Maximizing Stability of Human Stem Cells through Optimized Biopreservation Media Solutions

BioLife Solutions
POWERING THE PRESERVATION SCIENCES

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Introduction

A significant level of academic and commercial interest has been generated by the potential clinical benefits of regenerative medicine cell- or tissue-based products. Several challenges remain, related to the quality, transportation, storage, and cryobanking of the source material and cells used in these development stage products. With the number of applications and variety of stem cell lineages continually expanding, optimized biopreservation methods are critical. Suboptimal biopreservation systems result in significant limitations in transportation and storage stability and reduced clinical efficacy due to preservation-induced cell yield loss.

Typically, commercial and home-brew (formulated in-house) biopreservation media consist of a carrier solution such as cell growth/culture media, saline, or other physiologic buffers. Additional components including serum and protein (animal origin in many cases) are often added to the carrier media to enhance preservation efficacy. Unfortunately, the addition of serum or protein to the biopreservation media restricts potential use and widespread implementation of these cells in clinical applications. The purpose of this study was to investigate current methods and commonly used biopreservation media to store stem cells under both short-term (hypothermic preservation) and long-term (cryopreservation) conditions. In this study, commonly used standard serum-containing culture media and commercially available, fully defined, serum-free and protein-free preservation solutions were evaluated for preservation efficacy.

The results of this study demonstrate that the utilization of HypoThermosol® during hypothermic storage of stem cells improves post-preservation recovery and significantly extends stability compared to the other media tested. For cryopreservation of stem cells, CryoStor™ improved post-thaw recovery and viability when compared to similar home-brew media cocktails. Unlike traditional home-brew or leading commercial products, pre-formulated HypoThermosol and CryoStor are serum-free and protein-free and formulated to balance the intracellular state at low temperature, resulting in broad-based improvement in preservation efficacy, while offering a robust quality and regulatory footprint. Development of effective biopreservation methods permits extended stability for collection, transportation, processing, testing, and banking and may enable widespread clinical application of stem cells.

Methods

Cell Culture
Human mesenchymal stem cells (hMSC; Lonza, Walkersville MD) and human dental pulp stem cells (DPSC; New England Cryogenic Center, Newton MA) were cultured according to manufacturer recommendations.

Hypothermic Storage
To test the efficacy of hypothermic storage preservation media, cells were plated in 96-well culture plates and grown to confluence. The respective solutions were added to the cells and cultures were stored at 2-8°C for 1-7 days. Following hypothermic storage, solutions were removed and replaced with fresh culture media and the cells were allowed to recover for 1 day prior to assessment.

Cryopreservation
To investigate the efficacy of various cryopreservation media, standard cryopreservation methods were performed. For home-brew media preparation, DMSO (10%) only or DMSO and fetal bovine serum (FBS) were added to the base media. The CryoStor CS10 (10% DMSO) is pre-formulated with DMSO and does not contain serum or protein. Briefly, cells (3.25×10^6 cells/ml) were resuspended in 0.5ml of the respective media and placed into cryovials. Samples were cooled at roughly 1°C per minute then stored at -80°C for 3 hours and then transferred to LN_2 for 24 hours. Samples were thawed in a 37°C water bath, immediately resuspended in culture media (1:10 dilution), plated, and allowed to recover for 1 day.

Testing
Performance was tested using alamarBlue® (AbD Serotec) to determine relative cell viability. Cell cultures exposed to either hypothermic storage or cryopreservation were assessed for relative cell viability 1 day post-preservation. Efficacy of test solutions were compared to non-preserved 37°C controls. Error bars represent standard error of the mean (SEM).

To obtain fluorescent micrographs, cultures were labeled and fixed 1 day post-preservation with MitoTracker Red (red - mitochondria), AlexaFluor 488 phalloidin (green - actin cytoskeleton), and Hoechst (blue - nucleus) according to manufacturer instructions (Invitrogen, Carlsbad CA). Images were taken using a Zeiss Axiovert inverted microscope (Carl Zeiss, Germany).

Hypothermic Storage – hMSC

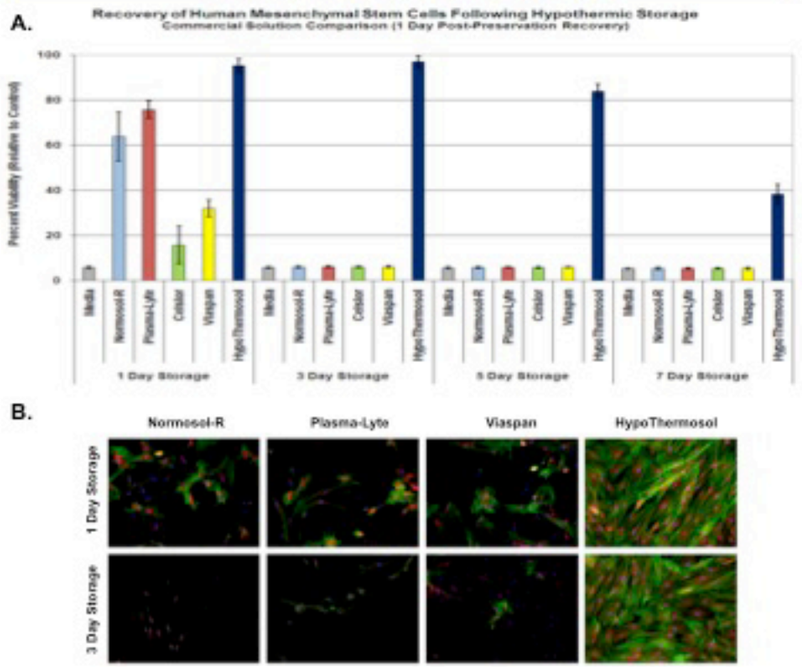

Figure 1: Recovery of hMSC following extended hypothermic storage. (A) Post-preservation viability of cells after 1-7 days of storage and 1 day of recovery. (B) Representative fluorescent micrographs of cells stored for 1 or 3 days and 1 day of recovery. HypoThermosol provides improved viability and extended stability.

Hypothermic Storage – DPSC

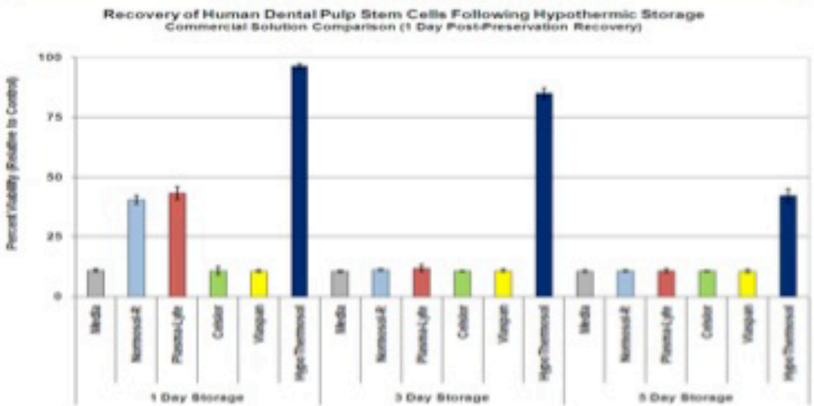

Figure 2: Recovery of DPSC following extended hypothermic storage. (A) Post-preservation viability of cells after 1-5 days of storage and 1 day of recovery. HypoThermosol provides improved viability and extended stability.

Cryopreservation – Home-Brew Comparison

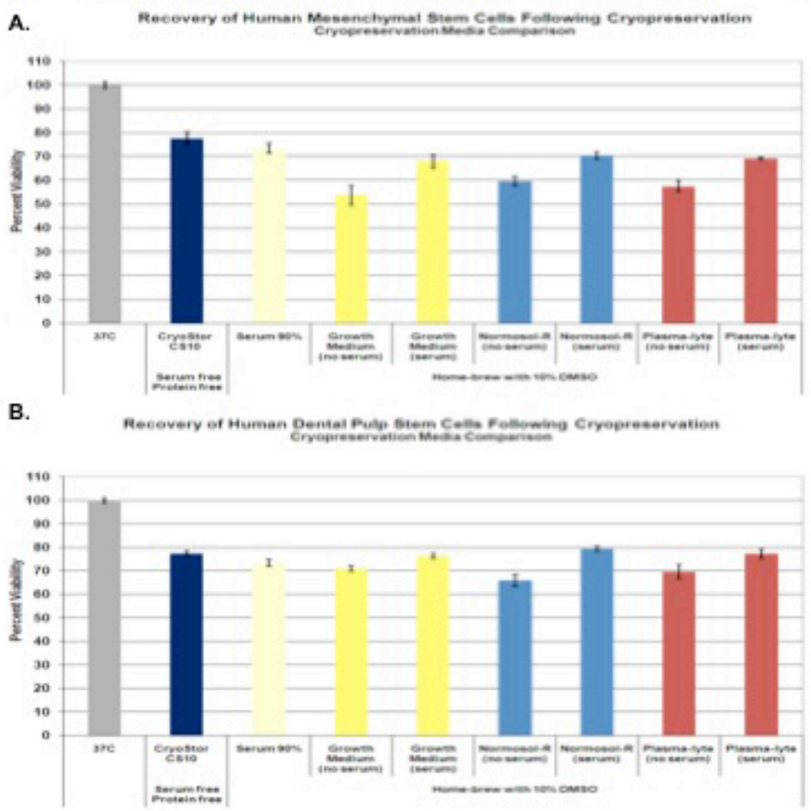

Figure 3: Post-thaw viability of hMSC and DPSC following cryopreservation in CryoStor compared to a variety of home-brew cocktails. (A) 1 day post-thaw viability comparison of hMSC. (B) 1 day post-thaw viability comparison of DPSC. CryoStor results in improved recovery without the need for protein or serum.

Summary of Results

- Hypothermic storage of hMSC and DPSC
  - Highest post-preservation recovery achieved using HypoThermosol when compared to all other solutions evaluated and all time points tested
  - HypoThermosol enables extended stability options
- Cryopreservation of hMSC and DPSC
  - Addition of serum to home-brew media improves post-thaw recovery
  - CryoStor (serum-free, protein-free) media provides improved post-thaw recovery compared to all serum-free media tested
- HypoThermosol and CryoStor:
  - Are pre-formulated serum-free and protein-free biopreservation solutions formulated for improved osmotic protection and reduced apoptotic and necrotic death of cells during hypothermic storage and cryopreservation
  - Reduce media formulation time and improves product consistency
  - Extend stability and improve post-preservation recovery
  - Are supported by FDA Master Files