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Engineered microenvironments for MSCs storage at ambient temperature in alginate core-shell capsules

O.S. Hubenia [1, 2], S. Leal-Marin [2], N.A. Trufanova [1], B. Glasmacher [2], O.Yu. Petrenko [1, 3]
[1] Institute for Problems of Cryobiology and Cryomedicine, National Academy of Sciences of Ukraine, Kharkiv, Ukraine
[2] Institute for Multiphase Processes, Leibniz University Hannover, Garbsen, Germany
[3] V.N. Karazin Kharkiv National University, Kharkiv, Ukraine

Human mesenchymal stromal cells (MSCs) hold great promise for regenerative medicine, but their effective storage remains a challenge. Traditional cryopreservation methods, while effective, require specialised infrastructure and cryoprotectants that can compromise cell integrity and function. In contrast, ambient temperature (AT) storage offers a simplified and cost-effective alternative that eliminates freezing stress and allows for immediate application. However, maintaining MSC viability and functionality at AT remains a significant challenge.

This study investigates the use of alginate-based core-shell encapsulation as a strategy to preserve human bone marrow-derived MSCs at 22 °C. MSCs were cultured in Dulbecco's Modified Eagle Medium (D-MEM) supplemented with 10% (v/v) fetal bovine serum, and with fibroblast growth factor-2 (FGF-2) or FGF-2 free. MSCs were stored either as monolayer cultures or core-shell alginate capsules containing blood plasma with CaCl2 (BP) or MSC culture medium (CM). Capsules were fabricated using a coaxial electrospray system and 2.5% (w/v) low-viscosity alginate. Cell viability (Trypan Blue, FDA/EthD dual staining) and metabolic activity (Resazurin assay) were assessed on day 0, 3, and 7 of AT storage. Rheological analyses of alginate and BP solutions were performed to refine encapsulation conditions.

Encapsulation preserved cell viability, with encapsulated MSCs maintaining over 75% viability in all encapsulated groups at day 3 of AT, compared to 57% in monolayers. By day 7, all encapsulated cells retained over 70% viability, *versus* 33% in monolayers. In FGF-2-supplemented CM, metabolic activity was highest in CM capsules (52.5%), followed by BP capsules (35.4%) and monolayers (27%) up to day 7. On the other hand, FGF-2-free BP capsules sustained the highest metabolic activity (49%) on the same day. Rheological analysis revealed alginate's shear-thinning and BP's shear-thickening behaviour, influencing MSC organisation into fibroblast-like or homogeneously distributed structures.

Overall, alginate/BP core-shell encapsulation effectively preserves MSC viability and function at ambient temperature, offering a promising alternative to cryopreservation. This platform has practical advantages for clinical translation, particularly in settings with limited cold-chain logistics.

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Effect of pre-chilling on ovarian tissue

E. Teas, K. Fowler, N. Kuscu, P. Kilbride, S.A. Williams
Nuffield Department of Women's and Reproductive Health, University of Oxford, Oxford, UK

The centralized 'Danish model' of ovarian tissue cryopreservation (OTC) aims to improve patient access by consolidating cryobank facilities. However, transport and processing logistics often delay the cryopreservation of ovarian cortical tissue collected for future fertility and hormone restoration. The effects of these delays on tissue quality post-thaw remain poorly understood, leading to uncertainty and potential variability in patient outcomes. However sheep ovarian tissue has been shown to have improved follicle survival post-thaw when subjected to a 48-hour period at 4 °C prior to cryopreservation, compared to immediate processing. We hypothesized that prechilling may precondition tissue by reducing metabolic activity, thereby enhancing their resilience to cryoinjury and ischemia.

We investigated the impact of pre-chilling on mouse ovaries, assessing mitochondrial metabolism via TOM20 (translocase of the outer mitochondrial membrane 20) as a marker of oxidative phosphorylation. Ovaries from 19-day-old F1 (C57B/6xCD1) mice were either immediately fixed (0h) or stored at 4 °C for 12, 24, 36, or 48 h before fixation. TOM20 expression was detected by DAB immunohistochemistry, and staining intensity in granulosa cells and oocytes of secondary follicles was quantified using ImageJ. Data are presented as mean ± SD, with significance of $p \leq 0.05$.

TOM20 levels in granulosa cells significantly decreased after 24 h (32.15 ± 11.91, $n = 21$), 36h (33.38 ± 10.72, $n = 15$), and 48 h (32.52 ± 15.83, $n = 17$) of cooling compared to 0 h controls (56.54 ± 11.97, $n = 19$; $p < 0.001$). In contrast, TOM20 expression in oocytes remained stable across all time points. These results suggest that pre-chilling alters mitochondrial metabolism in granulosa cells from 24h onwards, while oocyte metabolism appears unaffected within this timeframe. Our preliminary findings provide new insight into follicular metabolism under low-temperature conditions and highlight the potential impact of processing delays on ovarian tissue quality.

Further studies are needed to explore whether similar metabolic changes occur in other follicular stages and to determine long-term effects on oocyte health. These results could inform the optimization of clinical protocols for fertility preservation.