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Міноги України як перспективний об'єкт кріобіологічних досліджень

Д. Острась[1], Г. Гончаров[2], К. Міксон[3]

[1]НДІ біології, Харківський національний університет імені В.Н. Каразіна, м. Харків, Україна

[2] Харківський національний університет імені В.Н. Каразіна, м. Харків, Україна

[3] Інстиут проблем кріобіології і кріомедицини НАН України, м. Харків, Україна

Lampreys of Ukraine as a Prospective Object of Cryobiological Research

D. Ostras[1], H. Honcharov[2], K. Mikson[3]

[1]Research Institute of Biology, V.N. Karazin Kharkiv National University, Kharkiv, Ukraine

[2]V.N. Karazin Kharkiv National University, Kharkiv, Ukraine

[3]Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Kharkiv, Ukraine

Lampreys represent one of the most ancient vertebrate groups, one of the two extant lineages of jawless fish that retain archaic morphological features for about 360 million years. Due to its unique evolutionary position against all modern vertebrates, *Petromyzontiformes* as 'living fossils' is a popular model object for various fields of biological sciences [Docker, 2015]. Modern lampreys rank in the monotypic superclass *Petromyzontomorphi* (order: *Petromyzontiformes*) and are represented by 3 families and about 40 described species [Nelson, 2016]. Lampreys are distributed antitropically in both hemispheres, preferring water bodies of temperate or subarctic latitudes. In Ukrainian rivers, two lamprey species, the Ukrainian *Eudontomyzon mariae* and the Carpathian *Eudontomyzon danfordi*, are described in modern literature [Shandikov, 2008].

Lamprey refers to a limited group of species in Ukrainian fish, preferring to spawn at cold water (up to 10°C), which also include burbot (1–3°C), pike (6–10°C), perch (8–10°C), roach (10–14°C) [Marenkov, 2016]. In this group, lamprey and burbot spawning are the most cold-dependent, with spawning temperatures for lamprey ranging from 4–5°C according to our data. Based on the data from 10-year monitoring of *E. mariae* population of the 'Sviati Gory' [Ostras, 2021; Shandikov, 2012] National Nature Park (Sviatohirsk, Ukraine), it is important to study the adaptations associated with changes in the temperature regime of their biotope (associated with gradual changes in the hydrological regime regionally). The uniqueness of this population is regular and mass spawning in a single location, at the southeastern edge of the species range. During the monitoring of this population (2012–2021), we observed 10-fold fluctuations in the number of spawning individuals. Such fluctuations can be caused by changes in hydrological conditions (damming by beavers, anthropogenic impact) of the creek and, consequently, the possible change in temperature regime of the water body. *E. mariae* is included to the IUCN Red List and suggests that their population is threatened to extinction [Shandikov, 2008]. Conversely, the requirement of new model organisms for experimental studies is actual, primarily because of the specific phylogenetic position of lampreys [Docker, 2015; Katkov, 2012]. The effect of low temperatures on embryonic development, metamorphosis and maturation of gametes, spawning, including artificial conditions, is of research interest. Obtaining such, data, combined with the development of methods for cryopreservation of embryos and germ cells, will also solve the applied conservation task and examine the possibility to use local lampreys as model objects for cryobiology research.

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Сприятливий вплив інкапсуляції мезенхімальних стромальних клітин людини в альгінат на зберігання за кімнатної температури в умовах гіпоксії

О. Губеня[1], Ю. Кот[2], Н. Труфанова[1,2]

[1] Інститут проблем кріобіології і кріомедицини НАН України, м. Харків, Україна

[2] Харківський національний університет імені В.Н. Каразіна, м. Харків, Україна

Human Mesenchymal Stromal Cells Alginate Encapsulation Beneficial Effect for Storage at Ambient Temperature Under Hypoxic Conditions

O. Hubenia[1], Y. Kot[2], N. Trufanova[1,2]

[1]Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Kharkiv, Ukraine

[2] V.N. Karazin Kharkiv National University, Kharkiv, Ukraine

Mesenchymal stromal cells (MSCs) possess high proliferative potential, the ability to multilinear differentiation and low immunogenicity. Efficient methods for MSCs storage are required for their implementation in clinical and laboratory practice. Cell storage at ambient temperatures is an alternative to cryopreservation in case of short-term storage to simplify the transportation of samples and escape the disadvantages of cryopreservation techniques.

Here we study the viability, metabolic activity, and cell cycle of MSCs under culture and during storage at ambient temperature in the form of a monolayer, suspension, and encapsulated in alginate microspheres (AMS). Human dermal MSCs were isolated from skin samples obtained from adult donors after their informed consent and cultured in alfa-MEM supplemented with 10% of fetal bovine serum under standard conditions. MSCs storage was performed in sealed containers at 22°C. Viability (FDA/ EB dual staining), and metabolic activity (Alamar blue and MTT-tests) were assessed on 0, 3, 7, 10 and 14 days of storage. For cell analysis the MSCs were transduced with the Premo™ FUCCI Cell Cycle Sensor, then cultured in monolayer or in AMS with live-cell imaging of cell cycle progression with confocal laser scanning microscope Olympus FV10i-LIV and Olympus cell Sense Software. Abcam Cellular ROS Assay Kit (Deep Red) was used for the assessment of ROS level.

Viability by FDA/EB decreased during MSCs storage in form of monolayer by 70%, in suspension by 40% to day 7 compared with initial viability. Metabolic activity reduced even more sharply. During storage of MSCs in the form of a suspension, spheroid formation was revealed at times. On 7th day of MSCs storage, the encapsulation supported the viability and metabolic activity of 85% and 55% of initial indices, correspondingly. Metabolic activity of cells after 24 hrs of culture in AMS decreased by 40% compared to MSCs in monolayer. Cell cycle analysis showed that MSCs were completely arrested in the G1 phase 48 hrs after encapsulation. ROS sensor fluorescence which directly reflected real-time intracellular ROS level was 32.8 ± 5.2 and 2.32 ± 0.25 RFU/cell in intact monolayer and AMS, respectively. After 2 hrs incubation with 3 mM hydrogen peroxide the fluorescence of ROS sensor was 154.5 ± 7.8 RFU/cell in monolayer, and 11.78 ± 0.44 RFU/cell in AMS. Therefore, encapsulation of MSCs in AMS decreased both basal and induced ROS levels.

The benefits of encapsulation in alginate microspheres for the MSCs short-term storage and transportation under ambient temperature were shown. Changed rates of metabolic and cell cycle processes, and increased oxidative stress resistance of encapsulated MSCs, which played a great role in resistance to storage at ambient temperature, were revealed.

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