490g Cryopreservation of Human Platelets with a Trehalose-Based Formula

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Human platelets have extremely important medical applications, including uses in trauma situations, surgery, chemotherapy, bone marrow transplants, and treating immune system diseases including AIDS. Platelets, which are extremely sensitive to cold, are usually stored in blood banks at 22°C for only five days. An effective cryopreservation protocol would enable longer term storage and reduce the chronic shortage of platelets. Currently, 6% dimethyl sulfoxide (DMSO) is considered to be the most effective cryoprotectant for platelets, but DMSO must be washed away from thawed cells before transfusion due to cytotoxicity concerns. This additional processing causes more damage to the cells and lengthens the transfusion procedure. Therefore, researchers have been searching for methods to totally replace DMSO, or at least to decrease DMSO concentration by combining it with other cryoprotectants. Trehalose, a glucose disaccharide synthesized by many species of anhydrobiotic organisms, has significant promise as a possible nontoxic replacement for DMSO. The effectiveness of trehalose as a protectant has been demonstrated in the cryopreservation and lyophilization of proteins, bacteria, and some mammalian cells. The mechanism by which trehalose protects cells from cryopreservation and desiccation stresses is still under investigation. The "water replacement hypothesis" states that trehalose is able to take the place of water in forming hydrogen bonds with proteins and membrane lipids during water loss to prevent membrane fusion and protein denaturation. Another hypothesis suggests that trehalose participates in the formation of a protective amorphous glassy matrix instead of the formation of rigid ice crystals during cryopreservation. The protective ability of trehalose can be enhanced by phosphate, which was demonstrated in previous studies on Lactobacillus acidophilus preservation. It is hypothesized that phosphate could form a cross-linked network with trehalose through hydrogen bonding, which is consistent with the increases in viscosity and the glass transition temperature (T_g) of the trehalose solution upon addition of phosphate. We evaluated the effectiveness of a trehalose-phosphate formulation in protecting platelet structure and function following cryopreservation. An annexin V binding assay was used to quantify the efficacy of the trehalose-phosphate formulation in suppressing platelet activation during cryopreservation. Of the platelets cryopreserved with only trehalose 9.8% ± 0.26% were nonactivated; with the trehalose-phosphate formulation 23% ± 1.2% were nonactivated, comparable with the efficacy of 6% DMSO. The presence of both trehalose and phosphate in the cryopreservation medium was critical for cell survival, and preincubation in trehalose-phosphate solutions further enhanced viability. The optimal cooling rate for trehalose-phosphate-protected platelets, determined by annexin V binding assay, was 1-5°C/min. Measurements of platelet metabolic activity using an alamarBlue assay also established that trehalose-phosphate was superior to trehalose alone. Finally, platelets protected by the trehalose-phosphate formulation exhibited a virtually normal aggregation response upon thrombin addition similar to fresh platelets, but an increase of cytosolic calcium concentration upon thrombin addition was not observed in the cryopreserved platelets.