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Вплив спільного культивування нейральних клітин з МСК на формування сфероїдів та їх відновлення після кріоконсервування

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Effect of Co-Culturing Neural Cells with MSCs on Spheroid Formation and Their Recovery After Cryopreservation

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It is known that mesenchymal stem cells (MSCs) can interact with neural cells (NCs) and provide their protection through the secretion of neurotrophic, immunomodulatory and anti-apoptotic factors. These effects of MSCs suggest that their inclusion into spheroids (SPhs) formed by NCs will contribute to an increase in the survival rate and cell recovery rate after cryopreservation.

The study was aimed to evaluate the effect of MSC on the formation of NC spheroids and the efficiency of their recovery after cryopreservation.

NCs were obtained after pre-cultivation of the primary suspension of neonatal rat brain cells (P0). MSCs were derived from rat fetal liver cells (15 ED). All cells were cultured in a CO_2 incubator in DMEM/F12 supplemented with 10% fetal bovine serum. Spheroids were formed by the hanging drop method during the cultivation of NCs and NCs + MSCs. The drop volume was 20 µl and contained 4 x 10^3 cells. MSCs were co-cultured with NCs in a 1:1 ratio. Cells in the drops were cultured for 3 days. SPhs were cryopreserved under the protection of 10% DMSO at an initial cooling rate of 1 deg/min down to –80°C, followed by transfer into liquid nitrogen. The samples were thawed at 38°C and washed from DMSO to a final concentration of 0.1%. Some of the initial and thawed SPhs were cultured on the adhesive surface to test their viability.

On the 1st day of cultivation, the formation of one or more SPhs with a size of 20–30 µm was observed in the droplets. On the 3rd day, SPhs increased in size. If there were several SPhs, they merged into one, acquiring a spherical shape and dense packing of cells in it. At the same time, the average diameter of SPhs formed during the co-cultivation of NCs with MSCs was significantly larger (282 ± 2.64 µm) compared to SPhs formed by NCs alone (219 ± 8.6 µm). Under adhesive conditions, the initial SPhs, regardless of the presence of MSCs, attached after 2–3 hours of cultivation, their cells actively migrated, forming areas of the monolayer consisting of cells with neuronal and glial morphology. Thawed SPhs formed by co-cultivation of NCs with MSCs as well as NCs alone were attached to the adhesive surface after 6 and 24 h of cultivation, respectively. After SPhs attachment, as in the control, we observed the formation of monolayer areas by cells of neuronal and glial morphology. Thus, MSCs, on the one hand, increase the efficiency of NC SPhs formation, and, on the other hand, accelerate the recovery rate of cryopreserved NCs in the SPhs.

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Короткочасне зберігання МСК в капсулах типу ядро-оболонка при температурі навколишнього середовища

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Short-Term Storage of Core-Shell Encapsulated MSCs at Ambient Temperature

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Human mesenchymal stromal cells (MSCs) are a promising cell type for tissue engineering and regenerative medicine. Nonetheless, maximising the utility of MSCs requires the development of effective storage methods. The most popular option is cryopreservation, but storing these cells at ambient temperatures could simplify transportation, mitigate the limitations associated with cold temperatures and overcome disadvantages of cryopreservation with toxic cryoprotective agents.

This work was aimed to study the efficacy of human bone marrow MSCs storage at 22°C in α-MEM with 10% (v/v) foetal bovine serum in five different forms: monolayer, suspension, encapsulated in alginate microspheres (AMS), and core-shell alginate capsules with the addition of fresh porcine blood plasma or human amniotic membrane (hAM) extract and to analyse metabolic activity and cell viability during and after storage at ambient temperatures. Porcine blood plasma was obtained from a slaughterhouse according to German ethical standards. The placenta used to obtain hAM extract was sourced from Hanover Medical School after active donors' consent.

Blood plasma AMS and capsules were fabricated by electrospraying with 2.5% (w/v) low-viscosity alginate. Viability (Trypan Blue, FDA/EthD dual staining) and metabolic activity (Alamar blue) were assessed on day 1, 3, 5 and 7 of storage. Viability after 7 days of storage was 33% in monolayer, 58% in suspension, 84% in AMS, 87% in capsules with hAM extract, and 83% with porcine blood plasma. The metabolic activity of cells in AMS comprised 55%, in capsules with hAM extract – 58%, and in capsules with porcine blood plasma 64% from storage start level at the 7th day of storage, respectively. Metabolic activity in monolayer and suspension decreased sharply and was 27 and 16% on the 7th day. The protein-supplemented encapsulation techniques investigated in this study provide efficient storage of MSCs at ambient temperature for transport and subsequent use.

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