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Цитотоксичність та кріозахисна ефективність розчинів полівінілового спирту при заморожуванні еритроцитів людини

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Cytotoxicity and Cryoprotective Efficiency of Polyvinyl Alcohol Solutions During Freezing of Human Erythrocytes

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Suppression of ice recrystallization and growth reduction of already formed ice crystals, is one of the main effects of natural antifreeze proteins, along with the ability to be adsorbed on the surface of ice crystals, modify their growth and shape [Kristiansen E. 2005; Pertaya N. 2008]. However, there is a number of crucial challenges of their use in the cryopreservation practice: the very small amount of these compounds can be obtained from natural resources, the complexity of the complete chemical synthesis of analogues [Tachibana Y. 2004; Peltier R. 2010; Wilkinson B.L. 2012]; conflicting data on the efficiency of these compounds application in the cryopreservation of biomaterial [Koshimoto C. 2002]; cytotoxicity and immunogenicity of natural antifreeze proteins [Liu S. 2007].

A promising way to solve this problem is the development of cryopreservatives, in which, along with traditional cryoprotectants, the synthetic antifreeze compounds are used. From the list of these compounds, the synthetic polymer - polyvinyl alcohol (PVA) deserves special attention [Gibson M.J. 2010; Congdon T. 2013; Weng L. 2018].

The aim of the work was to investigate the cytotoxic effect and cryoprotective properties of polyvinyl alcohol solutions during freezing of human erythrocytes.

The research material was the erythrocyte concentrate obtained from human donor blood, prepared with the hemopreservative "Glugicir" in the Kharkiv Regional Blood Transfusion Center. This concentrate was stored for not longer than 48 hrs at a temperature of (4 ± 2) °C.

PVA (MW 9 and 31 kDa) (Sigma-Aldrich, USA) was used in the research. The concentration of free and total hemoglobin was determined by the hemoglobin-cyanide method using the "Hemoglobin SpL-2000" (Ukraine), hematocrit was done with a CM70 centrifuge, the percentage of hemolysis was calculated according to standard laboratory formulae. Morphology was analyzed by the immersion method of microscopic observation with a confocal laser scanning microscope "LSM S10 META Carl Zeiss" (Germany).

The studies showed that exposure of erythrocytes to PVA changed the shape of cells with a predominant number of spheroechinocytes both for 9 and 31 kDa.

Taking into account all the studied indices of preservation, the least cytotoxic cryoprotective solutions in relation to human erythrocytes are 0.2 and 1% PVA solutions with MW 31 and 0.5 kDa as well as 1% solutions with MW 9 kDa.

Thus, the perspective of using the PVA with MW 9 and 31 kDa was confirmed during cryopreservation of human erythrocytes.

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Вибір методів отримання та оцінки органотипових культур печінки для подальшого кріоконсервування

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Optimization of Methods for Obtaining and Evaluating Organotypic Liver Cultures for Further Cryopreservation

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Organotypic liver cultures are promising objects in biology, pharmacy and medicine. Their advantage is the preservation of the natural relationship of cells under the conditions of isolated cultivation. There are many methods of obtaining and evaluating organotypic cultures with different effectiveness indicators.

The aim of the study was to search for the most effective methods for obtaining and evaluating the morphofunctional properties of organotypic liver cultures for further cryopreservation.

For the study, we used the liver of BALB/c mice, from which slices were made using a vibrating microtome Leica VT1000 S. Low-melting and ordinary agarose were used in concentrations of 1–10%, phosphate buffer and culture medium for pouring blocks at different temperatures of pouring and slicing. The thickness of the sections varied from 50 to 500 μm. The methods of trypan blue staining, MTT test, resazurin reduction test, neutral red uptake test, histological study with paraffin embedding, preparation of cryo-microtome sections with hematoxylin-eosin staining, biochemical studies, confocal microscopy with staining with fluorescein diacetate, ethidium bromide and propidium iodide were used.

It was found that a 4% gel of ordinary agarose was optimal for obtaining liver slices of 300–400 μm. Thinner slices do not allow for preserving the integrity of the liver structures. Low-melting agarose allows for slightly lowering the temperature and facilitating the process of pouring the liver into blocks, but due to its fragility, the quality of the sections decreases. When making slices, the temperature of 37°C using a DMEM culture medium was determined as the optimal condition. The MTT test with formazan extraction by ethanol, resazurin reduction method, propidium iodide and fluorescein diacetate staining with confocal microscopy were selected as the preferred methods for functional evaluation. Kinetic methods of biochemical analysis (glucose level, LDH, urea in the medium) are preferred over other methods due to the staining of the culture medium. Morphological studies are better performed using a cryo-microtome instead of making paraffin blocks due to the small size of the objects.

Thus, for the application of the technique of organotypic cultivation, it is optimal to make vibratome sections of 300–400 μm using agarose, culture medium at a temperature of 37°C. To assess vital staining in confocal microscopy, MTT test, kinetic biochemical methods and cryo-microtome sections for light microscopy are used. A set of selected methods can be effectively applied in research.