Combination of Biopreservation Solution and a Gel Component Improves Stability and Recovery of Cells

DM Clarke, AJ Mathew

BioLife Solutions, Inc., 3303 Monte Villa Parkway Bothell, WA 98021

Introduction

Hypothermic and cryopreservation maintenance and storage of cells, tissues and organs is commonly performed in liquid media. Success is limited, in many situations, due to damage that occurs during shipment (transport), most often associated with mechanical trauma. Preservation and transport of cells and tissues has traditionally been achieved through "suspension" in a liquid preservation medium. While some preservation solutions offer improved preservation and stability, the liquid environment does not provide a physical support network for the cell and tissue during transport and subsequent preservation. Due to this lack of physical support, cells and tissues are exposed to numerous physical stresses during transport and storage. These stresses can include, but are not limited to, sedimentation, mechanical "jarring", compaction in a liquid column, shaking, vibration, shearing forces, and ice damage. As a result of these mechanical stresses plus additional biochemical stresses inherently associated with preservation in liquid, a significant level of cell death due to apoptosis and necrosis is initiated during and following the preservation interval.

The purpose of this study was to investigate the utility and efficacy of combining a gelling component with a biopreservation solution for both hypothermic storage and cryopreservation of cells. To simulate the potential damaging effects accumulated during transport of cellular products, a mechanical damage (stress) model system was developed and tested. Commonly used standard serum-containing culture media and commercially available, fully defined, serum-free and protein-free preservation solutions were used in the study.

In summary, the results of this study demonstrate that the incorporation of a gel during preservation can improve the stability and recovery of cells. Methods to improve transport and preservation of cells and tissues may ultimately improve the overall integrity of the cellular product.

Methods

Cell Culture

Normal human dermal fibroblasts (NHDF) and human mesenchymal stem cells (hMSC; Lonza, Walkersville MD) and human sperm cells were cultured following standard methods.

Preservation

Gel solutions for hypothermic storage/transport were prepared by combining gelatin to a 2% final concentration with either complete cell culture medium or HypoThermosol®-FRS (HTS; BioLife Solutions, Bothell WA). Cells were combined with either culture media or HTS with or without gelatin and maintained at 2-8°C for a specified number of days depending on the cell type. For cryopreservation studies, cells were preserved in complete culture media containing 5% DMSO or CryoStor™ CS5 (5% DMSO; BioLife Solutions, Bothell WA) and compared to these solutions with the addition of 2% gelatin. To remove cells from gel solutions, gel samples were briefly (2-3 min) warmed at 37°C until liquefied. Following storage, cell solutions were combined and diluted with fresh culture media and plated.

Transport Stress Simulation

To simulate potential mechanical and physical induced damage (stress) during transport, non-gel and gel NHDF cell samples were placed into a Styrofoam shipping/ storage container and dropped on 3 separate occasions (10 drops/time point) during the hypothermic storage exposure.

Testing

Post-preservation recovery and viability of hMSC and NHDF cell samples were assessed 24 hours post-preservation using alamarBlue® (AbD Serotec, Raleigh NC) to assess metabolic activity and compared to non-preserved controls. Human sperm recovery was assessed by microscopy detection of directional motility and compared to non-preserved fresh samples.

Hypothermic Storage – Gel Impact

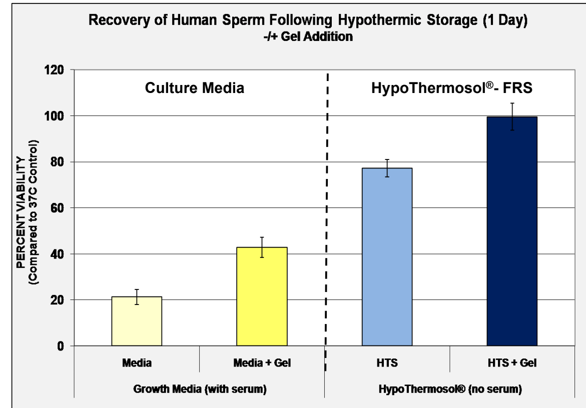

Figure 1: Recovery of human sperm following hypothermic storage in culture media or HTS with or without the combination of a gel. Sperm were stored for 1 day in standard liquid solutions or solutions combined with a gelling component. Sperm viability and motility were assessed 2 hours post-preservation and compared to non-preserved fresh controls.

Cryopreservation – Gel Impact

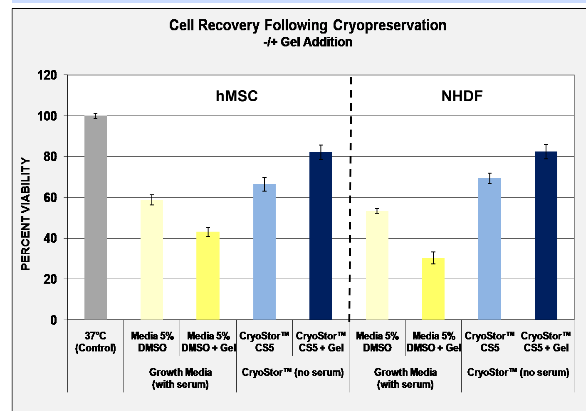

Figure 3: Post-thaw viability of hMSC and NHDF cells following cryopreservation in culture media (5% DMSO) or CS5 with or without the combination of a gel. HMSC were cryopreserved in standard liquid cryopreservation solutions or solutions combined with a gelling component. Following cryopreservation, cells were thawed, diluted in culture media, and plated. Cell recovery and viability were assessed 24-hours post-thaw and compared to non-preserved control cells.

Transport (Mechanical) Damage

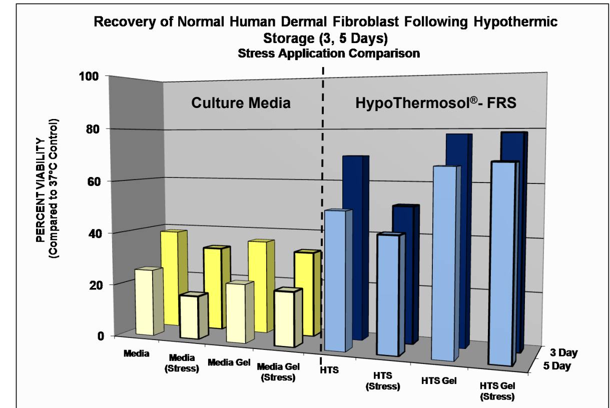

Figure 2: Recovery of NHDF cells following 3 and 5 days of hypothermic storage in culture media or HTS with or without the combination of a gel. NHDF cells. Stress conditions were applied to imitate conditions associated with cell/tissue shipping. Cell recovery and viability were assessed 24-hours post-preservation and compared to non-preserved control cells.

Summary of Results

- Recovery of functional human sperm was improved with the additional of a gel in both standard culture media and when combined with HTS;
  - The combination of a gel and HTS dramatically improved viability compared to a gel combined with culture media
- Applying simulated mechanical stress to NHDF cells during hypothermic storage resulted in decreased viability compared to non-stressed samples;
  - The combination of a gel reduced/prevented mechanical stress-induced loss observed with traditional liquid solutions
  - HTS improved stability and viability compared to media
  - The combination of a gel and HTS resulted in improved viability compared to media or HTS liquid solutions
- Addition of gel component for cryopreservation;
  - Improves viability of hMSC and NHDF when combined with CS5
  - Viability is reduced when gel is combined with culture media + DMSO
- Use of fully defined, serum-free, protein-free CryoStor and HypoThermosol-FRS biopreservation solutions offer;
  - Extended stability of cells
  - Improved post-preservation recovery and viability
  - Fully defined, serum-free and protein-free preservation reagents
- The combination of a gel and optimal preservation solution offers the potential to extend stability and improve recovery of cells and tissues during collection, transport, and storage