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Перспективи застосування кріоконсервованих мезенхімальних стовбурових клітин кісткового мозку в лікуванні неврологічних захворювань і травм

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Prospects for the Use of Cryopreserved Bone Marrow Mesenchymal Stem Cells in the Treatment of Neurological Diseases and Injuries

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We began to study the properties of the bone marrow mesenchymal stem cells (MSCs) and the possibility of their application in neurology and neurosurgery back in 2002 on the basis of the Cell Technologies Laboratory of VIROLA LLC and the Department of Neurosurgery of the Kharkiv National Medical University (KhNMU).

Over 20 years of joint research, we have developed technologies for the reproduction of mammalian bone marrow MSCs in culture, a method for cryopreservation and differentiation into neuroblasts, hepatocytes, osteoblasts, and insulin-producing cells.

The methods for the treatment of parkinsonism syndrome, stroke, spinal cord and brain injuries, Alzheimer's disease, and sciatic nerve injury have been developed on models of these diseases in laboratory animals according to state research programs of KhNMU.

Pilot clinical research on humans authorized by the Medical University Scientific Council have demonstrated the efficacy and safety of transplantation of autologous bone marrow MSCs in the treatment of diseases such as Parkinson's disease, multiple sclerosis, stroke, post-traumatic epilepsy, and brain injury. MSC therapy is safe, as it excludes the introduction of alien proteins and viruses into the patient's body. The effectiveness of this therapy can be explained by the fact that autologous MSCs not only stimulate tissue regeneration due to growth factors, but are also directly involved in the restoration of damaged tissues. The results of our research are published in 60 scientific articles and 14 patents of Ukraine.

Bone marrow MSCs are also effectively used in other areas of medicine, such as traumatology, cardiology, ophthalmology, combustiology, and dentistry. Therefore, it would be timely and promising to create a modern Cryobank of autologous MSCs in Ukraine, by analogy with cord blood banks, so that every adult has the opportunity to create the reserve of his own stem cells and use them at any time.

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Розробка електродів для кріоконсервування стовбурових клітин за допомогою електропорації з навантаженням сахарозою

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Development of Electrodes for Cryopreservation of Stem Cells by Electroporation-Assisted Loading with Sucrose

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The application of stem cells in regenerative medicine therapies has recently become one of the most promising strategies for the treatment of various diseases. Therefore, the need for an effective preservation method has increased. Currently, the cryopreservation of stem cells is the most commonly used method, utilizing dimethyl sulfoxide (DMSO) and fetal bovine serum (FBS) as a cryoprotective agent (CPA). However, due to the cytotoxic characteristics of DMSO an alternative is being investigated. In this regard, disaccharides like sucrose can be applied as a natural CPA. Since sucrose, due to the large size, is not able to diffuse trough the cell membrane, reversible electroporation can facilitate the incorporation of sucrose into the cell membrane.

For this purpose, new electrodes were developed and manufactured. The design is equivalent to parallel plate capacitors. Regarding the material, stainless steel and aluminum were investigated. The human bone marrow stem cells (hBMCs) were put in a buffer solution with a sucrose concentration of 450 mM and electroporated with a voltage of 300 V. 8 pulses were used with a duration of 100 µs and a frequency of 1 Hz. The distance between the electrodes was 2 mm. After the electroporation, the cells were frozen with a cooling rate of 1 K/min. After being stored in liquid nitrogen for at least seven days, the cells were thawed and the CPAs removed. Subsequently, the viability of the cells was investigated with trypan blue. Furthermore, the morphology, the viability, and the metabolism were evaluated after cultivating the cells again, using Alamar Blue, a fluorescence microscope, and a scanning electron microscope, respectively. The viability of the cells after thawing was 68.65% with the use of stainless steel for the electrodes and 36.58% with aluminum for the electrodes, compared to the viability of 84.51% for the cells that used 5% DMSO as CPA. The other experiments confirmed these findings. After cultivation, the specific growth rate of the cells electroporated with stainless steel electrodes was superior to the growth rate of the cells using DMSO as CPA.