Probl Cryobiol Cryomed 2023; 33(4):288
https://doi.org/10.15407/cryo33.04.288

Особливості формування та кріоконсервування сфероїдів з клітин головного мозку новонароджених щурів

С. Всеволодська, О. Сукач, О. Оченашко
Інститут проблем кріобіології і кріомедицини НАН України, м. Харків, Україна

Features of Formation and Cryopreservation of Spheroids From Brain Cells of Newborn Rats

S. Vsevolodska, O. Sukach, O. Ochenashko
Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Kharkiv, Ukraine

The development of effective methods for the formation and cryopreservation of brain cell spheroids is of great importance for their widespread use in biomedical research, pharmacology and biotechnology.

The aim of our work was to evaluate the peculiarities of spheroid formation by precultured neonatal rat brain cells and to determine their resistance to cryopreservation factors.

The primary suspension of neural cells (NCs) was obtained from the brain of newborn rats by the enzymatic-mechanical method. The number and viability of cells were counted in a Goryaev chamber. NCs were cultured at a concentration of 2×10^6 cells/ml in 24-well plates in DMEM/F12 with 10% serum at 37°C, in 5% CO_2 atmosphere, 95% air. After the formation of a 70% monolayer, the NCs were removed, washed, and resuspended in the culture medium. The resulted suspension at a concentration of 1×10^6 cells/ml was applied in the form of 25 μL drops to the inner surface of the Petri dish lid. The lid was placed with the drops down (hanging drop technique) and cultured according to the standard protocol. The spheroids were frozen at a rate of 1°C/min to –80°C, followed by transfer to liquid nitrogen and also by immersion in liquid nitrogen. As a cryoprotectant, 10% DMSO was used in culture medium with 10% serum. Spheroids were warmed at 38°C.

In the course of cultivation, the freshly obtained NCs attached, migrated, differentiated and spread out, forming the cell chains of various lengths and shapes as well as cell structures similar to a neuronal network. On day 4 of cultivation, the colonies of undifferentiated cells formed.

The beginning of spheroid formation was observed after 24 hours of cultivation of NCs in the drops. On the 4th day of cultivation, 1 spheroid of regular shape, 140–220 μm in diameter, was formed in all drops. Further cultivation did not lead to the formation of new spheroids or changes in the size of the formed ones. Reseeding of spheroids onto the adhesive surface led to their attachment, followed by the formation of monolayer sections by their cells.

Spheroids frozen by immersion in liquid nitrogen did not attach to the substrate and disintegrated into single cells during cultivation. Spheroids which were not washed from DMSO after cryopreservation at 1°C/min did not attach to the substrate and eventually their cells died. Spheroids washed from DMSO by a stepwise reduction in its concentration attached to the substrate on days 2–3 of cultivation with subsequent migration and flattening of their cells. However, such cells did not form the chains and structures similar to the neuronal network.

Probl Cryobiol Cryomed 2023; 33(4):288
https://doi.org/10.15407/cryo33.04.288a

Вплив низьких температур на морфологію альгінатних мікроносіїв

С. Нарожний[1], О. Боброва[1,2], І. Коваленко[1]
[1] Інститут проблем кріобіології і кріомедицини НАН України, м. Харків, Україна
[2]Науково-дослідний інститут рослинництва, м. Прага, Чеська Республіка

Effect of Low Temperatures on Morphology of Alginate Microcarriers

S. Narozhnyi[1], O. Bobrova[1,2], I. Kovalenko[1]
[1]Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Kharkiv, Ukraine
[2]Crop Research Institute, Praha, Czech Republic

Microbeads based on natural biopolymers are widely used in medicine for the targeted drug delivery. One such delivery system is microcarriers derived from sodium alginate. Cryopreservation of such systems can increase the availability and expand the scope of application of drugs based on them. Important characteristics of targeted delivery systems are parameters that affect the rate of release of encapsulated substances from microcarriers. Currently, the main mechanism for the drug release from alginate microcarriers is the diffusion process, so the rate of release of encapsulated substances depends on the contact area of the semipermeable membrane of the carriers with the solvent. Therefore, the aim of this study was to analyze the changes in the shape of sodium alginate microbeads after freezing and rewarming.

Microcarriers based on sodium alginate were obtained by electrospraying. For this, a 2% solution of sodium alginate was pumped out from the metal capillary with an inner diameter of 0.2±0.05 mm attached to the syringe into 2% wt/vol calcium chloride solution at a flow rate of 10 ± 0.1 ml/h using the syringe pump. The obtained microbeads were frozen at rates of 3-4 deg/min and 300 deg/min, then rewarmed in a water bath at 37°C. The shape of the microbeads before and after cooling was evaluated by analyzing microphotographs obtained with an Axio Obzerver Z1 confocal microscope (CarlZeiss).

For the experiment, two groups of microbeads with different average diameter of the equivalent sphere were obtained. It was 1222 ± 12 μm for the first group and 277 ± 67 μm for the second group. The largest change in shape was observed in the second group of microbeads, for which the roundness parameter decreased by 50.82% with slow cooling and by 37.80% with fast cooling. The change in this parameter in the first group was less significant and amounted to 21.30% for slow cooling and 5.42% for fast cooling.

The results of the study showed that the decisive factor influencing the shape of alginate microspheres during freezing is the cooling rate.