

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 α -MEM supplemented with 10% (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 μ g/ml. Viability (Trypan Blue, FDA/EthD dual staining), metabolic activity (Alamar blue) were assessed on 1, 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 FV10i-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 1. 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 indexes. 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 G1 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.