

5PS-33-04

LONG-TERM STORAGE OF PERIPHERAL BLOOD PROGENITOR CELLS (PBPC) AT -80°C RESULTS IN A PRONOUNCED DECREASE OF MEMBRANE INTEGRITY AND CLONOGENIC POTENTIAL COMPARED TO STORAGE AT <-170°C

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Autologous PBPCs for therapeutic use are usually stored at or below -170°C directly in or over liquid nitrogen. In some cases, e.g., potentially infectious units, the PBPCs are often stored at -80°C in mechanical refrigerators. Storage time varies from a few weeks to several months and even longer. The aim of this study was to investigate the above-mentioned storage temperatures with regard to WBC recovery, membrane integrity and colony forming potential (CFU) in semisolid culture assays after storage of PBPCs for three and six months.

Study material: Fifty-two frozen reference samples (1.5 ml) of PBPC concentrates were obtained from thirteen patients. All samples contained 10% (v/v) Me2SO and had been cooled down to -90°C at 1.5°C/min in a controlled rate liquid nitrogen programmed freezer. After storage for three and six months at -80°C and below -170°C, respectively, they were thawed within three minutes using a shaking water bath at 37°C. The samples were randomly separated into one of four groups. Terminology: Group 1: <-170°C/3 months, Group 2: -80°C/3 months, Group 3: <-170°C/6 months, Group 4: -80°C/6 months. After three and six months storage, paired samples with identical storage times (but different storage temperatures!) were thawed together and analyzed in parallel by the same person on the same day.

Numerical WBC recovery of Groups 2 and 4 (-80°C storage) were significantly lower (Student's t test, $p < 0.05$) compared to Groups 1 and 3 (< -170°C storage). Membrane integrities (in terms of trypan blue exclusion) differed significantly as well: $70.2 \pm 10.9\%$ (Group 1) vs. $59.0 \pm 12.3\%$ (Group 2) and $80.9 \pm 12.6\%$ (Group 3) vs. $67.9 \pm 15.2\%$ (Group 4), respectively. There was also a significant difference in the colony forming potential (CFU-GM + CFU-Mix + BFU-E): namely, $21.2 \pm 13.5 \times 10^5$ CFU/kg b.w. (Group 1) vs. $7.6 \pm 9.6 \times 10^5$ CFU/kg b.w. (Group 2) and $20.9 \pm 23.7 \times 10^5$ CFU/kg b.w. (Group 3) vs. $5.2 \pm 5.8 \times 10^5$ CFU/kg b.w. (Group 4), respectively.

Storage of cryopreserved PBPC in mechanical refrigerators at -80°C leads to a highly significant loss of cell membrane integrity and clonogenic potential after three and six months when compared to storage for an equivalent amount of time in the vapor phase over liquid nitrogen (<-170°C). However, due to the well-known variability of the viability tests from day to day and from one institution to another, a comparison of the results is only possible between samples processed in parallel by the same individual on the same day. This may be the major reason why others, to date, have failed to detect differences in WBC recovery, membrane integrity and colony forming potential of PBPCs stored at -80°C versus below -170°C.

In conclusion, these results strongly support the fact that storage of cryopreserved PBPCs at -80° is detrimental in as little as three months, when compared in parallel to storage at below -170°C. Consequently, storage only at below -170°C is recommended unequivocally.