MESENCHYMAL STROMAL CELLS STORAGE AT AMBIENT TEMPERATURE

Hubenia O.S.[1], Kot Yu.H.[2], Trufanova N.A.[1,3]

[1]Department of Cryobiochemistry, Institute for Problems of Cryobiology and Cryomedicine, NAS of Ukraine, Kharkiv, Ukraine

[2]Department of Biochemistry, School of Biology, V.N.Karazin Kharkiv National University, Kharkiv, Ukraine

[3]IPC&C of the National Academy of Sciences of Ukraine, Kharkiv, Ukraine

shalinaaleksandra@gmail.com

Background. Mesenchymal stromal cells (MSCs) possess high proliferative potential, the ability to multilinear differentiation, low immunogenicity and immunomodulatory properties. MSCs are increasingly used in various fields of biology, tissue engineering, etc. Short-term storage at ambient temperatures may both solve problem of cell injury associated with cryopreservation and simplify transportation between clinical centers.

Aim. To study viability, metabolic activity, and cell cycle of MSCs under culture and during storage at ambient temperature in form of monolayer, suspension, and encapsulated in alginate microspheres (AMS).

Methods. The experiments were performed on human dermal MSCs derived after adult donors informed consent. Storage was performed in sealed containers at 22°C in alfaMEM supplemented with 10% of fetal bovine serum. Viability (FDA/EB dual staining), metabolic activity (Alamar blue-test) were assessed on 0, 3, 7, 10 and 14 days of storage. For cell cycle analysis the FUCCI Cell Cycle Sensor was used, live-cell imaging of cell cycle progression of MSCs cultured in monolayer or in AMS was performed with confocal laser scanning microscope. Abcam Cellular ROS Assay Kit (Deep Red) was used for assessment of ROS level, basal and induced by incubation with hydrogen peroxide.

Results. Viability by FDA/EB decreased during MSCs storage in form of monolayer for 70%, in suspension for 40% at 7 day 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 encapsulation supported the viability and metabolic activity of 85% and 55% of initial indexes, correspondently. Metabolic activity of cells after 24 hr of culture in AMS decreased 40% compared to MSCs in monolayer. Cell cycle analysis showed that MSCs were completely arrested in G1 phase in 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 MSC in AMS decreased both basal, and induced ROS level.

Conclusions. Encapsulation of MSCs in alginate microspheres was shown as benefit approach for short-term storage and transportation under ambient temperature. Changed metabolic activity rate, cell cycle reversible arrest, and increased oxidative stress resistance of encapsulated MSCs were revealed as factors of resistance to storage at ambient temperature.

Acknowledgements. This research was supported by the joint Ukrainian-Czech R&D project “Cryobiology profile of 3D mesenchymal stem cells spheroids”. M/55 – 2022 from 25.05.2022.