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Збереження мезенхімальних стромальних клітин людини в альгінатних капсулах, збагачених білком

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Preservation of Human Mesenchymal Stromal Cells in Protein-Supplemented Alginate Capsules

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Human mesenchymal stromal cells (MSCs) are promising cell type for biomedical research and clinical medicine. Cell storage at ambient temperatures may simplify transportation and overcome disadvantages of cryopreservation.

Here we study the cell cycle, viability, metabolic activity of MSCs during storage at ambient temperature in five different forms: monolayer, suspension, encapsulation in alginate capsules (AMS), and AMS with addition of fresh porcine blood plasma or human amniotic membrane (hAM) extract. The extract was obtained from hAM after dissection of the placenta, followed by freeze-drying and membrane digestion. The experiments were performed on human bone marrow MSCs provided by Clinic for Orthopedic at Hannover Medical School and cultured in sealed containers at 22 °C alfa-MEM supplemented with l0% (v/v) of fetal bovine serum. Alginate 2.5% (w/v) low-viscosity was used for the AMS production. The protein concentration of AMS with hAM extract or porcine blood plasma was standardized (BCA Protein Assay Kit, Bradford Assay) to 32.6 µg/ml. Viability (Trypan Blue, FDA/EthD dual staining), metabolic activity (Alamar blue) were assessed on l, 3, 5 and 7 days of storage. For cell cycle analysis, MSCs were transduced with the Premo™ FUCCI Cell Cycle Sensor, then cultured in monolayer or AMS and live-cell imaging of cell cycle progression with confocal laser scanning microscope Olympus FVl0i-LIV with Olympus cellSense Software.

The findings indicated that the metabolic activity of cells in AMS decreased by 40% after 1 day of culture, as opposed to MSCs cultured in a monolayer. AMS with the addition of hAM extract, or porcine blood plasma decreased to 52% and 45% compared to MSCs in monolayer, respectively. Viability assessed by FDA/EthD decreased 70% during MSCs storage in monolayer, 40% in suspension, 87% in AMS with hAM extract, and 62% with porcine blood plasma at day 7 compared with initial viability after day l. Metabolic activity follows a similar decrease trend on the same days. On the other hand, at day 7 AMS without hAM or plasma presented viability of 85% and metabolic activity of 55% from initial indices. Furthermore, AMS with hAM extract and porcine blood plasma had metabolic activity of 53% and 62% of initial indexes, correspondingly. Cell cycle analysis showed that MSCs were completely arrested in Gl phase 2 days after encapsulation.

The benefits of AMS for the MSCs short-term storage and transportation under ambient temperature were shown with a correlation between the decrease of cell metabolic activity and cell viability.

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Кріорезистентність автотрофних організмів з полярних регіонів, визначена методом флуоресценції хлорофілу

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Cryoresistance of Autotrophic Organisms From Polar Regions Sensed by Chlorophyll Fluorescence Method

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Lichens are symbiotic organisms constituting great number of species in polar regions. They are well adapted to low temperature and considered cryoresistant. Interspecific differences in their cryoresistance, however, occur according to their degree of adaptation and severity of the environment.

In our study, by applying linear cooling technique, we evaluated interspecific differences in their cryoresistance. We selected thalli segments of 7 different lichen species: *Umbilicaria antarctica, Nephroma antarctica, Placopsis contortuplicata, Lasallia pustulata, Xanthoria elegans, Umbilicaria cylindrica, Usnea sphacelata*, and 1 species of a representative of extremophilic cyanobacteria *Nostoc commune.* These species were exposed to a constant-rate cooling from 20 to –35°C (rate of 2°C min^{-1}). Simultaneously, we measured chlorophyll fluorescence (ChlF) parameters in 30 s intervals: potential (Fv/Fm) and effective quantum yield of primary photochemical processes in PSII (ΦPSII). Data obtained were plotted against sample temperature showing the ChlF decline with temperature decrease, *i. e.* temperature response curves.

The curves of Fv/Fm and ΦPSII formed a typical S-curves that were species specific. They have a triphasic shape: (1) plateau (the temperature range decreasing from 20 to –5°C), (2) S-shaped decline, and (3) a shoulder reaching the critical point (temperature, Tc) at which ChlF values reached 0. Tc ranged between –25 and –28°C, suggesting that the above-specified experimental lichen species had a high resistance to sub-zero temperatures. Among the selected species, *L. pustulata, U. antarctica,* and *U. cylindrica* were the most cryoresistant in terms of photosynthetic processes below zero temperature. Tc (Fv/Fm) for them were –44.l, –43.6 and –43.0 ± 0.2°C respectively, while for other experimental species it varied in the range of –23…–39°C.

The method of linear cooling used in this study has proven its applicability in ecophysiological studies of Antractic lichens since it is sensitive enough for the evaluation of species-specific differences in cryoresistance. This study describes different parameters that can be derived from the S-curves and discuss their proper use in ecophysiological and stress physiology studies.