Probl Cryobiol Cryomed 2022; 32(4):304
https://doi.org/10.15407/cryo32.04.304

Розробка ефективних протоколів заморожування гемопоетичних стовбурових клітин

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Development of Effective Hematopoietic Stem Cell Freezing Protocols

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Hematopoietic stem cell (HSC) therapy is widely used to treat an increasing number of oncohematological diseases. The HSCs cryopreservation is almost the only way for their preservation. The researchers are faced with the problems, requiring a balanced selection of substances, their concentrations, compatibility and correlation with each other, and the most thorough compliance with physical and chemical medium conditions in cell procurement and storage. Therefore, we here aimed to test the efficiency of the developed technique for HSC freezing/thawing, and adapt it for clinical conditions.

The ratio of living and dead cells in donor HSC samples was analyzed. The composition of HSC subpopulations by CD45 and CD34 markers was also determined. The $CD34^+$ is a marker of HSC subpopulation, which is one of the most precise indices, enabling to predict the transplant engraftment. The measurements were performed using FACSAria III flow cytometer (BD, USA) after sample thawing.

As a result, the number of living HSCs in peripheral blood of patient's autologous specimens, subjected to uncontrolled rapid freezing and maintained in liquid nitrogen at –196°C ranged from 88.4% to 85.7%. The specimens, frozen slowly at –80°C had an average of 85.9% of living cells. The samples that did not undergo erythrocyte depletion had only 37.2% of living cells. The expression of $CD34^+$ in the above groups had a similar proportional dependence on the percentage of living $SD45^+$ cells. The samples, subjected to rapid uncontrolled freezing and maintained in liquid nitrogen at –196°C had from 16.7% to 19.1% of $CD45^+$ / $CD34^+$ cells. But the samples, frozen slowly down to –80°C had 18.8% of $CD45^+$ /$CD34^+$ cells. In case of absence of erythrocyte depletion and under slow freezing at –80°C, the percentage of $CD45^+$/$CD34^+$was only 6.9%. Further efficiency of HSC transplantation was demonstrated by restored hematopoiesis in patients. Thus, blood counts were recovered to days 10–13 after transplantation. These findings show that the HSC freezing using the designed protocol can be performed slowly at –80°C and rapidly down to –196°C. In both cases, the HSC viability is maintained at 80–85%, and the percentage of $CD34^+$ cells is 17–19% of the total mononuclear cell population. This complies with the international standards and has already been introduced into the practice of National Specialized Children's Hospital 'Ohmatdyt'.

Probl Cryobiol Cryomed 2022; 32(4):304
https://doi.org/10.15407/cryo32.04.304a

Токсичність кріопротекторів для клітин мікроводоростей *Chlorococcum dissectum*

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Toxicity of Cryoprotectants to *Chlorococcum dissectum* Microalgae Cells

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The success of cryopreservation depends on a wide range of factors, one of which is the choice of cryoprotective agent (CPA) and its concentration. The selection of the optimal CPA requires an individual approach taking into account the conditions of the experiment, in particular such factors as temperature, time of interaction with the CPA, the choice of CPA carrier solution. The use of penetrating CPAs due to mass transfer processes eliminates intracellular ice formation, which increases the safety of cells after heating, but can cause damage due to excessive dehydration. At the same time, the direct toxic effect of CPA on cells, which, in general, remains poorly understood, is not excluded [Best, 2015; Day, 2018].

Therefore, the aim of the study was to investigate the effect of different concentrations of CPAs on the metabolic activity of *Chlorococcum dissectum* microalgae cells.

Cells were grown on nutrient medium BG-11 [Al-Rikabey, 2018] until the beginning of the stationary phase of growth without aeration at a temperature of (25 ± 2)°C under round-the-clock illumination with white fluorescent light 3 kLux. The following solutions were used in the work in the appropriate final concentrations: Me_2SO (5, 10, 20%); glycerol (10, 15%); ethanol (5, 10%); ethylene glycol (5%). The cultures were incubated for 60 minutes with subsequent washing from the CPAs. The cell concentration within the experiment was the same and made 153 × 10^6 cells/ml. The possible damaging effect of CPA on microalgae cells was determined by assessing the metabolic activity of cells by Alamar Blue (AB) test [Chernobai, 2021]. Cells that were not exposed to CPA were used as controls.

Data analysis showed that the exposure of *Ch. dissectum* cells with solutions of the studied CPAs reduced the AB fluorescence. The solution of 5% ethylene glycol had the highest damaging effect, the value of the studied index decreased by almost 47%. The lowest decrease in metabolic activity (approximately 20%) was observed after exposure to 10% glycerol. The results obtained when exposed to other CPAs did not differ significantly, the fluorescence decreased by an average of 34%.

Thus, the obtained data can be used to select an effective CPA in the further development of a protocol for cryopreservation of *Ch. dissectum* microalgae cells.