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**AMBIENT TEMPERATURE STORAGE OF ENCAPSULATED CELLS IN ALGINATE
MICROSPHERES AND CORE–SHELL CAPSULES**

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Abstract. *The development of reliable short-term storage strategies is essential for the clinical translation of cell-based therapies in regenerative medicine, particularly in settings where cryogenic infrastructure is limited or transport distances are substantial. The aim of the research was to evaluate the influence of three-dimensional culture systems (alginate microspheres and core–shell capsules with different core compositions) and fibroblast growth factor (FGF) supplementation on the preservation of viability and metabolic activity of human bone marrow–derived mesenchymal stromal cells and HeLa cells during ambient temperature storage. MSCs and HeLa cells were encapsulated in alginate microspheres or in core–shell capsules containing culture medium, gelatin, or porcine plasma as the core component using electrospraying and coaxial electrospraying techniques. After 17 days of cultivation, constructs were stored at +22 °C for up to 7 days. Cell viability was assessed using trypan blue exclusion and fluorescent FDA/EthD-1 staining. Metabolic activity was determined by the Alamar Blue assay and normalised to baseline values at the onset of ambient storage. Statistical evaluation was performed using two-way ANOVA and mixed-effects modelling. A significant time-dependent decrease in viability and metabolic activity was observed across all groups during ambient storage ($p < 0.0001$); however, the extent of decline varied depending on cell type and microenvironmental conditions. Three-dimensional encapsulation markedly improved cellular stability relative to monolayer culture, particularly by Day 7. The most consistent preservation of functional parameters across both cell types was achieved in alginate microspheres and in core–shell capsules with a culture-medium core. Capsules containing gelatin or plasma cores showed greater variability and more pronounced reductions in functional readouts, especially under FGF-deprived conditions. The beneficial effect of FGF supplementation was more evident in MSCs, whereas HeLa cells exhibited limited responsiveness. The type of 3D carrier and capsule core composition are critical determinants of cell preservation during ambient storage. Alginate microspheres and culture medium–based core–shell capsules represent promising platforms for short-term storage of cell-based products under non-cryogenic conditions. FGF supplementation enhances MSC resistance to storage-associated stress and may serve as a modulatory component in optimised ambient preservation strategies.*

Keywords: mesenchymal stromal cells, HeLa, alginate microspheres, core–shell capsules, fibroblast growth factor, ambient storage, three-dimensional culture, encapsulation, alginate

The advancement of regenerative medicine has intensified the need for reliable systems for storage, transportation, and functional maintenance of cellular therapeutics. Particular attention is given to mesenchymal stromal cells (MSCs) due to their multipotent differentiation capacity, immunomodulatory properties, and ability to secrete trophic factors that promote tissue regeneration (Galipeau, 2018; Jovic, 2022). However, the clinical efficacy of MSC-based therapies is significantly limited by the decline in cell viability and functional stability during storage and transportation (Cheng, 2024; Pogozhykh, 2015; Wang, 2024). Cells are highly sensitive to fluctuations in temperature, pH, osmotic stress, and mechanical forces, which may lead to apoptosis activation and loss of metabolic activity (Mand, 2024; Veronesi, 2014).

Storage at ambient temperature (AT) is particularly attractive for regenerative medicine applications, especially under resource-limited or emergency conditions, since it does not require specialised cryoprotective agents or sophisticated equipment. The development of effective ambient storage strategies could significantly simplify the distribution of cell therapies and expand their use in clinical settings.

One promising approach to overcoming these limitations is cell encapsulation within biocompatible hydrogel matrices. Hydrogels provide mechanical protection, facilitate the diffusion of

nutrients and gases, and create a microenvironment that resembles the extracellular matrix (Cheng, 2024; Andersen, 2015). Among natural polymers, sodium alginate occupies a prominent position. This anionic polysaccharide derived from brown algae forms hydrogels via ionic crosslinking with divalent cations according to the “egg-box” model (Sarker, 2015; Simó, 2017). Alginate-based matrices are characterised by high biocompatibility and mild gelation conditions, enabling the encapsulation of viable cells without exposure to cytotoxic agents (Andersen, 2015).

However, native alginate lacks intrinsic cell-adhesive motifs required for cell-matrix interactions. To enhance biological functionality, alginate is frequently combined with gelatin, a denatured form of collagen, which contains arginine–glycine–aspartic acid sequences that promote cell adhesion and proliferation (Nooeaid, 2017; Alizadeh Sardroud, 2017; Ghaleh, 2021). The combination of alginate and gelatin enables the development of hybrid hydrogels with tunable mechanical and diffusion properties, suitable for supporting long-term cell viability.

Further advancements in encapsulation technologies have led to the development of core–shell structures, which provide spatial separation between the internal cellular microenvironment and the external protective layer (Barron, 2017; Gryshkov, 2021). Such systems enable regulation of shell thickness, diffusion rates, and capsule mechanical stability, which are critical for preserving cells under stress conditions, including ambient storage. The coaxial electrospraying technique allows the fabrication of homogeneous capsules with controlled size and high reproducibility (Barron, 2017; Mehregan Nikoo, 2016; Nguyen, 2015).

An important factor in maintaining proliferative activity is the presence of growth factors, particularly fibroblast growth factor (FGF), which stimulates cell proliferation and metabolic activity, especially in stem cells such as MSCs (Schäfer, 2016). However, the effect of FGF under conditions of restricted diffusion and reduced metabolic exchange during ambient storage remains insufficiently investigated. Understanding these mechanisms is essential for translating encapsulation-based storage systems into clinically applicable regenerative medicine strategies.

To standardise encapsulation parameters, the use of model cell lines is advisable. HeLa cells, the first immortal human cell line, are characterised by high proliferative capacity and resistance to stress factors (Przybylo, 2016). Owing to their biological stability, HeLa cells represent a convenient model for evaluating the effects of capsule composition, diffusion limitations, and growth factors prior to transitioning to more sensitive therapeutic cells such as MSCs.

Therefore, it is relevant to investigate the influence of the encapsulation system type (alginate microspheres versus core–shell capsules with varying core composition) and the presence of FGF on the viability and metabolic activity of MSCs and HeLa cells during ambient storage.

The aim of study was to evaluate the effects of the core medium composition in alginate core–shell capsules and fibroblast growth factor supplementation on the viability and metabolic activity of MSCs and HeLa cells under ambient temperature storage conditions.

Materials and Methods. *Experimental groups and study design.* To assess cell viability and metabolic activity of MSCs or HeLa cells during AT storage, the following spatially organised systems were investigated: conventional monolayer culture, alginate microspheres (AMS), and alginate core–shell capsules.

For the core-shell capsules, different core compositions were used, including culture medium (CM), gelatine (Gel), or plasma (BP) from porcine blood. All experimental groups were analysed both with and without FGF.

Cell viability and metabolic activity were evaluated after 17 days of cultivation, followed by ambient storage at room temperature for 0, 3, and 7 days.

Cell culture of human bone marrow-derived mesenchymal stromal cells. Human bone marrow-derived mesenchymal stromal cells (bmMSCs) were obtained from the biobank of the Institute for Multiphase Processes (Garbsen, Germany). All procedures were conducted in accordance with applicable legislation and institutional ethical requirements.

Cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM; Bio&SELL, Germany) supplemented with 10% (v/v) fetal bovine serum (FBS; Bio&SELL, Germany), 1% (v/v) penicillin–streptomycin (100 U/mL penicillin, 0.1 mg/mL streptomycin, 0.25 µg/mL amphotericin B; Sigma-Aldrich, USA), 1% L-glutamine (Carl Roth, Germany), 25 mM HEPES (Bio&SELL, Germany), and 2 µg/L recombinant human fibroblast growth factor-2 (FGF, PeproTech, USA).

Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO₂ (95% relative humidity) in cell-adhesive flasks (TPP, Switzerland). The CM was replaced every 2–3 days. Cells were

passed at approximately 80% confluency using 0.05% trypsin/EDTA solution (Biochrom, Germany), followed by neutralisation with complete medium and centrifugation at 1000 rpm for 5 min.

Cell counting was performed using an automated cell counter (Vi-Cell XR, Beckman Coulter, Germany) with standard bmMSC settings (cell diameter range 10–50 μm), applied consistently across all experiments.

Cell culture of HeLa cells. HeLa cells were obtained from the biobank of the Institute for Multiphase Processes (Garbsen, Germany), and all procedures complied with institutional and ethical regulations.

Cells were cultured in DMEM supplemented with 10% FBS, 1% penicillin–streptomycin, 0.25 $\mu\text{g}/\text{mL}$ amphotericin B, and 1% L-glutamine at 37 °C in 5% CO_2 . The medium was changed every 2–3 days. Cells were passaged at approximately 80% confluency using 0.05% trypsin/EDTA, followed by neutralisation and centrifugation (1000 rpm, 5 min).

Cell numbers were determined using a Vi-Cell XR cell counter with a diameter range set to 10–25 μm .

Encapsulation of MSCs and HeLa cells in alginate microspheres and core-shell capsules. Homogeneous alginate microspheres (AMS) were produced by electrospraying as previously described (Trufanova, 2025). MSCs or HeLa cells were suspended in alginate prior to encapsulation.

Core-shell alginate capsules with varying core compositions were fabricated using a modified coaxial electrospraying protocol previously described (Gryshkov, 2021).

A 2.5% (w/v) sodium alginate solution (Sigma-Aldrich, A2158) was prepared in sterile HEPES buffer (154 mM NaCl, 10 mM HEPES, pH 7.4) under continuous stirring (350 rpm) for at least 14h and sterilised by sequential filtration (0.65, 0.45, and 0.22 μm).

For BP-containing cores, fresh BP was supplemented with 0.1 M CaCl_2 to a final concentration of 1% (v/v). Gelatin solutions (2.5% w/v) were prepared in HEPES buffer at 37 °C. Prior to electrospraying, all solutions were equilibrated to 37 °C.

Cells were resuspended in the respective core solutions (Gel, BP, or CM) at a concentration of 0.5×10^6 cells/mL.

Core-shell capsules were generated using a coaxial electrospraying system consisting of a dual-channel syringe pump, a 21G blunt-end coaxial needle, a high-voltage generator (10 kV, 0.014 mA), and a grounded gelling bath containing 100 mM CaCl_2 . The shell (alginate) flow rate was set to 40 mL/h; the core flow rates were: Gel – 8 mL/h; BP – 8 mL/h; CM – 10 mL/h. AMS were fabricated using a similar electrospraying setup without core formation at a flow rate of 4 mL/h. Following formation, capsules were incubated in 100 mM CaCl_2 for 30 min, washed, and transferred to culture medium.

Capsules were cultured for 17 days in 150-mm dishes (TPP, Switzerland) in either FGF-supplemented CM (CM + FGF-2) or medium without FGF (CM – FGF), with medium change every 2–3 days.

After cultivation, capsules were transferred to 2-mL cryovials, hermetically sealed, and stored at ambient temperature (+22 °C) for up to 7 days (0, 3, 5, and 7 days).

Assessment of cell viability. After 17 days of cultivation under physiological conditions, the viability of encapsulated cells was evaluated using trypan blue exclusion and fluorescein diacetate (FDA) / ethidium homodimer-1 (EthD-1) staining.

For trypan blue exclusion, cells were released from the alginate matrix by incubation in sodium citrate until the hydrogel dissolved completely. The resulting suspension was mixed 1:1 with 0.4% trypan blue (Sigma-Aldrich, USA), and viable (unstained) and non-viable (stained) cells were quantified using a Vi-Cell XR cell counter. Viability was expressed as a percentage of the total cell number.

For fluorescence-based assessment, capsules were incubated for 20 min at ambient temperature in the presence of FDA (2 μM) and EthD-1 (4 μM). After washing with PBS, the number of viable cells was counted under a fluorescence microscope (Axiovert 200M, Carl Zeiss).

All experiments were conducted in at least three independent replicates.

Assessment of metabolic activity. Metabolic activity was evaluated using the Alamar Blue assay (Thermo Fisher Scientific, USA).

Capsules were transferred into 24-well plates containing culture medium supplemented with 10% (v/v) Alamar Blue reagent and incubated for 4 h at 37 °C. Supernatants were collected, and fluorescence intensity was measured using a multimode plate reader (BioTek Synergy HTX) at excitation/emission wavelengths of 560/590 nm.

Section 1

Fluorescence values were normalised to baseline values at Day 0 of AT storage and expressed as percentages.

Statistical analysis. Statistical analyses were performed using GraphPad Prism (GraphPad Software, USA). Data are presented as mean \pm SD.

A two-way repeated-measures ANOVA with a Geisser–Greenhouse correction was used to evaluate the effects of storage time and experimental conditions on viability and metabolic activity. When appropriate, a mixed-effects model (REML) was applied. Post hoc comparisons were performed using Šídák, Dunnett, or Tukey tests depending on the experimental design.

Metabolic activity was normalised to Day 0 (100%). Differences were considered statistically significant at $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), $p < 0.0001$ (****).

Experiments were performed in 2–3 independent series ($N = 2–3$), with 3–6 samples per condition ($n = 3–6$).

Results. *Viability of mesenchymal stromal cells during ambient storage in AMS and core-shell alginate capsules.* At the onset of ambient storage (Day 0), MSC viability remained high ($\geq 98\%$) across all experimental groups, regardless of the culture system and the presence or absence of FGF (Fig. 1).

By Day 3 of ambient storage, MSCs' viability in monolayer culture was $96.0 \pm 2.5\%$ in the presence of FGF, but decreased markedly to $68.6 \pm 0.8\%$ in its absence. In AMS, viability remained high, reaching $90.1 \pm 2.7\%$ (+FGF) and $82.0 \pm 2.1\%$ (–FGF). In core–shell capsules with CM, viability at Day 3 was $98.0 \pm 0.4\%$ (+FGF) and $92.2 \pm 4.2\%$ (–FGF), exceeding the corresponding values in monolayer culture and AMS. In core–shell capsules with a Gel core, viability was $95.1 \pm 1.9\%$ (+FGF) and $88.3 \pm 2.8\%$ (–FGF). In core–shell capsules with BP, viability at Day 3 reached $94.6 \pm 2.2\%$ (+FGF), whereas in the absence of FGF, it decreased to $86.9 \pm 3.1\%$, indicating an early detrimental effect of growth factor withdrawal during ambient storage.

By Day 7, MSCs' viability in monolayer culture declined sharply to $34.7 \pm 0.8\%$ (+FGF) and $38.3 \pm 5.4\%$ (–FGF). In contrast, core–shell capsules maintained substantially higher viability. In CM, viability remained at $84.1 \pm 1.0\%$ (+FGF) and $78.7 \pm 9.0\%$ (–FGF). In Gel, viability at Day 7 was $82.0 \pm 1.8\%$ (+FGF) and $69.0 \pm 6.4\%$ (–FGF). The lowest viability among the encapsulated systems was observed in BP under –FGF conditions ($62.0 \pm 4.2\%$), whereas supplementation with FGF improved MSCs preservation, increasing viability to $77.3 \pm 0.6\%$.

Overall, AMS, and particularly core–shell capsule systems, maintained MSC viability at $\sim 70–85\%$ after 7 days of ambient storage, particularly under +FGF conditions, which was markedly higher than in monolayer culture.

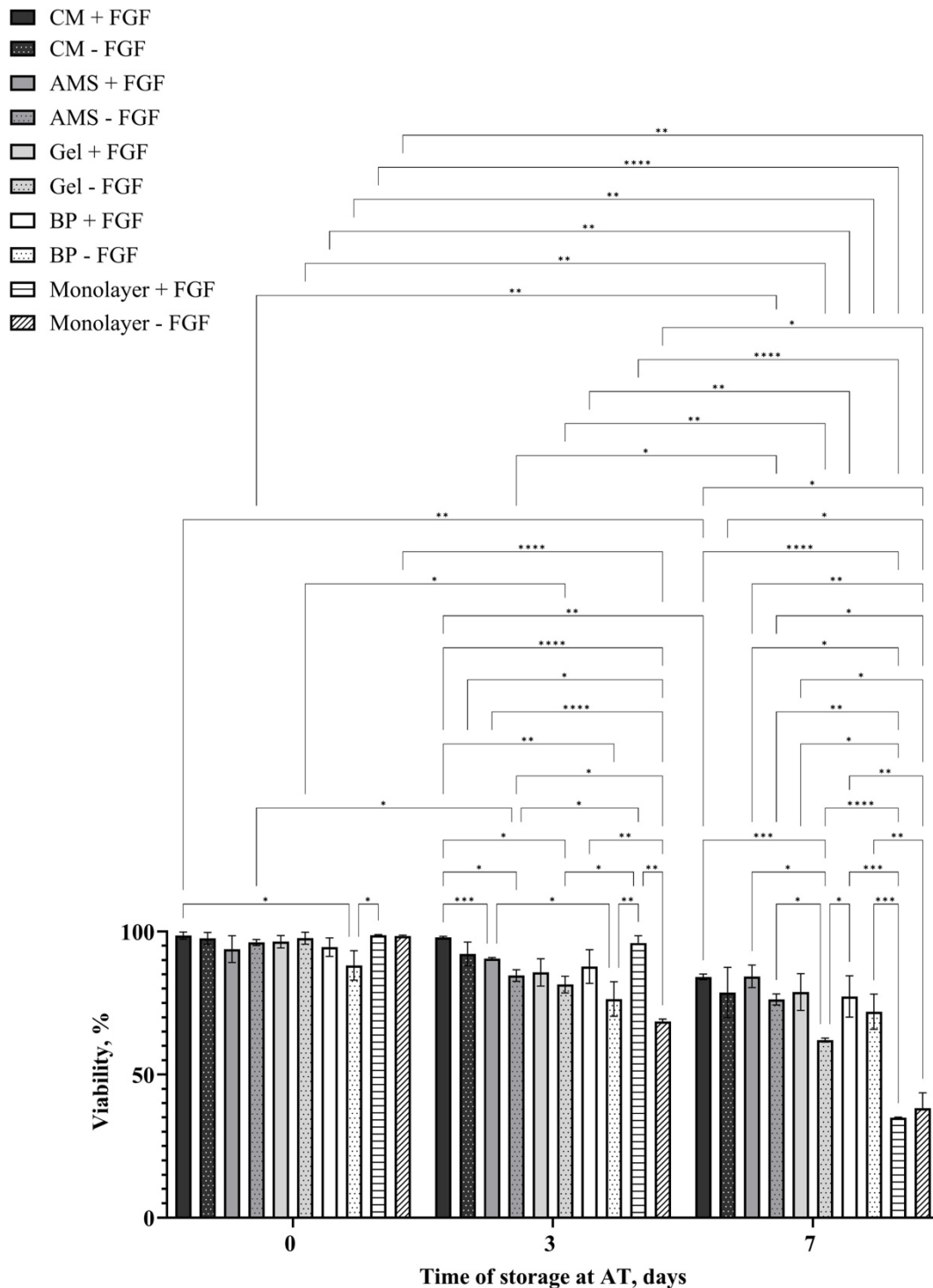


Fig. 1. MSC viability during ambient temperature storage

MSC viability at Day 0, 3, and 7 of ambient storage in monolayer culture, AMS, and core-shell capsules with cores composed of CM, Gel, or BP, in the presence of FGF or absence of FGF. Data are presented as mean \pm SD ($n = 3$, $N = 3$). Statistical analysis: two-way RM ANOVA. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$

Viability of encapsulated HeLa cells during ambient storage. HeLa cell viability was assessed on Days 0, 3, and 7 of ambient storage. At baseline (Day 0), viability was high across all groups (90–100%) with no pronounced intergroup differences (Fig. 2).

- CM + FGF
- CM - FGF
- AMS + FGF
- AMS - FGF
- Gel + FGF
- Gel - FGF
- BP + FGF
- BP - FGF
- Monolayer + FGF
- Monolayer - FGF

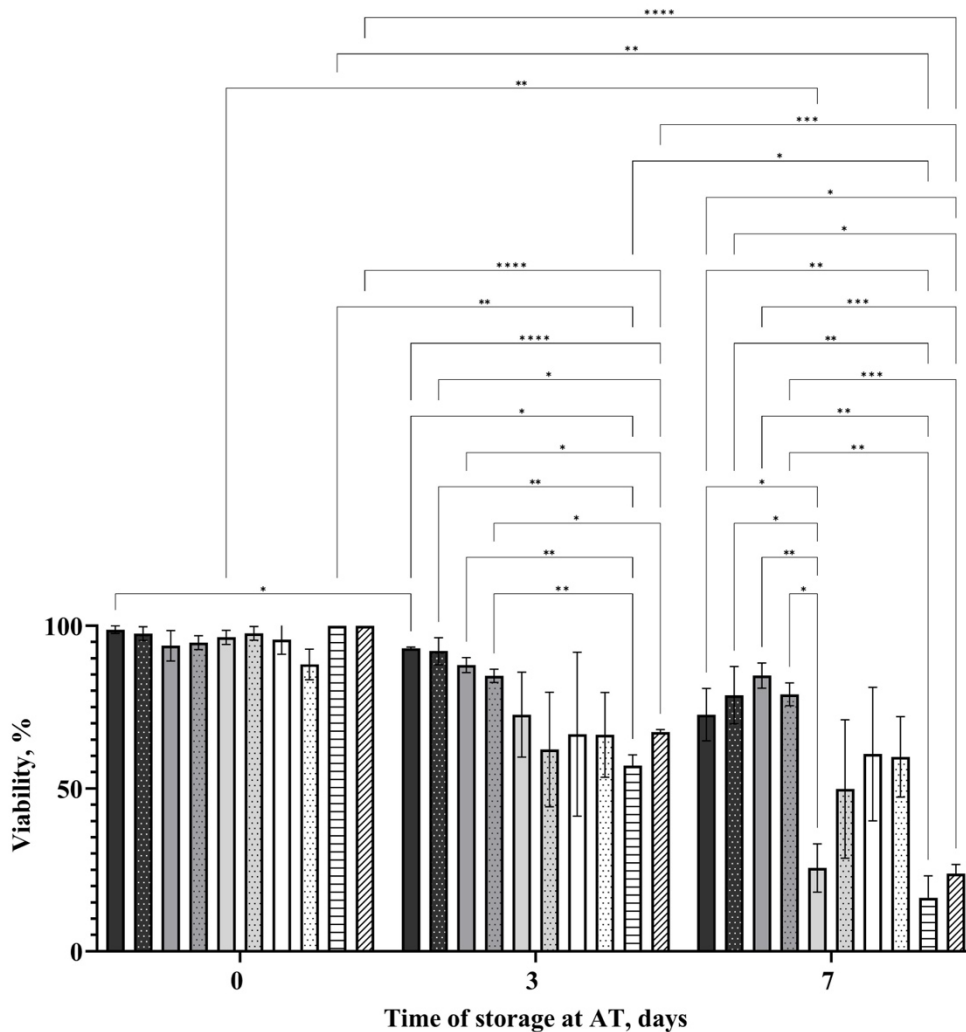


Fig. 2. HeLa cell viability during ambient storage

HeLa viability at Day 0, 3, and 7 during ambient storage in monolayer culture (AMS) and in core-shell capsules with CM, Gel, or BP cores, in the presence or absence of FGF. Data are presented as mean ± SD (n = 3, N = 2). Statistical analysis: mixed-effects model (REML) with Geisser-Greenhouse correction; significant effects of time, condition, and interaction (p < 0.0001). *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001

Mixed-effects analysis revealed a statistically significant effect of time (p < 0.0001), culture/encapsulation conditions (p < 0.0001), and their interaction (time × condition, p < 0.0001), indicating that viability changes depended on both storage duration and the specific microenvironment.

On Day 3, viability in monolayer culture decreased to 57.0 ± 3.3% (+FGF) and 67.3 ± 0.8% (-FGF). In AMS, viability remained higher at 87.9 ± 2.3% (+FGF) and 84.6 ± 2.0% (-FGF). In CM,

viability was $93.1 \pm 0.4\%$ (+FGF) and $92.2 \pm 4.2\%$ (-FGF). In Gel, viability decreased to $72.7 \pm 13.1\%$ (+FGF) and $62.0 \pm 17.6\%$ (-FGF), with greater variability under -FGF conditions. In BP, viability values were $66.7 \pm 25.2\%$ (+FGF) and $66.5 \pm 13.0\%$ (-FGF), suggesting early instability of this system.

By Day 7, viability decreased further in most groups. In monolayer culture, viability dropped to $16.4 \pm 6.7\%$ (+FGF) and $23.8 \pm 2.8\%$ (-FGF). In AMS, viability remained substantially higher at $84.7 \pm 3.9\%$ (+FGF) and $79.0 \pm 3.5\%$ (-FGF), indicating a pronounced protective effect of encapsulation. In CM, viability reached $72.7 \pm 8.1\%$ (+FGF) and $78.7 \pm 8.8\%$ (-FGF). In Gel, viability declined sharply to $25.6 \pm 7.4\%$ (+FGF) and $49.9 \pm 21.3\%$ (-FGF). In BP, viability varied within $60.6 \pm 20.5\%$ (+FGF) and $59.7 \pm 12.4\%$ (-FGF).

Taken together, HeLa viability during ambient storage was strongly dependent on encapsulation type and capsule core composition. The most stable viability over 7 days was observed in AMS, whereas monolayer culture exhibited the most pronounced and progressive decline. The significant time \times condition interaction further supports that viability dynamics are determined by the specific properties of each microenvironment.

Metabolic activity of mesenchymal stromal cells during ambient storage. MSC metabolic activity was assessed using the Alamar Blue assay and normalised to Day 0 of ambient storage (Day 0 = 100%). On Day 3, a pronounced decrease in metabolic activity was observed in monolayer culture to $54.7 \pm 1.2\%$ (+FGF) and $53.0 \pm 0.8\%$ (-FGF) (Fig. 3). In AMS, metabolic activity remained higher at $83.8 \pm 24.7\%$ (+FGF) and $78.5 \pm 21.2\%$ (-FGF). In CM, values were $82.0 \pm 19.6\%$ (+FGF) and $79.6 \pm 20.1\%$ (-FGF). In Gel, metabolic activity was $61.0 \pm 3.6\%$ (+FGF) and $48.2 \pm 1.9\%$ (-FGF). In BP, metabolic activity on Day 3 was $69.6 \pm 11.9\%$ (+FGF), but decreased to $32.34 \pm 11.2\%$ under -FGF conditions.

By Day 7, metabolic activity in monolayer culture further decreased to $28.5 \pm 2.2\%$ (+FGF) and $18.2 \pm 3.2\%$ (-FGF). In AMS, metabolic activity remained comparatively high at $93.6 \pm 27.9\%$ (+FGF) and $61.0 \pm 11.9\%$ (-FGF). In CM, metabolic activity reached $56.3 \pm 13.0\%$ (+FGF) and $44.1 \pm 14.1\%$ (-FGF). In Gel, values were $52.3 \pm 2.5\%$ (+FGF) and $31.0 \pm 1.6\%$ (-FGF). The most pronounced decrease among the encapsulated systems was observed in BP core-shell capsules under -FGF conditions ($14.9 \pm 5.0\%$), whereas +FGF partially preserved metabolic activity ($72.2 \pm 9.7\%$).

Overall, MSC metabolic activity decreased over time in all systems ($p < 0.0001$), however, the extent of preservation depended strongly on encapsulation type and the presence of FGF.

Metabolic activity of HeLa cells during ambient storage. HeLa metabolic activity was assessed by Alamar Blue with normalisation to the initial level (Day 0 = 100%). On Day 3, metabolic activity in monolayer culture decreased to $57.06 \pm 5.04\%$ (+FGF) and $55.23 \pm 1.88\%$ (-FGF) (Fig. 4). In AMS, activity remained higher at $67.96 \pm 6.05\%$ (+FGF) and $67.66 \pm 2.90\%$ (-FGF). In CM, values were $64.96 \pm 2.80\%$ (+FGF) and $60.40 \pm 0.85\%$ (-FGF). In Gel, metabolic activity reached $55.04 \pm 7.19\%$ (+FGF) and $34.14 \pm 25.55\%$ (-FGF), accompanied by substantial variability in the absence of FGF. In BP, values were $68.82 \pm 5.79\%$ (+FGF) and $51.09 \pm 3.53\%$ (-FGF).

By Day 7, monolayer metabolic activity decreased further to $18.95 \pm 2.51\%$ (+FGF) and $17.05 \pm 2.27\%$ (-FGF). Among encapsulated groups, outcomes depended on capsule design and core composition. In AMS, metabolic activity remained the highest at $63.35 \pm 1.92\%$ (+FGF) and $62.52 \pm 3.89\%$ (-FGF). In CM, values were $47.24 \pm 2.50\%$ (+FGF) and $46.56 \pm 4.55\%$ (-FGF). In Gel, metabolic activity declined to $34.99 \pm 5.43\%$ (+FGF) and $19.47 \pm 14.28\%$ (-FGF). The most pronounced reduction was observed in BP, reaching $17.95 \pm 3.38\%$ (+FGF) and $18.85 \pm 3.70\%$ (-FGF).

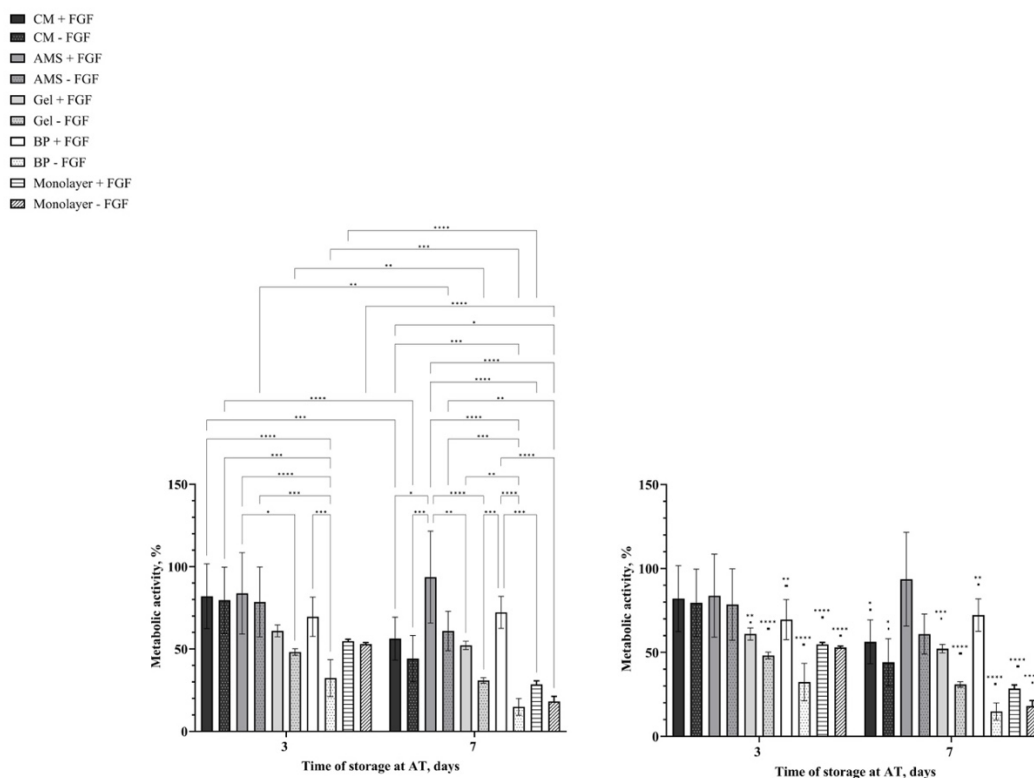


Fig. 3. MSC metabolic activity during ambient storage

MSC metabolic activity after 3 and 7 days of ambient storage in monolayer culture, AMS, and core-shell capsules with CM, Gel, or BP cores, in the presence or absence of FGF. Data are presented as mean \pm SD ($n = 3$, $N = 2$) and normalised to Day 0 (100%). The left panel indicates between-group differences at each time point (Day 3 and Day 7), and the right panel shows statistical significance relative to baseline (Day 0) within each group. Statistical analysis: two-way ANOVA. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$

Discussion

In this study, we evaluated how the presence of fibroblast growth factor (FGF) and the type of 3D carrier (AMS and core-shell capsules with different core compositions) affect the preservation of cell viability and metabolic activity during AT storage. Across both cell models, the overall trend was a time-dependent decline in readouts with increasing storage duration; however, the magnitude and kinetics of these changes depended strongly on cell type and microenvironment.

For MSCs, the contribution of FGF was more consistent and biologically expected. As primary cells, MSCs are sensitive to deprivation of trophic signals under stress conditions, including AT storage, restricted gas exchange, and metabolite accumulation. Under these conditions, FGF may support survival by activating pro-proliferative and anti-apoptotic pathways, stabilising the cytoskeleton, supporting survival, metabolism and increasing cellular stress tolerance (Nickle, 2024; Yun, 2010; Akl, 2016). This interpretation is in line with our observation that, across several encapsulated systems, FGF supplementation was associated with improved preservation of viability and/or metabolic activity at later storage time points. The effect was particularly pronounced in the plasma-core system, where the presence of FGF markedly improved both metabolic activity and viability during AT storage, suggesting that trophic support becomes critical in this microenvironment.

In contrast, the effect of FGF on HeLa cells was minimal or inconsistent. This can be explained by the nature of HeLa as a transformed cancer cell line with high growth autonomy, altered signalling networks, and reduced dependence on exogenous growth factors. In addition, such cell lines frequently rely on autocrine growth-support mechanisms and metabolic adaptations that increase tolerance to fluctuations in medium composition. Therefore, any potential contribution of FGF is likely masked by the dominant effects of the microenvironment, including diffusion constraints, core composition, and accumulation of toxic by-products (Gannoun-Zaki, 1994). In other words, for HeLa cells, the key determinant was not the presence of FGF, but rather the storage system's ability to maintain stable conditions (substrate availability, removal of metabolic waste, and mitigation of micro-stress).

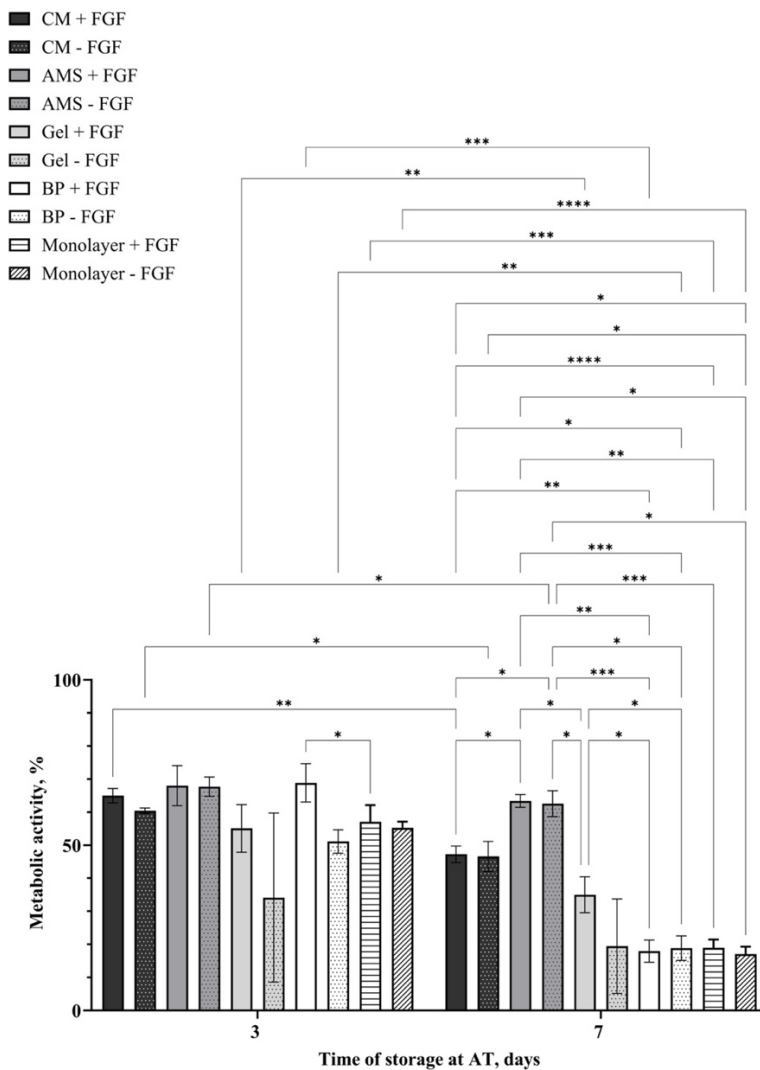


Fig. 4. HeLa metabolic activity during ambient storage

*HeLa metabolic activity after 3 and 7 days of ambient storage in monolayer culture, AMS, and core-shell capsules with CM, Gel, or BP cores, in the presence or absence of FGF. Data are presented as mean \pm SD ($n = 3$, $N = 2$) and normalised to Day 0 (100%). Statistical analysis: two-way ANOVA with a mixed-effects model (REML). Significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. All groups vs Day 0: **** $p < 0.0001$*

The lack of a clear FGF effect in HeLa cells, particularly under encapsulated conditions, may further indicate that other processes become rate-limiting, most notably mass transport (e.g., diffusion of glucose and oxygen into the construct and lactate export) (Edmunds, 1989; Kasinskis, 2014). When diffusion is limiting, the addition of a growth factor cannot compensate for substrate depletion, and maintaining basic cell survival becomes more relevant than stimulating proliferation under stress (Place et al., 2017). Moreover, microenvironmental stressors such as pH shifts, osmolarity changes, and localised accumulation of metabolites may exert a stronger influence on cellular function than growth signalling (Diener, 2016). Consequently, the carrier design and core composition emerged as the primary determinants of HeLa performance during AT storage (McCarthy, 1956).

It was shown that encapsulation generally provided better protection than monolayer culture, particularly at Day 7, when monolayers showed the most pronounced decline in both viability and metabolic activity. This observation is consistent with the high vulnerability of monolayer cultures during AT storage to rapid nutrient depletion, local accumulation of toxic products, and mechanical and/or oxidative stress, whereas hydrogel-based systems can partially buffer these fluctuations and provide a more stable microenvironment (Tarusin, 2016; Trufanova, 2025).

Across both cell types, AMS demonstrated the most stable performance or one of the most favourable time courses. This may reflect an advantageous balance between protection and mass transfer, including (i) sufficient matrix permeability to essential substrates, (ii) reduced mechanical stress, (iii) spatial distribution of cells with limited excessive aggregation, and (iv) improved

microenvironmental stability. This interpretation is consistent with previous reports showing that encapsulated MSCs exhibit greater resistance to adverse conditions (e.g., oxidative stress or nutrient limitation) than monolayer cultures or cell suspensions (Trufanova, 2025; Souza, 2021).

For core–shell capsules, the core composition was a critical variable (Arumugam, 2026). A CM core yielded consistently high MSC viability and generally supported stable performance, likely because it provides a predictable environment in terms of ionic composition, buffering capacity, and substrate availability (Gryshkov, 2021; Nebel, 2022). Gel-core capsules produced more variable outcomes, particularly for HeLa at early time points, which may be attributed to the more complex behaviour of Gel during AT storage. Gel can alter viscosity and diffusion, change core mechanics, and act as a source or sink for proteins, thereby modulating the availability of growth factors and metabolites (Yu, 2015). High variability may additionally indicate heterogeneity of the gel phase and/or differences in local cell distribution (Hettiaratchi, 2018; Yu, 2015).

BP-core capsules appeared to be the most challenging system in terms of stability, especially under eptanse FGF conditions. BP is a biologically active, multicomponent solution containing enzymes, coagulation factors, adhesion-related proteins, and other components that may modify both the matrix and cellular responses (Sonker, 2015; Chisini, 2017). During AT storage, this complexity may promote undesirable processes such as protein aggregation/denaturation, viscosity changes, and microenvironmental heterogeneity, ultimately increasing variability (Chisini, 2017). The observation that FGF improved MSC preservation in plasma-core capsules suggests that trophic deprivation becomes particularly critical in this environment, and that FGF can partially compensate for stress (Aizman, 2015).

Notably, viability and metabolic activity were not always reduced in parallel. In several conditions, particularly within encapsulated systems, viability remained relatively high, whereas metabolic activity declined more rapidly. This pattern is typical of a “stress-survival” mode, in which cells maintain membrane integrity but downshift metabolic activity (Khatib, 2020; Vackova, 2022). From a practical perspective (e.g., logistics of cell products and short-term storage prior to administration), such a combination may be acceptable or even advantageous, provided that cells can regain functional activity upon return to optimal culture conditions (Corwin, 2014; Trufanova, 2025).

Overall, our findings support two key conclusions. First, the effect of FGF is a strong cell-type-specific modulator: for MSCs, it acts as an important trophic support under stress, whereas for HeLa cells, its contribution is substantially smaller due to the intrinsic autonomy of this transformed cell line and the dominant influence of the microenvironment. Second, the type of 3D system and, for core-shell capsules, the core composition are decisive factors for maintaining cellular function during AT storage. The most stable outcomes were achieved with AMS and culture-medium core-shell capsules, whereas gelatin- and especially plasma-core systems showed increased variability and, under certain conditions, a stronger decline in functional readouts.

This study was performed at room temperature under controlled laboratory conditions. In real-world transport scenarios, cells may experience temperature fluctuations, mechanical loads, and changes in gas composition, all of which could further impact cell state (Gostage, 2025). Therefore, an important next step is to model realistic transport conditions and assess the robustness of encapsulated cells under such stressors. This will help optimise AT storage strategies and facilitate the translation of the proposed systems for practical use in regenerative medicine, particularly in resource-limited settings and during emergency conditions.

Conclusions. During ambient-temperature storage, the viability and metabolic activity of MSCs and HeLa cells declined over time ($p < 0.0001$); however, the magnitude of the decline was strongly dependent on the culture system and capsule core composition.

Three-dimensional encapsulation provided significantly greater cellular stability compared with monolayer culture. The most pronounced reduction in viability and metabolic activity was observed in monolayers at Day 7 of storage.

Among the encapsulated systems, alginate microspheres and core–shell capsules with a culture medium core demonstrated the most stable viability and metabolic activity profiles for both cell types.

Core composition had a substantial impact on cell preservation. Core–shell capsules containing gelatine or porcine plasma exhibited greater variability and lower functional readouts, particularly at later storage time points and in the presence of fibroblast growth factor-2.

For the first time, a positive effect of fibroblast growth factor supplementation on cell viability and metabolic activity during ambient temperature storage was demonstrated. This effect was cell-type-

specific and was more pronounced in mesenchymal stromal cells, whereas in HeLa cells, the contribution of FGF was less evident.

Overall, these findings provide a basis for improving the logistics of cell-based therapeutics and may contribute to the development of clinically feasible ambient-storage solutions for regenerative medicine. Implementing such strategies could enhance the accessibility and scalability of cell therapies in routine clinical practice.

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ЗБЕРІГАННЯ КЛІТИН В АЛЬГІНАТНИХ МІКРОСФЕРАХ ТА КАПСУЛАХ СТРУКТУРИ «ЯДРО–ОБОЛОНКА» ЗА АМБІЄНТНИХ ТЕМПЕРАТУР

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Резюме. Розробка ефективних систем короткострокового зберігання клітин є важливою передумовою впровадження клітинних технологій у регенеративній медицині, особливо в умовах обмеженої інфраструктури та під час транспортування клітинних препаратів. Метою дослідження було оцінити вплив типу тривимірної системи культивування (альгінатні мікросфери та капсули структури «ядро–оболонка» з різним складом ядра) та додавання фактора росту фібробластів (ФРФ) на збереження життєздатності й метаболічної активності мезенхімальних стромальних клітин кісткового мозку людини та клітин лінії HeLa під час зберігання за амбієнтної температури. МСК та клітини HeLa інкапсулювали в альгінатні мікросфери або в капсули структури «ядро–оболонка» з ядром на основі культурального середовища, желатину або плазми крові свиней методом електророзпилення та коаксіального електророзпилення. Після 17 діб культивування капсули зберігали за температури +22 °C протягом 7 діб. Життєздатність визначали методом виключення трипанового блакитного та флуоресцентним фарбуванням (FDA/EthD-1). Метаболічну активність оцінювали за допомогою тесту Alamar Blue з нормалізацією показників до початку амбієнтного зберігання. Статистичний аналіз проводили з використанням двофакторного дисперсійного аналізу та mixed-effects моделі. Встановлено, що зі збільшенням тривалості амбієнтного зберігання показники життєздатності та метаболічної активності достовірно знижувалися в усіх групах ($p < 0,0001$), проте ступінь змін залежав від типу мікрооточення та клітинної моделі. Тривимірна інкапсуляція забезпечувала більш стабільні показники порівняно з моношаровою культурою, особливо на 7-му добу зберігання. Найбільш сприятливі результати для обох клітинних типів отримано в альгінатних мікросферах та в капсулах із ядром на основі культурального середовища. Використання желатинового або плазмового ядра супроводжувалося більшою варіабельністю та вираженішим зниженням функціональних показників, особливо за відсутності ФРФ. Позитивний вплив ФРФ на збереження клітин був більш вираженим для МСК, тоді як для HeLa його ефект мав обмежений характер. Тип 3D-системи та склад ядра капсул є ключовими чинниками підтримання функціонального стану клітин за амбієнтного зберігання. Альгінатні мікросфери та капсули з ядром на основі культурального середовища демонструють найбільший потенціал для розробки ефективних технологій короткострокового зберігання клітинних продуктів за умов навколишнього середовища. Додавання фактора росту фібробластів підвищує стійкість мезенхімальних стромальних клітин до стресових умов та може розглядатися як додатковий модулюючий компонент у складі систем зберігання.

Ключові слова: мезенхімальні стромальні клітини, HeLa, альгінатні мікросфери, капсули «ядро–оболонка», фактор росту фібробластів, амбієнтне зберігання, 3D-культура, інкапсуляція, альгінат

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