Enhanced Survival of LNCaP Cells Following Cryopreservation Using the CryoStor™ CS5 Preservation Solution and Corning® CellBIND® Surface

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Application Note

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Abstract

Most cell cryopreservation techniques use a mixture of cell culture media, animal sera and DMSO as a freezing solution, which, combined with slow cooling rates, prevents ice crystal damage and other adverse effects associated with the freezing process[1]. However, the rapid recovery of viable, functional cells able to quickly attach and grow in culture is often a problem following cryopreservation. Cells that appear to be viable immediately upon thawing are often discovered to be floating or poorly attached after 24 hours incubation. These cells usually become necrotic or enter apoptosis and, as a result, recover very slowly, if at all. Additionally, these traditional freezing solutions usually contain a protein and/or serum component which can contribute to variability in cell survival, increase contamination risks associated with using animal-derived components and add significant cost to the process.

Here we demonstrate the benefits of using the Corning CellBIND surface combined with the protein-free BioLife CryoStor CS5 freezing solution to improve the survival of LNCaP cells, a human prostate cancer cell line that is difficult to preserve and maintain due to its slow growth and poor attachment properties. The CellBIND surface is created using a process that improves the cell attachment properties of polystyrene culture vessels. It has been demonstrated to improve the recovery and attachment of LNCaP cells following cryogenic storage[2]. The CryoStor CS5 freezing solution was developed by BioLife Solutions to improve cell survival and recovery during and following cryogenic storage[3,4]. It contains 5% DMSO and is based on a protein-free formulation originally developed for storage of tissues and organs under hypothermic (4°C to 10°C) conditions.

Methods and Materials

LNCaP cells (obtained from ATCC; CRL-1740) were cryopreserved following ATCC recommendations in either RPMI 1640 medium with 10% fetal bovine serum (FBS) and 5% DMSO (standard freezing medium) or CryoStor CS5 freezing solution. Cells were recovered from liquid nitrogen storage by thawing rapidly and plating into 25 cm^2 (T-25) flasks or 96-well microplates with either the Corning CellBIND surface or a standard Tissue Culture (TC)-treated surface. Growth medium was RPMI 1640 plus 10% FBS.

Results

After thawing and 24-hour incubation at 37°C in 5% CO_2, the cells were harvested from T-25 flasks and cell survival measured using a hemocytometer with viability determined by trypan blue exclusion (Figure 1). These experiments were done by Corning scientists. Cell survival (also 24-hours post-thaw) in 96-well microplates was determined based on metabolic activity using an almarBlue™ assay (Figure 2). These experiments were done by BioLife Solutions scientists. Each study was performed in triplicate and repeated three times at each study site.

Using the Corning CellBIND surface with standard freezing medium increased LNCaP survival by over 25% compared to the standard tissue culture surface. However, combining CryoStor CS5 freezing solution with the CellBIND surface showed a 58% increase in LNCaP survival over the standard cell culture surface (ANOVA, p = 0.0013). Similar results were observed when cultures were visualized using fluorescent microscopy; the CryoStor CS5 freezing solution, and CellBIND surface combination showed increased cell attachment compared to the standard freezing media and the standard cell culture surface (Figure 3).

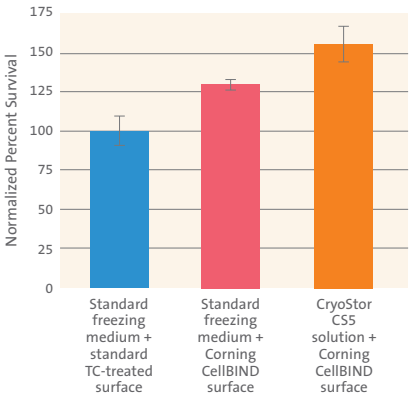

Figure 1. Survival and attachment of cryopreserved LNCaP cells in T-25 flasks 24 hours post-thaw. Survival was determined using direct cell counts (hemocytometers) with results normalized to cultures grown under standard conditions (blue column). Data is an average ± SD of 3 independent experiments performed by Corning scientists (N = 3 repetitions per group).

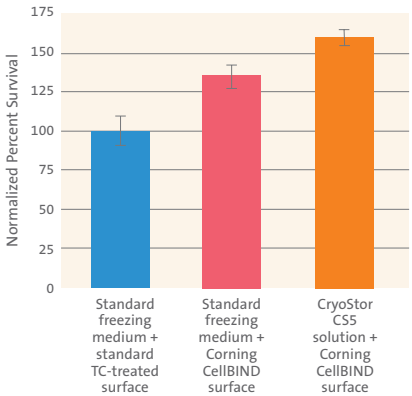

Figure 2. Survival and attachment of cryopreserved LNCaP cells in 96-well microplates 24 hours post-thaw. Survival was determined using an almarBlue assay and results normalized to cultures grown under standard conditions (blue column). Data is an average ± SD of 3 independent experiments performed by BioLife Solutions scientists (N = 3 repetitions per group).

Standard freezing medium plus standard TC-surface

Standard freezing medium plus Corning CellBIND surface

CryoStor CS5 freezing solution plus Corning CellBIND surface

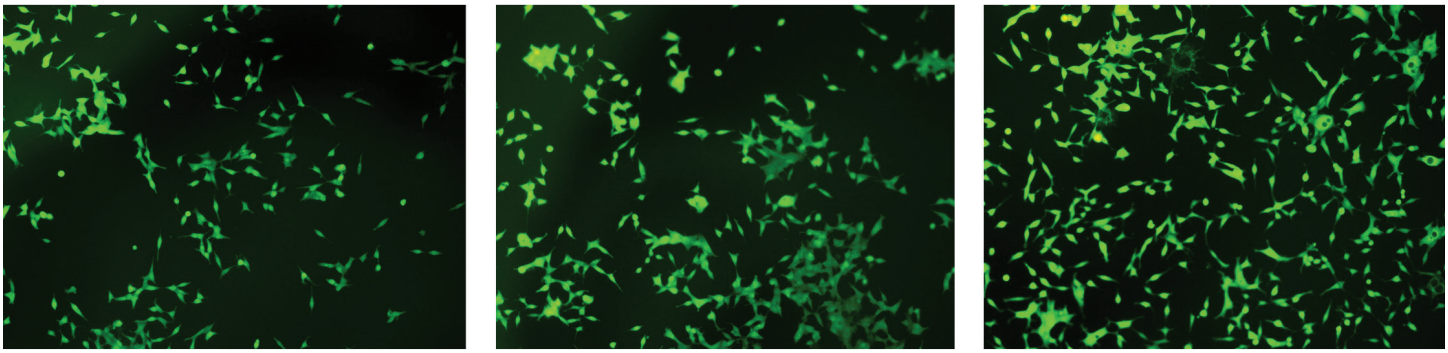

Figure 3. Fluorescent micrographs of cryopreserved LNCaP cells in 96-well microplates 24 hours post-thaw. Cells were cryopreserved in either traditional media + 5% DMSO or BioLife CryoStor CS5 (5% DMSO) then thawed and plated onto either Corning CellBIND surface or the standard TC-treated surface.

Conclusions

- Combining the Corning® CellBIND® surface with the BioLife Solutions CryoStor™ CS5 freezing solution resulted in significant increases in LNCaP cell survival following cryopreservation.
- CryoStor CS5 freezing solution combined with the Corning CellBIND surface eliminated the need for proteins or sera in the cryopreservation process, reducing both the costs and the risks associated with using animal-derived sera and proteins.

References

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4. Stylianou J, Vowels M, and Hadfield K. (2006) Novel Cryoprotectant Significantly Improves the Post-Thaw Recovery and Quality of HSC from CB. Cytotherapy. Vol. 8(1):57-61.

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