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Comprehensive study of hydrostatic pressure treated human umbilical cord blood cells via response surface method ☆

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ABSTRACT

Amelioration of the survival parameters of cryopreserved samples after thawing has already been addressed through several techniques including vitrification to avoid the formation of ice cores. However, this approach cannot be followed in the case of samples with higher volumes. Hydrostatic pressure (HP) treatment has been proven to increase some qualifying parameters (e.g., motility, insemination efficiency) of certain biological samples. Accordingly, the preparation of umbilical cord blood (UCB) samples through an active (mechanical) pre-stressing process to increase the survival rate of cryopreserved samples can be regarded as a novel strategy that calls for basic experimental studies. The goal of our study was to assess the effects of HP treatment on the qualifying parameters (DNA fragmentation by agarose gel electrophoresis and capillary electrophoresis, Total Nucleotide Cell (TNC) count, CD34+/CD45+ count, and superoxide dismutase activity (SOD) of human umbilical cord blood (UCB) derived cells). The experimental arrangement was set to provide data for response-surface analysis to take into account the common effects of the individual variables of pressure and time exposure. 3D visualization of experimental data revealed that 50-min long HP treatment at 12.5 MPa can significantly ($\alpha = 0.05$) enhance white blood cell (WBC) and CD34+/CD45+ cell counts. However no DNA fragmentation was observed even at higher pressures, SOD activity was triggered over 15.0 MPa. As a conclusion, HP treatment may contribute to the optimal cryopreservation of UCB cells by significantly increasing WBC and CD34+/CD45+ cell counts without adverse effects neither on DNA stability nor on triggering SOD activity.

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Introduction

Treatment of umbilical cord blood derived stem cells prior to cryopreservation and the effects of cryopreservation parameters have been extensively studied and documented in terms of cell viability [14,29]. Indeed, established SOP techniques exist to introduce Me_2SO without a significant loss in cell count, to deplete RBCs, to remove cell clothes, etc. [10,34]. However, methods for directed preconditioning of UCB cells to arrive at a possible graft with higher quality parameters have not been conciliated and no harmonization is foreseen, while efforts have been made at individual laboratory levels [20,22].

One of the possible treatment processes that might be implied in the case of stem cells is the use of hydrostatic pressure (HP). Basically, pressurization (also referred as a mode of mechanical loading) is a well established process in food preservation where the inactivation of infecting microbes is achieved in the pressure range of 200–1000 MPa [4,6]. Also, this high HP can be also applied, e.g., for decontamination of human blood plasma [1,28]. Indeed, the direct use of this high pressurization related to stem cell studies is limited to decellularization purposes [15].

The mode of action of high HP is partly related to the modification of protein structures, starting from changes in enzymatic activity and ending at irreversible denaturation [11,12,31]. The level of pressure to initiate any detectable change in activity may start at as low as 20 MPa, while even tetrameric structures may stay intact up to 320 MPa and single-chain proteins do not undergo denaturation at pressures below 400 MPa. This relatively high pressure range without denaturation events offers the possibility to apply HP based techniques for the dissociation of protein aggregates formed during the expression of recombinant proteins [30,32]. It should be also noted that both natural and synthetic DNA are stable and do not usually show any degradation up to pressure levels of at least 200–300 MPa [17].

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On the other hand, the application of medium HP has been propagated for decades in the field of cartilaginous tissue regeneration. As recently summarized by Delaine-Smith and Reilly [5], the functional development and osteogenic differentiation of mesenchymal stem cells can be enhanced by the hydrostatic treatment in the range of 0.1–10 MPa. The mechanism by which HP increases matrix production and affects the phenotypic stability is, however, not completely understood. Therefore, several factors influencing the possibly positive effects of hydrostatic treatment should be clarified, such as the mode of action (cyclic/intermittent pressure [2,8] vs. single treatment) [35], type of medium (pellet cultures vs. alginate/agarose matrix) [9,18], etc.

Contrarily to the pressure engineering of cartilage tissues, HP up to 40 MPa (that was defined high by the authors) [7] have been found to provide generally positive effects when applied prior to the cryopreservation of mammalian cells. It was shown that the same HP level (also up to 40 MPa) applied prior to cryopreservation could increase the viability and fertility of bull semen after thawing [26]. Also, pig oocytes showed a significantly increased resilience against cryopreservation after being treated with sub-lethal hydrostatic treatment [24].

The goal of our study was to examine the effect of HP treatment on human umbilical cord blood (UCB) derived cells. The monitoring of DNA fragmentation, Total Nucleotide Cell (TNC) count, CD34+/CD45+ count, and superoxide dismutase activity (SOD) were addressed. To the best of our knowledge, none of these parameters have been tested previously on stem cells under the circumstances of elevated but sub-lethal pressure, thus novel information is expected that might be considered in the quality aspects of cryopreservation policy.

Experimental

Samples and sample processing

UCB samples with a total volume of at least 60 ml (excluding citrate–phosphate-dextrose/CPD/anticoagulant) were acquired for the experiment in the Polish Stem Cell Bank (Warsaw, Poland). They were collected in Cord Blood Collection Kits/Ravimed Sp. z o.o.; Łajski, Poland/containing the CPD solution. The UCB samples were maintained and transported to Krio Institute Ltd. (Budapest, Hungary) according to established protocols at ambient temperature (4–30 °C). The samples were stored in refrigerators at 5 °C after acceptance and they were processed within 72 h after collection on the average.

The samples were divided in six subsamples with equal volumes and each subsample was filled in transfer bags (150 ml W/4; JMS Singapore PTE Ltd.; Singapore) and completed with additional CPD solution to contain about 29 ml of CPD. Afterwards the hematological data of the subsamples were recorded with a Diatron Abacus 5 hematology analyzer by a volumetric impedance method by counting cells passing through a small aperture (Diatron Group; Budapest, Hungary) to check the parallelity of the subsamples in terms of white blood cell (WBC) distribution.

UCB units were acquired through a manual cell enrichment technique using a standard protocol for red blood cell depletion (RBC) with hydroxyl-ethyl starch (HES) and volume reduction carried out on Teruflex ACS-201 separation stands (Terumo BCT, Inc., Lakewood, CO, USA) [33,34]. Volume reduction was followed by centrifugation at 1420 rpm for 15 min at 22 °C (Heraeus Cryofuge 5500i; Thermo Fisher Scientific Inc., Waltham, MA, USA), and the final volume was set to 21.0 ml on a balance by manually removing excess blood plasma. After this process the hematological data of the subsamples were recorded to provide data of reference. At the same time, CD 34+/CD45+ cell count parameters were measured with a FACScan flow cytometer (SC-201 AA; Becton, Dickinson and Company, NJ, USA) using the related diagnostic kits of the producer.

HP treatment

HP treatments were carried out in a HHP 1400 instrument (Cryo-Innovation Ltd., Hungary) [25] at 8 °C. In order to provide uniform hydrostatic treatments samples were degassed on Teruflex ACS-201 separation stands.

The experimental parameters were based on a response surface method (RSM) with the independent variables of hydrostatic pressure in the range of 4.4–15.9 MPa and exposure in the range of 9–51 min. Altogether, nine sets of pressure-exposure parameters were determined (Fig. 1). The processed UCB units were analyzed by two different approaches:

(1) In case only one set of pressure-exposure parameters was studied at a time, the six parallel subsamples were divided in two to provide three subsamples for HP treatment and three subsamples for control. In this approach, the subsamples were HP treated, analyzed for hematological, CD and SOD parameters, cryopreserved, stored, thawed and analyzed again for hematological, CD and SOD parameters immediately after thawing. Altogether with the data of reference, three groups of hematological and CD data were acquired for the HP treated samples (9×3 data of reference, 9×3 data after HP treatment and 9×3 data after thawing) and two groups for control samples (9×3 data of reference and 9×3 data after thawing). The results of this approach finally concerned the optimization of surviving WBC and CD counts in the function of HP exposure parameters and the SOD activity of UCB units, which are presented in Figs. 2–4 and 7.

(2) In case several pressure-exposure parameters were studied on the same set of the given six parallel subsamples, the six subsamples were 5:1 distributed to provide five subsamples for HP treatment and one for control. In this approach, the subsamples were HP treated and analyzed for hematological and CD parameters, but afterwards no cryopreservation was carried out, because the different time-span values between the end of the individual HP treatments and the start of the cryopreservation process would have significantly influenced the outcome of the cryopreservation. The results of this approach are presented in Figs. 5 and 6.

When cryopreservation was addressed, the subsamples were kept at 6 °C for 30 min after the HP treatment. Afterwards they were mixed with the freezing solution to reach the final dimethyl sulfoxide (Me_2SO; WAK-Chemie Medical GmbH, Steinbach, Ger-

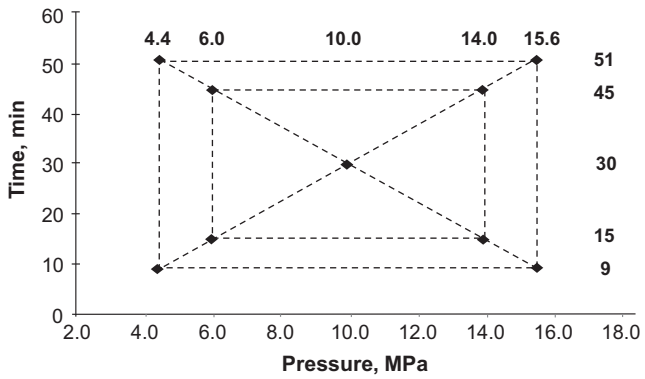

Fig. 1. Experimental arrangement for pressure-exposure parameters. The nine pressure-exposure sets are indicated with black tetragons, while the individual parameters are highlighted in bold for ease of presentation.

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