BIOPRESERVATION MEDIA ACCELERATED STABILITY CHARACTERIZATION AND PERFORMANCE

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Introduction

Stability studies, including accelerated or stressful conditions, are useful for determining whether accidental exposure to conditions beyond recommended usage (e.g., elevated temperatures, freeze-thaw cycles) is deleterious to biopreservation media products; specifically, their ability to provide a protective environment for biologic source material and finished cellular products throughout the manufacturing and clinical delivery processes. The knowledge gained by these studies is beneficial to both the manufacturer and their customers who use such biopreservation media products.

Forced degradation or stress testing studies are an important measure of the stability of a product or component. Typical testing of stressed or degraded samples is designed to :

- determine structural transformations of the substance or product
- detect concentrations of potential degradation products
- elucidate possible degradation path-ways
- identify degradation products generated during product storage and use
- facilitate improvements in the manufacturing process parallel with accelerated studies
- determine whether accidental exposure to conditions outside normal ranges are deleterious to the products
- determine if any of the conditions affect the overall intended performance or function of the product

In this study, HypoThermosol® (HTS-FRS) and CryoStor™ (BioLife Solutions, Bothell, WA) biopreservation solutions were exposed to a series of forced degradation conditions to determine whether exposures to temperatures other than normal storage ranges (2-8°C) are deleterious to the products. Specifically, do the aberrant temperature exposures influence the efficacy of the solutions to provide optimal protection of cells during hypothermic storage or cryopreservation? While long-term ICH compliant stability studies are underway, these data represent important stability characteristics of these products, which are currently used for biopreservation of a wide variety of source materials and derived cellular products.

Methods

Forced Degradation Testing
50ml aliquots of HTS-FRS and CryoStor biopreservation solutions were placed into sterile 60ml PETG Nalgene bottles. Samples were then exposed to a range of conditions: 1) 37°C for 1 week, 2) 37°C for 1 month, 3) 65°C for 1 week, 4) 10 freeze/thaw cycles to -80°C, 5) 2-8°C control standard. For the freeze/thaw condition, solutions were frozen for 20 minutes and then thawed in a 37°C water bath until all ice had melted. Following exposure to the specified conditions, solution samples were returned to 2-8°C and evaluated 24 hours post.

Hypothermic Storage
To test the efficacy of HTS-FRS degraded solutions, Normal Human Dermal Fibroblasts (NHDF; Lonza, Walkersville, MD) 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 5 days. Following hypothermic storage, solutions were removed and replaced with fresh culture media and allowed to recover for 1 day.

Cryopreservation
To test the efficacy of CryoStor force degraded solutions, standard cryopreservation testing was performed using NHDF cells. Briefly, cells (6.5×10^5 cells/ml) were resuspended in 0.5ml of the respective CryoStor solutions and placed into 1.2ml cryovials. Cryopreservation studies were performed using a Nalgene Mr. Frosty. Samples were 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), a metabolic activity indicator, 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 force degraded solutions were compared to standard solutions maintained at 2-8°C (recommended storage condition). Error bars represent standard error of the mean (SEM).

Results – Cryopreservation (CS10)

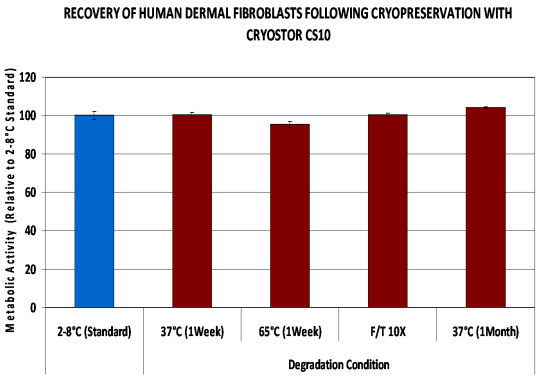

Figure 1: Post-thaw viability of NHDF cells following cryopreservation with CryoStor CS10 variants. Relative cell viability was assayed 1 day post-thaw. Efficacy of degraded solutions was compared to that of cells cryopreserved in the 2-8°C maintained standard solution. Of the degrading conditions investigated, none had any impact on the efficacy of CS10 to cryopreserve NHDF cells.

Results – Cryopreservation (CS5)

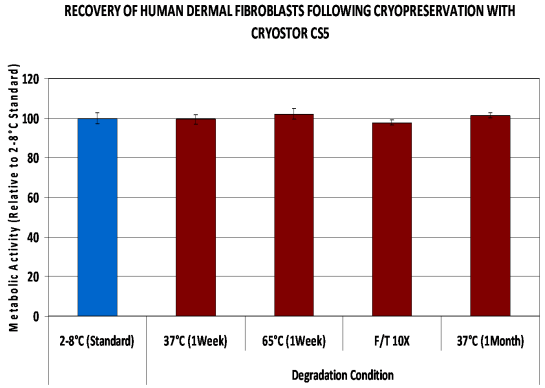

Figure 2: Post-thaw viability of NHDF cells following cryopreservation with CryoStor CS5 variants. Relative cell viability was assayed 1 day post-thaw. Efficacy of degraded solutions was compared to that of cells cryopreserved in the 2-8°C maintained standard solution. Of the degrading conditions investigated, none had any impact on the efficacy of CS5 to cryopreserve NHDF cells.

Results – Hypothermic Storage

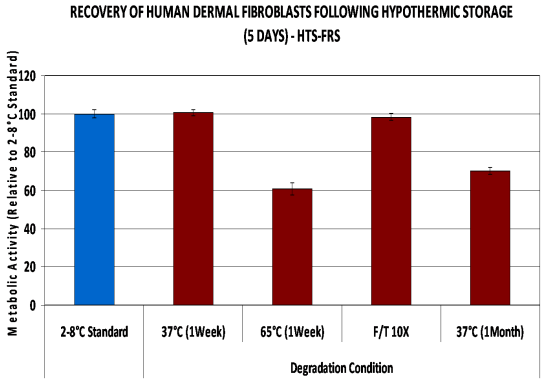

Figure 3: Post-preservation viability of NHDF cells following hypothermic storage with HTS-FRS force degraded solutions. Relative cell viability was determined 1 day post-preservation. Efficacy of degraded solutions was compared to that of cells preserved in the 2-8°C maintained standard solution. Excessively high temperatures for long periods of time reduced HTS-FRS efficacy in NHDF cells.

Analytical Observations

	Color	Sediment	pH
CryoStor CS10	Yellowish following 65°C exposure	White particulate following 65°C exposure	More acidic with excessive heat (65°C) and time
CryoStor CS5	Yellowish following 65°C exposure	White particulate following 65°C exposure	More acidic with excessive heat (65°C) and time
HypoThermosol HTS-FRS	Yellowish following 65°C exposure	No particulate observed with any condition tested	More acidic with excessive heat (65°C) and time

Summary of Results

- ❑ Cryopreservation efficacy of CryoStor biopreservation solutions for NHDF cells was unaffected by the force degradation conditions tested in this study
- ❑ Excessive heat significantly reduced HypoThermosol solution preservation efficacy
  - 65C for 1 week
  - 37C for 1 month
- ❑ Multiple freeze/thaw cycles do not effect CryoStor or HypoThermosol biopreservation
- ❑ Study suggests that some levels of exposure to temperatures not consistent with storage/shipment of CryoStor and HypoThermosol biopreservation products will not negatively impact product efficacy
- ❑ Long-term ICH compliant studies are underway