Cryopreservation of Adherent Cells: Strategies to Improve Post-Thawing Viability and Function

**Introduction**

Clinical and commercial availability of cell-based products for tissue engineering and regenerative medicine require effective methods for their long-term storage in cryobanks, which are not yet established for complex systems such as cell monolayers, tissues or biosynthetic constructs [1]. Cell entrapment in a gel is a promising cryopreservation strategy to improve post-thaw viability and function of cell monolayers which were shown to poorly survive the cryopreservation process [2-5]. In this work, combined strategies for the cryopreservation of adherent cells were investigated based on cell entrapment in clinical-grade, highly purified alginate of extremely high viscosity (9.1% w/v, viscosity in distilled water > 30 mPa.s) uniformly cross-linked with BSA [6]. As model systems, Neuroblastoma N2a and Caco-2 Colon Adenocarcinoma cell lines were used due to their specific characteristics, which makes them interesting lines for studying the cryopreservation of differentiated cells [1-5]. As the cryopreservation medium, serum-free Cryostor [7] (BioCell Solutions) solution was compared with culture medium supplemented with bovine serum, both containing 10% MeSO.

**Aim and Strategy**

Develop optimized methodologies for the cryopreservation of functional cell monolayers for cell-based therapies and in-vitro pharmacological studies.

**STRATEGY**

Monolayer’s entrapment beneath a layer of ultra-high viscous (UHV) alginate

**Methods**

- **Culture**: Caco-2 and N2a cells were cultured on 4-well plates in either a non-differentiated or fully differentiated state. Caco-2 cells spontaneous differentiation into enterocyte-like cells was achieved through long-time culture. Neurotrophic differentiation of N2a cells was induced through retinoic acid addition to bovine serum content medium. After 1 or 4 days post-inoculation, a thin layer of UHV alginate cross-linked by BSA-fluor was added over the cells on the plates.

- **Cryopreservation**: After 5 days of culture (or 21 days for differentiated Caco-2 cells), cells were frozen at ① -1°C to -80°C. inside the plates with either serum-supplemented culture medium or Cryostor [7]. Survival (50%) of both 10% MeSO, and stored at -80°C during at least 1 week.

- **Post-thawing characterization**: Cell viability was assessed through membrane integrity assay and the metabolic assay alamethicin [8]. The structural integrity and differentiation state of the cells was evaluated through scanning electron microscopy. Maintenance of cell differentiated state after thawing was assessed through biochemical and immunomorphological assays, respectively.

**Results**

- **Effect of alginate entrapment on cell growth and differentiation**

- **Post-thaw recovery of non-differentiated monolayers**

- **Post-thaw viability and differentiation state of differentiated monolayers**

- **CONCLUSIONS**

- Monolayer entrapment beneath an alginate layer improves cell recovery by avoiding detachment from the substrate and minimizing membrane damage and cell detachment after thawing.

- The use of Cryostor solution improves the cryopreservation process for both cells lines, allowing the maintenance of high post-thaw recovery of viability and differentiation state.

- Cryptostor solution allows full recovery of metabolic activity and initiation of proliferation within 24 hours post-thawing.

**References**


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