UDC 664.644.7:611.018.2/.6:615.014.41 https://doi.org/10.15407/biotech9.04.058

ENCAPSULATION OF MESENCHYMAL STROMAL CELLS IN ALGINATE MICROSPHERES

D. Tarusin
S. Mazur
N. Volkova
Yu. Petrenko
V. Zaikov
A. Petrenko

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

E-mail: tarusindmitriy@gmail.com

Received 29.04.2016

The research aim work was a comparative study of structural and functional state and capacity for differentiation of multipotent mesenchymal stromal cells, stored at various temperatures either as cell suspensions or within alginate microspheres. Storage was carried out in a culture medium based on α-MEM at temperatures of 4, 22 and 37 °C in sealed cryovials. After 1, 2 and 3 days the alginate microspheres were dissolved and viability (MTT test), the attachment properties and metabolic activity (AB test) were evaluated in monolayer cell culture. It has been shown that the storage of the mesenchymal stromal cells in suspension for 3 days at the indicated temperatures resulted in a decrease of the studied parameters. Mesenchymal stromal cells after storage within alginate microspheres at 22 and 37 °C showed a high viability (78 and 87%, respectively), kept the attachment properties (62 and 70%), metabolic activity (79 and 75%) and ability to differentiation. The results indicate that the mesenchymal stromal cells entrapped in alginate microspheres are more resistant to storage conditions than a suspension of mesenchymal stromal cells. Entrapment in alginate microspheres is a promising technological approach for a short-term storage of mesenchymal stromal cells at positive temperatures.

Key words: multipotent mesenchymal stromal cells, alginate microspheres, metabolic activity, induced differentiation.

Multipotent mesenchymal stromal cells (MSCs) are characterized by unique properties and high therapeutic potential. For effective application of MSCs it is necessary to develop the conditions of their storage and transportation ensuring the preservation of the viability and functional activity. A common method for cell storage is cryopreservation [1, 2]. It allows the storage of biological material during unlimited period of time, however, requires the use of special equipment and cryoprotectants which are as a rule of toxic effect [3].

For a short-term storage and transportation of cells as a cheap and technically simple alternative to deep cold (−196 °C) there are widely used the positive temperatures which are below of the physiological values inherent to homoiotherms. When developing the hypothermic storage conditions the major attention is concentrated to optimizing the composition of media [4, 5, 6], leaving out of the focus the spatial arrangement of cells in a liquid medium during storage. Natural sedimentation in the dispersion over time leads to a situation when individual cells occur to be in different conditions. The last circumstance can be avoided by continuously stirring the cell suspension, increasing the viscosity of the medium or formation of a specific cell environment with their entrapment in the hydrogel, for example alginate microspheres (AMSs).

The advantages of cell entrapment in hydrogel AMSs as a technological method which provides an increase in cell viability during storage have been previously identified when we studied the correlation of MSCs hypothermic storage on the compositions of the used media [7]. In present paper we set out to determine if this effect occurs without using the special media for hypothermic storage.

The aim of the work was a comparative study of the structural and functional

condition and ability to induce differentiation of MSCs stored at positive temperatures either in the form of cell suspensions or entrapped in alginate hydrogel microspheres.

Materials and Methods

Experiments were performed in human adult dermal MSCs, procured in accordance with the recommendations of the WMA Association of Helsinki, and the requirements of Bioethics Committee of the Institute for Problems of Cryobiology and Cryomedicine of NAS of Ukraine.

The monolayer of dermal MCSs was cultured in the culture medium (CM) of α-MEM (PAA, Austria) supplemented with 10% fetal bovine serum (FBS), 50 IU/ml of penicillin and 50 μg/ml streptomycin, at 37 °C, 5% CO_2 and 95% humidity. When the cells reached a 70% monolayer, they were then passaged by the standard technique using Trypsin/Versene mixture at 1:4 ratio and plated with 5,000 cells/cm^2 reseeding coefficient [9]. The MSCs cultures which underwent 4–8 passages were used for study.

Experiments on cell viability during storage in CM were performed using MSCs in suspension and entrapped in AMSs. For entrapment in AMSs the cells were suspended in 1.2% sodium alginate solution (Sigma-Aldrich, USA), prepared with Hank's solution (pH 7.4). The resulting mixture was then placed into a 1 ml syringe with a 0.33 mm needle diameter and added dropwise into 2% $CaCl_2$ solution, where it was left for 10 min to polymerize. The obtained AMSs, containing MSCs, were stepwise washed from an excess of calcium ions by buffer solution (0.15 M NaCl, 25 mM HEPES, pH 7,4) and placed in CM. The AMSs diameter made was 2.0±0.1 mm.

For the purpose of storage the MSCs in suspension of single cells or within AMSs was placed into 2 ml cryovials with a semicircular bottom (NUNC, USA). The dose of cells placed into a cryovial either as the suspension or entrapped in AMSs was about 100,000, total volume of medium made 1 ml. The sealed cryovials with samples were incubated at 4, 22 and 37 °C during 1, 2 or 3 days in the upright position. At each time point the experimental material in cryovials was a visually assessed. To evaluate MSCs survival was determined cell viability after washing of storage medium and dissolving the AMSs, and cell attachment, metabolic activity and ability to induce differentiation, after placing them under culture conditions. As the control MSCs in suspension and entrapped in AMSs were not subjected to storage (zero time point of storage).

For extraction of the cells from AMSs was added 1 ml of 50 mM sodium citrate, resulting in a gel depolymerization. Cells were washed with a buffer solution, centrifuged for 7 min at 150 g and resuspended in 1 ml of CM. Stored in suspension MSCs was also washed with buffer solution and suspended in a fresh portion of the CM.

The MSCs viability was assessed by MTT test. With this aim the 0.5 ml cell suspension was supplemented with 50 μl of a redox indicator MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium] bromide (Sigma-Aldrich) in 5 mg/ml concentration. After 2 hrs' incubation at 37 °C the samples were centrifuged, the sediment was suspended in saline and a number of cells was calculated in a Goryaev's chamber according to the standard technique [10]. The viability was determined as the ratio of MSCs accumulated formazan to the total number of cells and expressed as a percentage.

Cell attachment was assessed by the ability of MSCs to attach to the culture plastic. On 1 day after MSCs placement under cell culture conditions there was counted a number of non-attached cells according to total amount of the cells and the level of attachment was calculated by the following formula:

$$E = \frac{A - B}{A} \times 100\%,$$

where A — number of cells before plating (by MTT test);

B — number of not attached cells.

Metabolic activity of MSCs was evaluated by the cell ability to reduce the redox indicator AlamarBlue (AB) after 1 day of culturing. For this purpose, in the wells of the culture plate was added 0.5 ml of fresh CM containing 10% AB and fluorescence was measured at excitation wavelength 550 nm and emission 590 nm using a spectrofluorometer Tecan Genios (Australia) after 2 hours of incubation at 37 °C with 5% CO_2. The data were processed with program XFLUOR4 v/4/50 software (Tecan GENios) and expressed in relative fluorescence units (RFU). The calculation was performed using the formula:

$$RFU = \frac{S_x - S_b}{S_b},$$

where S_x — fluorescence of the sample;

S_b — fluorescence of the blank test (10% AB solution in CM without cells).

Adipogenic differentiation of MSCs was induced by culturing in α-MEM medium, containing 10% FBS, 0.5 mM 3-isobutyl-1-methylxanthine (Sigma-Aldrich), 1 μM dexamethasone (Sigma-Aldrich), 10 μg/ml insulin (Sigma-Aldrich), 100 μM indomethacin (Sigma-Aldrich). To consider a spontaneous differentiation the cells were cultured in α-MEM medium with 10% FBS without inducers. Media were changed 2–3 times per week. After 21 days the cell cultures were fixed for 30 min at 4 °C with 10% formalin, prepared on Hanks solution. To confirm adipogenic differentiation of MSCs the neutral lipids were stained by Oil Red O (Sigma-Aldrich). Fixed preparations of cell cultures were washed with 60% isopropyl alcohol (Macrochem, Russia) and kept in a freshly prepared solution of Oil Red O (30 mg/ml in 60% isopropanol) for 1 h at room temperature, then washed with distilled water [11].

Osteogenic differentiation of MSCs was induced following culture in α-MEM medium, containing 10% FBS, 100 nM dexamethasone (Sigma-Aldrich), 10 mM β-glycerophosphate (Sigma-Aldrich), 0.2 mM L-ascorbic acid-2-phosphate (Sigma -Aldrich) for 21 days. Calcium accumulation, assessed morphologically by staining using Alizarin Red S (Sigma-Aldrich), was considered as the confirmation of differentiation in this direction [12]. For this aim the fixed preparations of MSCs cultures were incubated for 2 min in a 1% Alizarin Red S dye solution at room temperature, and then thoroughly washed with distilled water.

Morphological studies of stained preparations were carried out by light inverted microscope CETI Inverso (CETI, Belgium).

The results were statistically processed using Excel (Microsoft, USA) and Past Statistic v/3/01 (Sweden). Depending on the pattern of data distribution the significance of differences between the indices was evaluated using either a parametric Student's t-test or nonparametric Mann-Whitney test. Differences between the samples were considered as significant at $P \leq 0{,}05$.

Results and Discussion

Human dermal MSCs in the form of suspension and entrapped in AMSs were kept in normo- and hypothermic conditions for 1–3 days in sealed vials with medium, which is common environment for their culture and maximally supporting their vital functions.

At the first stage of the study the suspensions of isolated MSCs during their storage at positive temperatures were visually controlled. At 4 °C after the first day the cells were sedimented with the formation flexible loose residue. On 3rd day of storage the sediment density was increased, but when gently shaking the vial a homogeneous cell suspension was formed. CM colour, reflecting the pH, did not change within 3 days of storage at 4 °C.

Exposure of MSCs suspension for 3 days under mild hypothermia conditions at 22 °C also resulted in the sediment formation of cells without changing the medium pH. However turning it to a suspended state did not allow the obtaining of homogeneous suspension. In cell suspension there were observed small sized cell aggregates.

Cell suspensions storage in normothermic conditions at 37 °C to day 1 formed relatively dense sediment. For turning MSCs into a suspension form required some effort. Obtained after sediment suspending the suspension consisted mainly of cell aggregates and non-cellular strands. There were observed the more dense and large-sized aggregates versus those at 22 °C. Furthermore by 2nd day of storage an acidification of the CM occurred.

When observing the state of AMSs with the MSCs stored at 4, 22 and 37 °C showed no changes in their integrity and CM up to the 3 days of the study: the entrapped in AMSs cells remained distinct, cell aggregates were not observed and colour of the storage CM did not differ if compared to control.

Assessment of the cell viability reflecting the general characteristics of their survival and morphological and functional status of MSC as attachment and metabolic activity confirmed the differences in cell state after storage, due to the features of their spatial arrangement, i.e. they have been in suspension or entrapped in the hydrogel AMSs.

Initial viability of human dermal MSCs in suspension estimated by MTT test made 97 ± 2%. MSCs within AMSs maintained viability at the same level.

The Fig. 1 has shown that MSCs storage in suspension at all the temperatures lead to gradual decrease in cell viability and significant differences were observed after 1 day. After 3 days of MSCs storage in suspension at 22 and 37 °C the viability was about 50%. In the case of hypothermic storage at 4 °C decrease in this parameter was more pronounced — 30%.

Entrapped in AMSs MSCs was more tolerated to storage. After the 1st day a significant decrease in viability was observed only in the case of cells stored in AMSs in hypothermic conditions at 4 °C. Use of the temperatures 22 and 37 °C allowed maintaining a level of MSCs viability close to the initial. A significant reduction in the viability of MSCs within AMSs during storage in mild hypothermic conditions at 22 °C was observed only on final periods of storage, but the percentage of cells accumulating formazan remained relatively high, in particular 78± 4%, that was significantly higher compare to viability of the MSCs in suspension. Storage of the MSCs in AMSs at 37 °C not resulted in reducing the viability up to three days resulting in a difference between the rates of MTT test for MSCs in suspension and within AMSs about 40% (87 ± 9% and 49 ± 10%, respectively).

Hypothermic storage of MSCs within AMSs at 4 °C resulted in a gradual decrease of viability during the observation period and at the end of this period the differences between cells in the suspension and AMSs were not observed. Advantage of MSCs storage within AMSs was revealed after two days when the viability of MSCs was about 20% higher if compared to viability of the cells in suspension (Fig. 1).

The ability of MSCs to attach to the culture plastic is one of the determining characteristics of these cells, which implemented by a complex cascade of responses and generally reflect the cell survival rate. MSCs storage in suspension at all the studied temperatures led to a gradual reduction in their attaching ability (Fig. 2). As a result by 3rd day only 20% of the cell suspension exposed at 4 and 37 °C were able to attach to the plastic. The use of mild hypothermia (22 °C) to a lesser extent influenced this parameter, more than 40% of cells kept the attachability ($P \leq 0.05$).

In general, changes in attachment of MSCs within AMSs during storage were similar to the changes' dynamic of viability obtained by MTT assay. The ability to attachment of the MSCs within AMSs stored at 22 and 37 °C significantly differs if compared to MSCs in suspension. Despite this parameter was lower than initial reference level after two days of storage, to the end of the experiment the percentage of capable to attachment MSCs was 62 ± 10% (22 °C) and 69 ± 7% (37 °C), which was significantly higher than the results obtained for MSCs in AMSs stored at 4 °C. Ability to attachment of MSCs in AMSs after hypothermic storage (4 °C) did not differ versus the MSCs stored in suspension.

Attached to the plastic MSCs were subjected to monolayer culture to confirm their morphological and functional integrity. In culture these cells was flattened, acquired fibroblast-like morphology, actively proliferated and thereafter could form a monolayer.

For integral evaluation of the MSCs state in culture was used AB test which reflects a

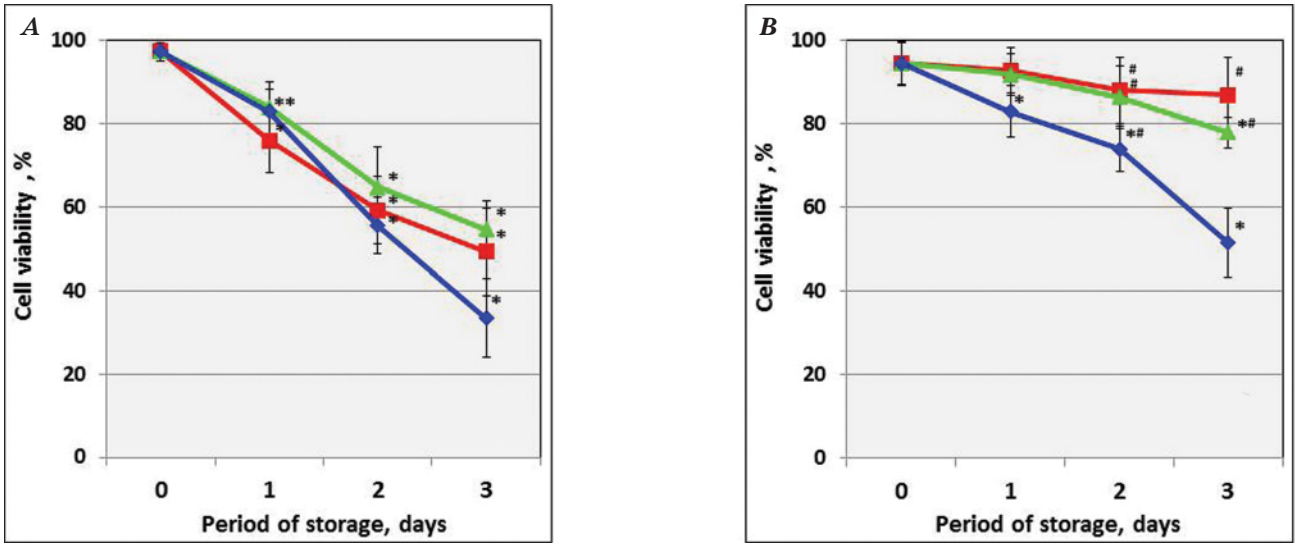

Fig. 1. **Comparison of multipotent mesenchymal stromal cells viability during storage in suspension (*A*) and within alginate microspheres (*B*) at 4 (♦), 22 (▲) и 37 (■) °C using MTT assay**
Hereinafter: $P \leq 0.05$ compared to the control (*) and to MSCs suspension (#)

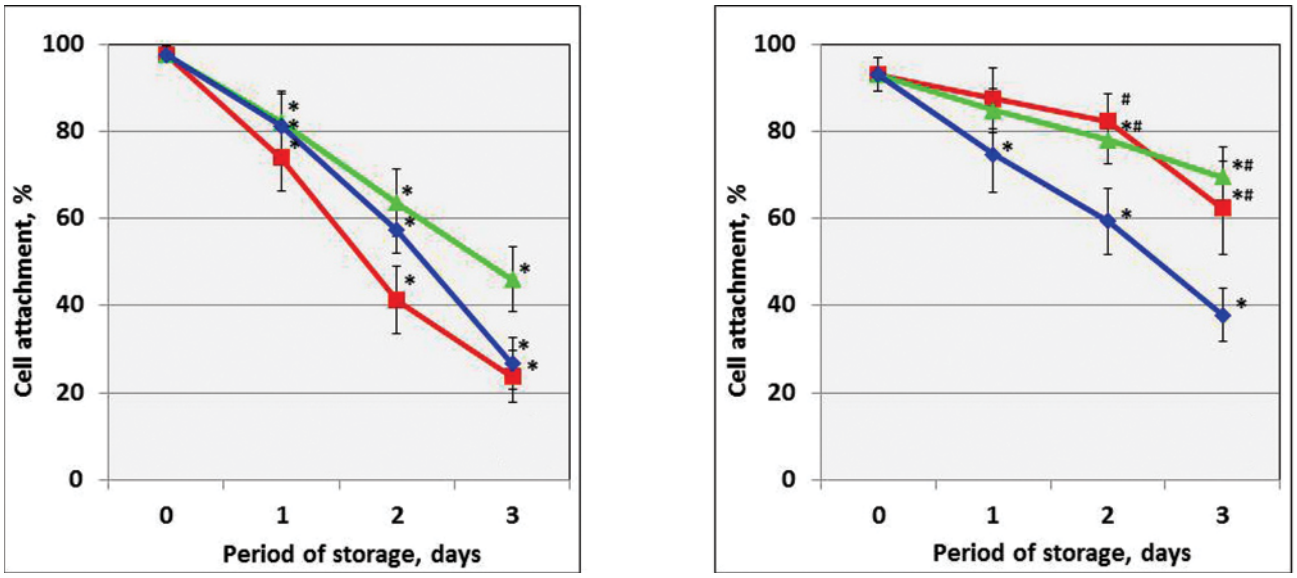

Fig. 2. **Comparison of attachment ability of multipotent mesenchymal stromal cells after storage in suspension (*A*) and alginate microspheres (*B*) at 4 (♦), 22 (▲) и 37 (■) °C**

metabolic activity of cells. Evaluations were made after first day of monolayer culture (Fig. 3). MSCs culture obtained after hypothermic storage at 4 °C in suspension and within AMSs was able much less to recover AB than the control. For MSCs cultures stored for three days the parameters were characterized at 0.99 ± 0.42 and 1.86 ± 0.55 RFU, while for the control they were 5.28 ± 0.69 and 5.4 RFU ± 0.79, respectively. Cell cultures obtained after MSCs storage at 22 and 37 °C in suspension were characterized by low levels of the AB test significantly different if compared to the control to day 2 of storage. Only for MSCs cultures which were stored within AMSs at 22 and 37 °C AB test parameters were on the level of control and were 4.04 ± 0.56 and 4.24 ± 0.52 RFU after three days.

A distinctive feature of MSCs is their ability to induced multilineage differentiation. Conditions of MSCs storage may be considered as satisfactory only if this ability is confirmed. For this MSCs after three days of storage within AMSs at 4, 22 and 37 °C were induced to differentiate into osteogenic and adipogenic directions. As you can see in Fig. 4 during culture in adipogenic medium the cells acquired a circular shape and accumulated intracellular lipid in the form of typical vacuoles that stained with Oil Red O dye in the red-pink colour. In shape and intensity of staining cells within AMSs subjected to storage did not differ versus the control.

Also, the ability of the MSCs to differentiate into osteogenic direction was confirmed (Fig. 5). After of the effect of appropriate inducers in cultures accumulated calcium resulting in specimens staining with Alizarin Red S in red.

The cells cultured in medium without inducers the accumulation of differentiation product was not observed. The ability of cells to the directed differentiation in monolayer culture conditions favours its functional utility.

The results show that entrapment in AMSs which provides a separate location and uniform cell distribution can prolong the safe storage of MSCs at positive temperatures. During storage within AMSs the cells largely remained viable, kept attachment properties, metabolic activity and differentiation potential versus those cells stored in suspension.

Uniform distribution of cells in the medium depth provides equal conditions of nutrient and oxygen intake and the removal of waste products. These conditions can significantly affect the survival of the cells during storage. One promising approach for uniform distribution of cells is their entrapment in microspheres made of natural and synthetic hydrogels. This approach is also being more widely used in biotechnology. For a material of natural origin used for the entrapment of living cells the one of the foremost is alginate, a linear polysaccharide composed of 1–4 beta-linked residues of beta-D-mannuronic and alpha-L-guluronic acids. Alginate is low toxic, under physiological conditions it can form a gel, capable of providing the cell

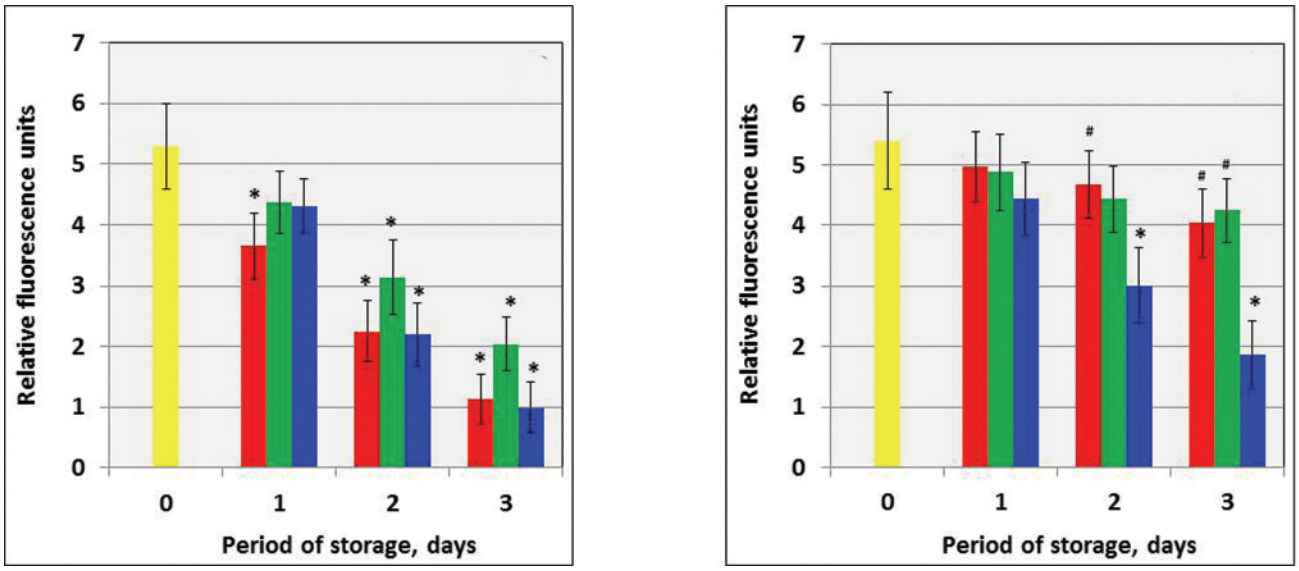

Fig. 3. **Comparison of metabolic activity of multipotent mesenchymal stromal cells after storage in suspension (*A*) and alginate microspheres (*B*) 4 (♦), 22 (▲) и 37 (■) °C after one day of culture using AB test**

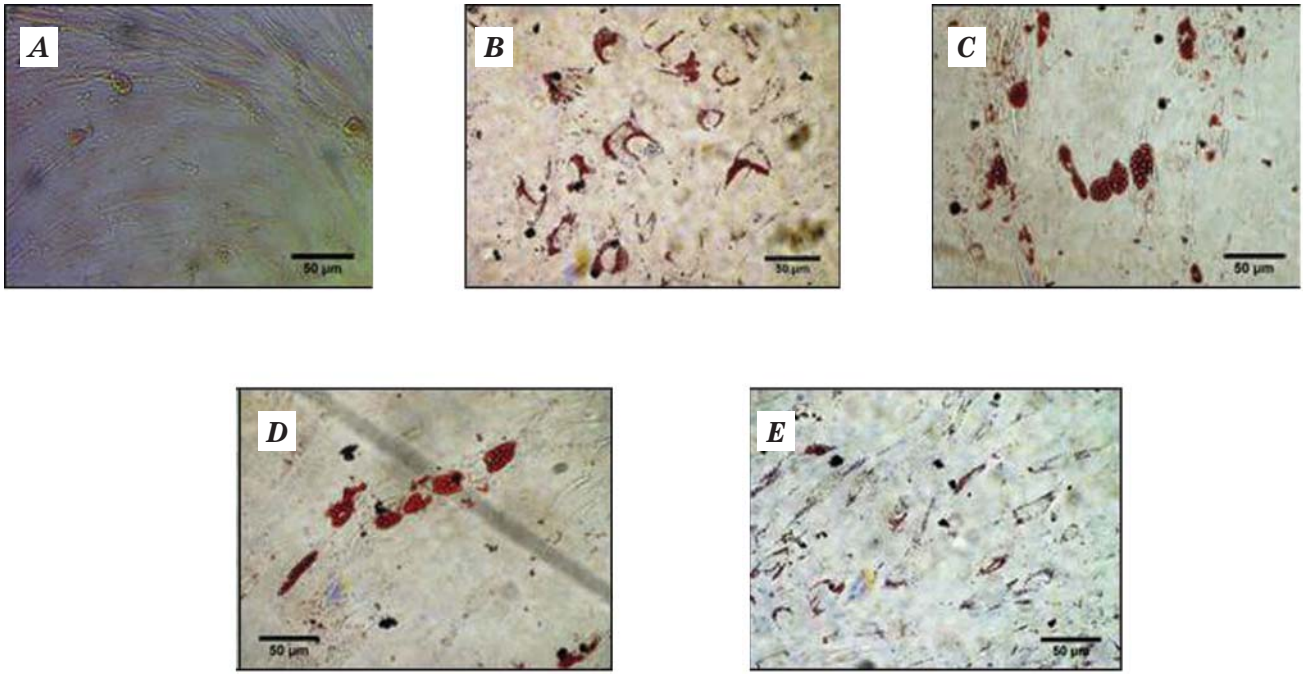

Fig. 4. **Adipogenesis *in vitro* of multipotent mesenchymal stromal cells in medium without (*A*) and with adipogenic inducers of differentiation before (*B*) and after three days of storage at 37 (*C*), 22 (*D*) and 4 (*E*) °C:** Oil Red O staining

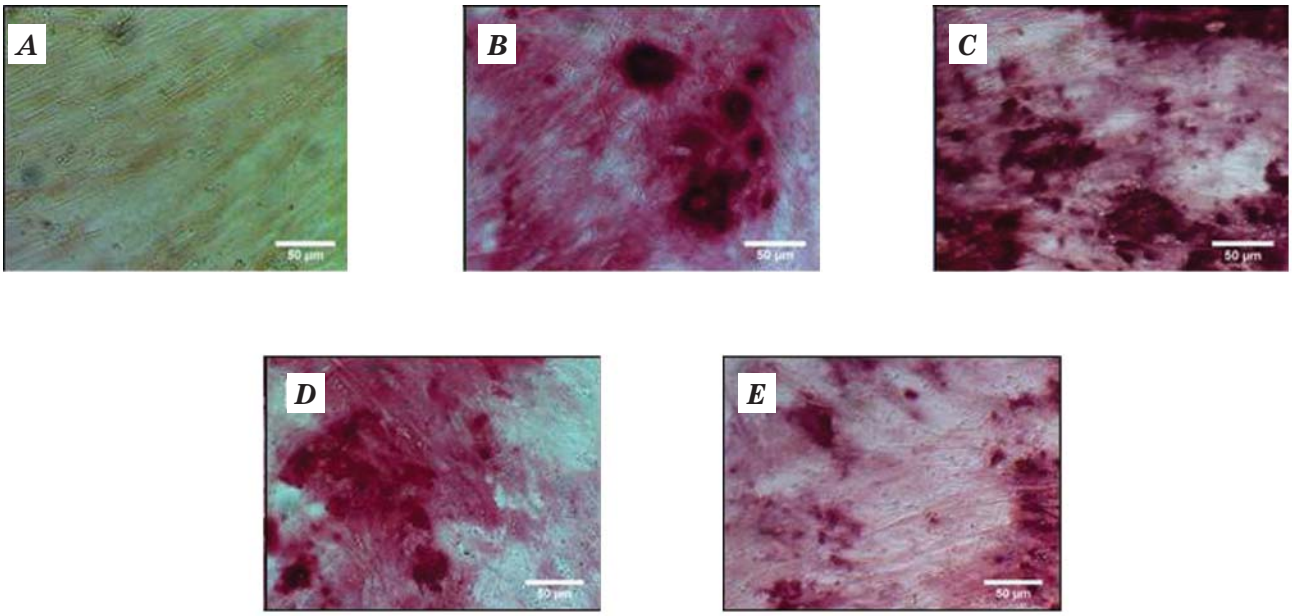

Fig. 5. **Osteogenesis *in vitro* of multipotent mesenchymal stromal cells in medium without inducers (*A*) and in the presence of osteogenic inducers of differentiation before (*B*) and after three days of storage at 37 (*C*), 22 (*D*) and 4 (*E*) °C:** Alizarin Red S staining

immobilization, transporting the nutrients thereto and removing decomposition products and immunoisolating the cells at transplantation. These properties allow to successfully use alginate hydrogels for entrapment of MSCs [13], pancreatic beta-cells [14], hepatocytes [15], genetically modified fibroblasts [16], embryonic stem cells [17] with the perspective of their subsequent transplantation.

The development of cell transplantation requires an improvement of various technological stages of this complex process. Mandatory step is the short-term storage and/or transportation. A common approach is the maintaining of MSCs suspension at described above conditions in a liquid medium, i.e. based on exposure of cell pellets, the density of which is storage temperature dependent. This leads to problems with the availability of nutrients for the cells under storage and maintaining an adequate gas exchange. We have previously studied the effect of the ionic composition of the medium at hypothermic storage of MSCs at 4 °C [7]. These results showed the importance of the maintenance of osmotic equilibrium in the "cell-its microenvironment" system to preserve viability. Sedimentation of the cells leads to virtually uncontrollable changes in the microenvironment of the system cellular elements. Entrapment of the MSCs in AMSs allows the isolation of the cell elements from each other by means of the alginate hydrogel that eliminated the mechanical contact and the formation of cell pellets. Here the storage conditions of individual cell components are likely getting more standardized and controlled. The result is an increase in survival of entrapped in AMSs MSCs compared to the cells in suspension.

Hypothermic temperatures (4 °C) should be noted to decrease the viability of MSCs and this was more pronounced than at mild hypothermia and normothermia. It reflects the development of the system further, destabilizing the state processes caused by this temperature control, the most important of which is probably the decrease in the ability of the cell to maintain ionic homeostasis [7].

Thus, an entrapment in alginate microspheres allows higher survival of MSCs during short-term storage at positive temperatures in comparison with the storage in the form of cell suspension. Entrapment in alginate microspheres can be considered in a new way, in particular, a technological method for storage and transportation of MSCs.

REFERENCES

1. *Goh B. C., Thirumala S., Kilroy G., Devireddy R. V., Gimble J. M.* Cryopreservation characteristics of adipose-derived stem cells: maintenance of differentiation potential and viability. *J. Tiss. Eng. Regen. Med*. 2007, 1 (4), 322–324. doi: 10.1002/term.35.
2. *Vemuri M. C., Chase L. G., Rao M. S.* Mesenchymal Stem Cell Assays and Applications. *Meth. Mol Biol.* 2011, V. 698, P. 3–8. doi: 10.1007/978-1-60761-999-4_1.
3. *Qi W., Ding D., Salvi R. J.* Cytotoxic effects of dimethyl sulfoxide (DMSO) on cochlear organotypic cultures. *Hear Res.* 2008, V. 1–2, P. 52–60. doi: 10.1016/j.heares.2007.12.002.
4. *Mathew A. J., Baust J. M., Van Buskirk R. G., Baust J. G.* Cell Preservation in Reparative and Regenerative Medicine: Evolution of Individualized Solution Composition. *Tiss. Eng.* 2004, 10 (11–12), 1662–1671. doi: 10.1089/ten.2004.10.1662.
5. *Mathew A. J., Van Buskirk R. G., Baust J. G.* Improved Hypothermic Preservation of Human Renal Cells Through Suppression of Both Apoptosis and Necrosis. *Cell Pres. Tech.* 2003, 1 (4), 239–253. doi: 10.1089/15383440260682071.
6. *Corwin W. L., Baust J. M., Baust J. G., Van Buskirk R. G.* Characterization and modulation of human mesenchymal stem cell stress pathway response following hypothermic storage. *Cryobiology*. 2014, 68 (2), 215–226. doi: 10.1016/j.cryobiol.2014.01.014.
7. *Tarusin D. N., Petrenko Yu. A., Semenchenko O. A., Mutsenko V. V., Zaikov V. S., Petrenko A. Yu.* Efficiency of the Sucrose-Based Solution and UW Solution for Hypothermic Storage of Human Mesenchymal Stromal Cells in Suspension or Within Alginate Microspheres. *Probl. Cryobiol. Cryomed*. 2015, 25 (4), 329–339.
8. *Vega Crespo A., Awe J. P., Reijo Pera R., Byrne J. A.* Human skin cells that express stage-specific embryonic antigen 3 associate with dermal tissue regeneration. *Bioresx Open Access*. 2012, V. 1, P. 25–33. doi: 10.1089/biores.2012.0204.
9. *Stover A. E., Herculian S., Banuelos M. G., Navarro S. L., Jenkins M. P., Schwartz P. H.* Culturing Human Pluripotent and Neural Stem Cells in an Enclosed Cell Culture System for Basic and Preclinical Research. *Jx Visx Exp*. 2016, V. 112, P. 1–9. doi: 10.3791/53685.
10. *Davis J.* Basic cell culture: a practical approach. *Oxford: Oxford University Press.* 2002, 408 p.
11. *You L., Pan L., Chen L., Gu W., Chen J.* MiR-27a is Essential for the Shift from Osteogenic Differentiation to Adipogenic Differentiation of Mesenchymal Stem Cells in Postmenopausal Osteoporosis. *Cell Physiol. Biochem.* 2016, 39 (1), 253–265. doi: 10.1159/000445621.
12. *Chen S., Ryan D. A., Dwyer M. A., Cashman J. R.* Synergistic effect of Wnt modulatory small molecules and an osteoinductive ceramic on C2C12 cell osteogenic differentiation. *Bone.* 2014, V. 67, P. 109–121. doi: 10.1016/j.bone.2014.06.032.
13. *Gryshkov O., Pogozhykh D., Hofmann N., Pogozhykh O., Mueller T., Glasmacher B.* Encapsulating non-human primate multipotent stromal cells in alginate via high voltage for cell-based therapies and cryopreservation. *PLoS One*. 2014, 9 (9), 1–12. doi: 10.1371/journal.pone.0107911.
14. *Safley S. A., Cui H., Cauffiel S., Tucker-Burden C., Weber C. J.* Biocompatibility and immune acceptance of adult porcine islets transplanted intraperitoneally in diabetic NOD mice in calcium alginate poly-L-lysine microcapsules versus barium alginate microcapsules without poly-L-lysine. *J. Diab. Sci. Technol.* 2008, 2 (5), 760–767.
15. *Ser-Mien Chia, Kam W. Leong, Jun Li, Xi Xu, Kaiyang Zeng, Poh-Nee Er, Shujun Gao, Hanry Yu.* Hepatocyte Encapsulation for Enhanced Cellular Functions. *Tiss. Eng*. 2000, 6 (5), 481–495. doi: 10.1089/107632700750022134.
16. *Hunt N. C., Smith A. M., Gbureck U., Shelton R. M., Grover L. M.* Encapsulation of fibroblasts causes accelerated alginate hydrogel degradation. *Acta Biomaterialia*. 2010, 6 (9), 3649–3656. doi: 10.1016/j.actbio.2010.03.026.
17. *Richardson T., Kumta P., Banerjee I.* Alginate Encapsulation of Human Embryonic Stem Cells to Enhance Directed Differentiation to Pancreatic Islet-Like Cells. *Tiss. Eng.: Part A*. 2014, 20 (23–24), 3198–3211. doi: 10.1089/ten.TEA.2013.0659.

ІНКАПСУЛЯЦІЯ МЕЗЕНХІМАЛЬНИХ СТРОМАЛЬНИХ КЛІТИН В АЛЬГІНАТНІ МІКРОСФЕРИ

Д. Н. Тарусін
С. П. Мазур
Н. А. Волкова
Ю. А. Петренко
В. С. Зайков
А. Ю. Петренко

Інститут проблем кріобіології і кріомедицини НАН України, Харків

E-mail: tarusindmitriy@gmail.com

Метою роботи було порівняльне вивчення структурно-функціонального стану і здатності до диференціювання мультипотентних мезенхімальних стромальних клітин, що їх зберігали за різних температур у вигляді клітинних суспензій або у складі альгінатних мікросфер. Зберігання проводили в культуральному середовищі на основі α-МЕМ за температур 4, 22 і 37 °С у герметично закритих кріопробірках. Через 1, 2 і 3 доби альгінатні мікросфери розчиняли, і життєздатність (МТТ-тест), адгезивні властивості та метаболічну активність (АВ-тест) клітин оцінювали у моношаровій культурі. Показано, що зберігання мезенхімальних стромальних клітин у вигляді суспензії протягом 3 діб за даних температур призводило до зниження досліджуваних показників. Мезенхімальні стромальні клітини після зберігання в альгінатних мікросферах при 22 °С і 37 °С виявляли високу життєздатність (78 і 87%, відповідно), збереження адгезивних властивостей (62 і 70%), метаболічну активність (79 і 75%) і здатність до диференціювання. Результати свідчать, що мезенхімальні стромальні клітини, інкапсульовані в альгінатні мікросфери, стійкіші до умов зберігання, ніж суспензія мезенхімальних стромальних клітин. Інкапсуляція в альгінатні мікросфери є перспективним технологічним прийомом для короткострокового зберігання мезенхімальних стромальних клітин за позитивних температур.

Ключові слова: мультипотентні мезенхімальні стромальні клітини, альгінатні мікросфери, метаболічна активність, індуковане диференціювання.

ИНКАПСУЛЯЦИЯ МЕЗЕНХИМАЛЬНЫХ СТРОМАЛЬНЫХ КЛЕТОК В АЛЬГИНАТНЫЕ МИКРОСФЕРЫ

Д. Н. Тарусин
С. П. Мазур
Н. А. Волкова
Ю. А. Петренко
В. С. Зайков
А. Ю. Петренко

Институт проблем криобиологии и криомедицины НАН Украины, Харьков

E-mail: tarusindmitriy@gmail.com

Целью работы было сравнительное изучение структурно-функционального состояния и способности к дифференцировке мультипотентных мезенхимальных стромальных клеток, хранившихся при разных температурах в виде клеточных суспензий или в составе альгинатных микросфер. Хранение проводили в культуральной среде на основе α-МЕМ при температурах 4, 22 и 37 °С в герметически закрытых криопробирках. Через 1, 2 и 3 суток альгинатные микросферы растворяли, и жизнеспособность (МТТ-тест), адгезивные свойства и метаболическую активность (АВ-тест) клеток оценивали в монослойной культуре. Показано, что хранение мезенхимальных стромальных клеток в виде суспензии в течение 3 суток при указанных температурах приводило к снижению изучаемых показателей. Мезенхимальные стромальные клетки после хранения в альгинатных микросферах при 22 °С и 37 °С проявляли высокую жизнеспособность (78 и 87%, соответственно), сохранность адгезивных свойств (62 и 70%), метаболическую активность (79 и 75%) и способность к дифференцировке. Результаты показывают, что мезенхимальные стромальные клетки, инкапсулированные в альгинатные микросферы, более устойчивы к условиям хранения, чем суспензия мезенхимальных стромальных клеток. Инкапсуляция в альгинатные микросферы является перспективным технологическим приемом для краткосрочного хранения мезенхимальных стромальных клеток при положительных температурах.

Ключевые слова: мультипотентные мезенхимальные стромальные клетки, альгинатные микросферы, метаболическая активность, индуцированная дифференцировка.