Institute of Biomedical Technology

Biological Packaging for Preservation of Cells and Tissues

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Introduction and Background

Within the biomedical engineering subspecialties, there is a need for reversible physiological/developmental arrest of cells and tissues (biopreservation) in support of global distribution followed by on-demand restoration of cellular function. Two avenues utilized to achieve biopreservation is that of hypothermic (4°C) storage (short-term - hours to days) and cryopreservation (-196ºC) (long-term – months to years). Both methods can result in a significant loss of cell viability and/or function. As a result, there is a growing need for improved methods for storage/transportation in areas such as cellular and tissue (engineered and native)-based therapies for clinical and pharmaceutical applications.

Recently we have identified the activation of apoptotic and necrotic cell death pathways as a critical factor in determining the preservation window for a given biologic. Accordingly, we have developed a new platform of hypothermic preservation solution, HypoThermosol® (HTS), and cryopreservation solution, CryoStor™, designed to buffer the overall physiological and biochemical response of cells to the preservation process as well as specifically modulate both the apoptotic and necrotic cascades. This has led to the establishment of BioLife Solutions, Inc, a university incubator specializing in the development of "packaging" systems for biologics for improved preservation. The HypoThermosol® and/or CryoStor™ family of solutions has been shown to improve preservation of an engineered human skin equivalent, human skeletal muscle cells, human liver cells, and pancreatic islets.

These improved preservation solutions are being utilized or evaluated by groups engaged in:

- Cellular cardiomyoplasty for the repair for cardiac damage (BioHeart, Inc.)
- The development of bioartificial liver assist devices (one company in California and a separate company in the Netherlands)
- Pancreatic islet transplantation for the treatment of diabetes (University of Miami, Columbia University)
- Cosmopharmaceutical preservation of engineered skin
- Stem cell research both in the United States and overseas.

In addition, BioLife Solutions has been awarded numerous NIH/NSF grants. BioLife Solutions has developed from an academic incubator concept to the manufacture and sale of commercial products, established contract services, and employs and/or provides graduate assistance for 14 people.

Examples of Successful Applications

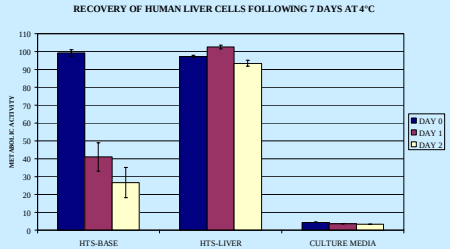

Figure 1. HypoThermosol-LIVER was custom developed for the preservation of liver cells within a bioartificial Liver Assist Device and was able to maintain viability of these cells for one week.

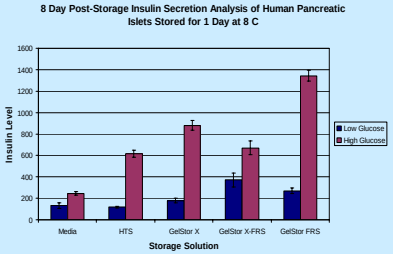

Figure 2. Pancreatic islets preserved in GelStor™ exhibit greater insulin regulation in comparison to conventional media. GelStor™ is a semi-solid preservation solution developed to protect cell types that are extremely sensitive to mechanical stress, such as pancreatic islets. Improved preservation of islets allows for improved pancreatic islet transplantation for the treatment of Type I Diabetes. This research is in collaboration with The University of Miami Diabetes Research Institute and Columbia University

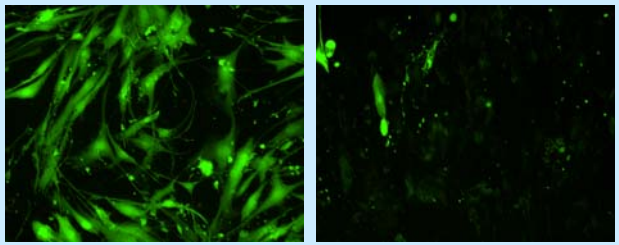

Figure 3. Human skeletal muscle cells following 5 days at 4ºC in HypoThermosol-FRS (left panel) or conventional storage media (right panel) – stained with the fluorescent integrity indicator Calcein-AM. HypoThermosol-FRS is being utilized by BioHeart, Inc. for the shipping and preservation of skeletal myoblasts for cellular cardiomyoplasty, a new treatment for repairing heart damage.

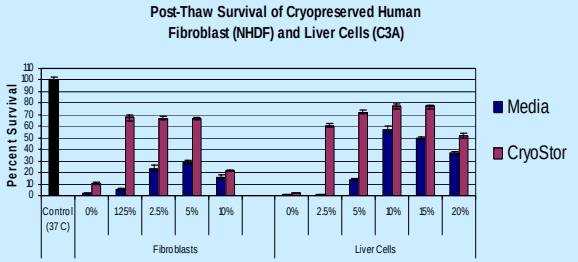

Figure 4. Twenty-four hour post-thaw cell viability analysis of human fibroblast (NHDF) and human hepatocarcinoma (C3A) cells following cryopreservation. Utilization of traditional CP methodologies (Media + DMSO) resulted in a CPA dependent survival limit (cap) in the cell systems tested. Evaluation of survival allowed for the determination of an "optimal" CPA concentration, yielding maximal cell survival. Further, utilization of CryoStor resulted in a substantial elevation in cell survival over the media-based CP survival cap and facilitated the successful cryopreservation at significantly reduced CPA levels.

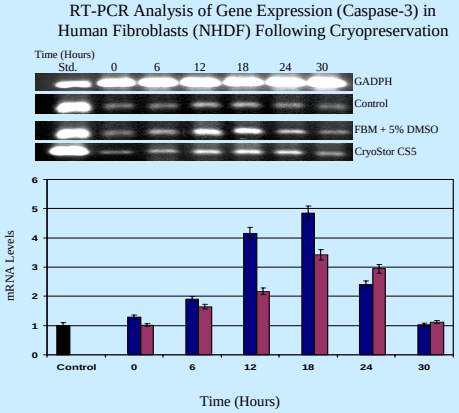

Figure 5. Post-thaw time course RT-PCR analysis of gene expression (caspase-3) in human fibroblast cells following cryopreservation in either media + 5% DMSO or CryoStor CS5. Caspase-3 expression increases significantly following thawing, peaking at 18-hours post-thaw and returning to that of controls by 30-hours. Preservation of fibroblasts in CryoStor resulted in a reduction in the extent of caspase-3 upregulation in comparison with media + DMSO samples. Caspase-3 expression levels were normalized internally to a matched GADPH standard then compared directly to matched time point non-frozen controls (37°C) and converted to graphical format for comparative purposes.

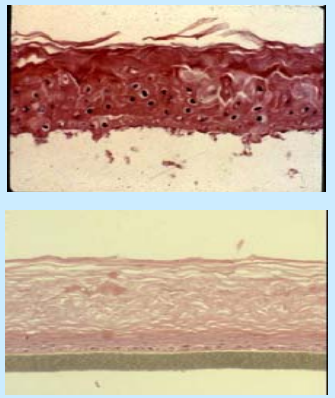

Figure 6. The engineered human skin equivalent EpiDerm™ was stored in HypoThermosol® (Bottom Panel) and compared histologically to EpiDerm™ stored in KGM (Top panel). Note that the EpiDerm™ preserved in HypoThermosol® appears to have a normal histology whereas this is not the case when EpiDerm™ is stored in conventional media (KGM). EpiDerm™ preserved in culture media have detached from the basement membrane. EpiDerm™ preserved in HypoThermosol® maintain tissue integrity and attachment to substrate.

- Hypothermic preservation is utilized for short-term (hours to days) preservation of biologics. Cryopreservation is utilized for long-term (months to years) preservation.
- Increasing the time and quality of preservation is needed for the shipping/storage of cellular and tissue products in the growing fields of regenerative medicine, tissue engineering, and pharmaceuticals.
- Custom hypothermic solutions, such as the HypoThermosol® and CryoStor™ solutions, can be developed for the improved shipping and storage of engineered human cells and tissues used in the Regenerative Medicine and In Vitro Toxicology arenas so that these important products are better able to reach a global marketplace.