6) bag, from NPBI the PSV 3277 standard PVC (n=15) and DPL-110 TOTM (n=16) and from Terumo the old (n=18) and new (n=14) molded Teruflex bags were examined.

The Biotest 763, the NPBI DPL-110 and the Terumo new molded Teruflex are recommended for platelet storage.

The plasma volumes averaged 63 ml with a range of 39 to 81 ml. Platelet concentrations were comparable in all types of bags with an overall mean of 0.89±0.33 x10^11/ml. The number of contaminating leukocytes and erythrocytes never exceeded 10^6 per unit. The initial pH was between 6.98 and 7.19. After 7 days storage the pH in all bags remained above the critical value of 7.0 with a range of 6.56 to 7.38. No decrease of pH was observed during storage, on the contrary a significant (P<0.05) increase in the PSV 3277 and the new molded Teruflex bags was measured. Still the PCO_2 diminished equally in all bags also indicating adequate gas exchange. Good morphology scores of the platelets (greater than 98%) were observed in 98% of the PC after 5 day storage and in 83% after 7 days. These data indicate that PC can be equally well stored in normal PVC bags as in special platelet storage bags, provided that the PC are leukocyten poor.

The plasma pH decreased distinctly different populations of particles: the predominant one had diameters in the range of 0.1 to 0.4 um and was particle diameters of 100 x 10^3 cell/ul were stored in the bag containing citrate-phosphate-dextrose at 22°C for 24 to 72 hours. The bags were either kept in a flat position without agitation or continuously stirred by a tumbler agitator (Trprmp), or a flat bed rotator (30rpm). At the beginning of each experiment, fresh platelets separated from healthy donor were used as control group. The remaining platelets were washed twice with the 0.2% sodium-citrate dissolved in the IIMB of PBS. After incubation of the suspended platelets in the Tyrode’s buffer solution at 37°C for 30 minutes, they were fixed with 1% paraformaldehyde at 4°C for 2 hours, and were then incubated with anti-human GP IIb/IIIa mouse monoclonal antibody (Sag/ml) at 37°C for 1 hour. The platelets, thus treated with primary antibody, had undergone further incubation with fluorescein-conjugated goat anti-mouse IgG (H+L) antibody. Analysis by flow cytometry (FCM) provided the advantage of quantifying the number of particles.

Quantitation of Microparticles in Platelet Suspensions by Flow Cytometry

Quantification of microparticles in platelet concentrates (PC) was performed by using a FACSCalibur (Becton-Dickinson) flow cytometer to study the distribution of small microparticles previously shown to be present in citrated plasma and serum (D.T. Hiller et al., Blood 60: 834, 1982). Analysis by flow cytometry offers the advantage of discrimination of populations of particles by light scattering and immunofluorescent properties. We found two distinctly different populations of particles: the predominant one had diameters in the range of 0.1 to 0.4 um and was moderately autofluorescent (AF); the other was equally AF with particle diameters of 1.0 to 3.0 um and probably included a few intact platelets. By adding a precise quantity of highly fluorescent beads of 0.9um diameter to each sample, relative concentrations of particles (small and/or large) could be quantified in platelet suspensions after various treatments using native particle and bead counts. The lowest concentration of particles was found in samples from whole blood collected into CPDA-1 with POE-I and theophylline plus sodium azide (CPD-Az). Blood in CPDA-1 alone had twice the number of small and large particles; serum had a 20X higher particle concentration. A much larger number of particles was found in platelet concentrates (PC) stored for transfusion. Fresh PC had approx. 150X higher particle concentration than CPD-Az, rising to over 200X by the eighth day of storage at 22°C. Also, we noted a shift in distribution between particle populations in stored PC toward the larger size. The concentration of larger particles alone rose from 100X relative to CPD-Az to 350X after 3 days of storage. Similar changes in supernatant platelet factor 3 (PF3) activity were noted in stored PC in another study (A.P. Bode and D.T. Hillier, Vox Sang 51: 299, 1986).

Also, we noted a significant rise in supernatant platelet factor 3 activity in stored PC toward the larger size. This technique of examining and quantifying particles in platelet preparations by flow cytometry will facilitate and expand the characterization of platelet vesiculation and the released particles.