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FEATURE ARTICLE

"HOME-BREW" BIOPRESERVATION SOLUTIONS: QUALITY IMPLICATIONS AND CONSIDERATIONS

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WEB RESOURCES

www.aabb.org | *American Association of Blood Banks*

www.bestcollaborative.org | *Biomedical Excellence for Safer Transfusion (BEST) Collaborative*

www.celltherapy.org | *International Society for Cellular Therapy*

www.cordbloodforum.org | *Cord Blood Forum*

www.marrow.org | *National Marrow Donor Program*

UPCOMING EVENTS

Phacilitate Cell & Gene Therapy Forum *http://www.phacilitate.co.uk/pages/cgtherapy/index.html*	Washington, D.C.	Jan 25-27, 2010
Biobanking Conference 2010 *http://www.bioportfolio.com/cgi-bin/acatalog/Biobanking-Conference-2010.html*	London, UK	Feb 4-5, 2010
ISBER 2010 Annual Meeting & Exhibits *http://www.isber.org/2010.html*	Rotterdam, Netherlands	May 10-14, 2010
ISCT Annual Meeting *http://www.celltherapysociety.org/Annual_Meeting/*	Philadelphia, PA	May 22-26, 2010
ISSCR 8th Annual Meeting *http://www.isscr.org/*	San Francisco, CA	June 16-19, 2010

EDITOR'S CORNER

Mike Rice, Chairman & CEO, BioLife Solutions, Inc.

Happy new year from Phacilitate's 6th annual Cell & Gene Therapy Forum in Washington, D.C.,

We're pleased to exhibit and present at Phacilitate for the fourth consecutive year. The cell therapy and regenerative medicine markets hold great promise to improve clinical outcomes of numerous disorders and diseases via the delivery of novel cell-based and tissue-based biologic therapies. BioLife Solutions holds a unique position in these growing clinical markets by supplying critical best-in-class, GMP grade biopreservation media products that are used by an expanding customer base to improve the clinical quality and biopreservation economics of cell therapy product development and commercialization.

The feature article of our Winter 2010 issue of BioPreservation Today® was authored by Dominic Clarke, Ph.D., BioLife's Director of Research & Development. Dr. Clarke reports the results of comparative cell preservation efficacy and stability experiments using traditional "home-brew" storage and freeze media as well as commercial, pre-formulated products including our own serum-free/protein-free CryoStor™ and HypoThermosol®.

Also in this issue, Erik Woods, Ph.D., President of General Biotechnology in Indianapolis, reports the results of cryopreservation experiments with mesenchymal-like stem cells derived from human endometrium. These 'Endometrial Regenerative Cells' are the subject of a clinical trial for multiple sclerosis. Dr. Woods evaluated our CryoStor™ CS10 with the novel CellSeal™ Cryogenic Container, a sterile cryovial designed for clinical applications.

For those readers attending Phacilitate this year, please stop by our corporate exhibit in space 17. To learn more about Phacilitate, please visit http://www.phacilitate.co.uk/pages/cgtherapy/index.html.

Thank you for your interest in our products and I hope you find this issue of BioPreservation Today interesting and helpful.

Best regards,

Mike

"HOME-BREW" BIOPRESERVATION SOLUTIONS: QUALITY IMPLICATIONS AND CONSIDERATIONS

by Dominic M. Clarke, Ph.D., Director of Research and Development, BioLife Solutions, Inc.

Biopreservation of cells, tissues, and organs is a frequently applied and required practice used to extend the stability and viability in both short-term and long-term storage of samples for research and clinical applications. Outside of storing cells at normothermic temperatures (37°C growth conditions), most practitioners utilize either hypothermic preservation or cryopreservation methods. Hypothermic preservation is typically performed at temperatures above 0°C and usually between 2-10°C, but certainly below ambient (around 22°C) or normothermic conditions. It is used for short-term transport and storage, during which metabolic activity is greatly reduced but still present at some level. Cryopreservation, or frozen storage, involves long-term storage of biologics at or below -80°C (typically below -140°C) in conditions of metabolic arrest. A wide array of preservation media solutions, biologic packaging products, and methods are available to the user, which can be selected based on the specific requirements of the biologic of interest.

Biopreservation is an important step for transport and storage of biologics including the multitude of products being developed for cellular therapy indications. But preservation is often regarded as a standard process with minimal optimization applied to the procedure or the reagents used. For non-clinical applications, most if not all of the attention is focused on the simplest and least costly method to ensure some level of post-preservation recovery. Unfortunately, a similar thought process is often used for potential clinical cellular applications. Again, a means to reduce the cost and time to develop optimal preservation methods are the focus. By doing so, how will cutting these corners impact the post-preservation recovery, viability, and the anticipated function of the biologic? Furthermore, when considering clinical cell therapy products, what are the additional quality and regulatory components one must consider? What is the true cost? This article focuses on biopreservation media optimization.

Traditional biopreservation media typically fall into two broad groups: home-brew (solutions made up in-house) or commercial, pre-formulated products. Generally, home-brew media solutions, are prepared in-house by combining a number of components of varying quality and under varying levels of cleanliness, while commercially available solutions consist of ready-to-use media solutions that do not require the addition or mixing of components prior to use. Home-brew solutions are often the first choice of practitioners for their preservation needs, since these solutions are assumed to be both effective and more cost effective compared to commercial solutions. Home-brew preservation media can consist of any number of components, but are generally comprised of cell growth (culture) medium or other physiologic buffers, serum and/or protein, and a cryoprotectant such as DMSO if cryopreservation is required.

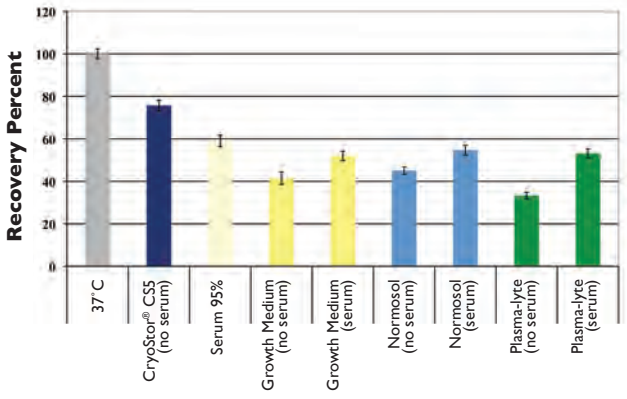
Since cell culture media and other physiologic buffers (extracellular-like) are designed for growing or maintaining biologics rather than preservation, additional components including serum and protein are frequently added

to the culture media to enhance preservation efficacy. As demonstrated in Examples 1A and 1B below, normal human dermal fibroblasts (NHDF) and human mesenchymal stem cells (hMSC) were cryopreserved in a variety of preservation media and assessed for post-thaw viability following one day of recovery at 37°C. A selection of home-brew cryopreservation solutions were prepared with and without the addition of serum, and compared to the commercially available GMP, completely defined, serum-free and protein-free CryoStor™ freeze media. The results indicate that the inclusion of serum in each of the home-brew solutions improved post-thaw viability, compared to the respective solutions without serum; but none of these solutions were found to be as effective as CryoStor, which has ionic concentrations that effectively balance the intra-cellular environment at hypothermic and cryopreservation temperatures. Finally, as compared to the other serum-free solutions, CryoStor enabled considerable improvement in viability of both the NHDF and hMSC, which is an indication of the preservation efficacy provided by the other components in this proprietary formulation.

Hypothermic transport and storage of biologics, like cryopreservation, incorporates an array of home-brew and commercial preservation solutions. Biologics are harvested and frequently transported or stored at refrigerated temperatures in culture media (with/without serum) or other commercially available isotonic and electrolyte solutions (i.e. saline, Normosol®-R, Plasma-Lyte®, Celsior®). Again, these solutions are ideal for maintenance of cells at normothermic temperature in cell culture conditions that mimic native environments. However, they do not balance cells when subjected to low temperature preservation, as opposed to commercially available intracellular-like solutions (i.e. HypoThermosol®-FRS, Viaspan). One day of storage for overnight shipping is often considered to be sufficient, but what happens if extended stability options are required for instances when shipments get delayed or tissues for procurement arrive outside of normal hours?

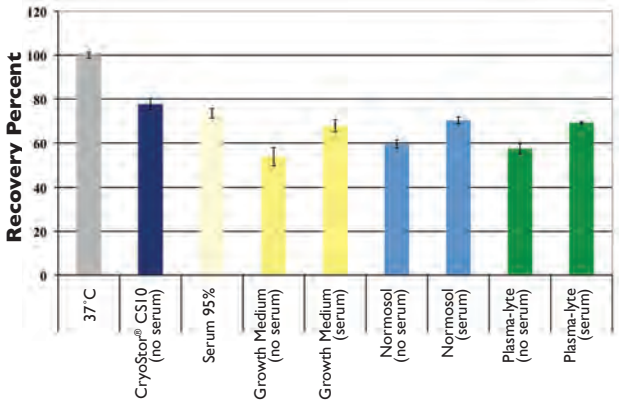
A critical issue affecting commercialization of new cell therapy and regenerative medicine products is minimal storage and transport stability that limits geographic clinical distribution and may result in costly redundant manufacturing facilities to support worldwide product availability. In Examples 2A and 2B, NHDF and hMSC cultures were exposed to hypothermic conditions for one to five days in a selection of home-brew and commercially available preservation solutions. Fluorescent micrographs were obtained following one day of recovery at 37°C to assess cell recovery and preservation solution efficacy. At first glance, each of the solutions tested provide complete protection to NHDF cells during one day of hypothermic storage (Example 2A). However, when the storage time was extended to three

Recovery of Normal Human Dermal Fibroblasts Following Cryopreservation Compared to 37˚C Control (1 Day Recovery)
5% DMSO In all Conditions



Example 1A
Cryopreservation of Normal Human Dermal Fibroblasts (NHDF)

Recovery of Human Mesenchymal Stem Cells (hMSC) Following Cryopreservation Compared to 37˚C Control (1 Day Recovery)
10% DMSO In all Conditions



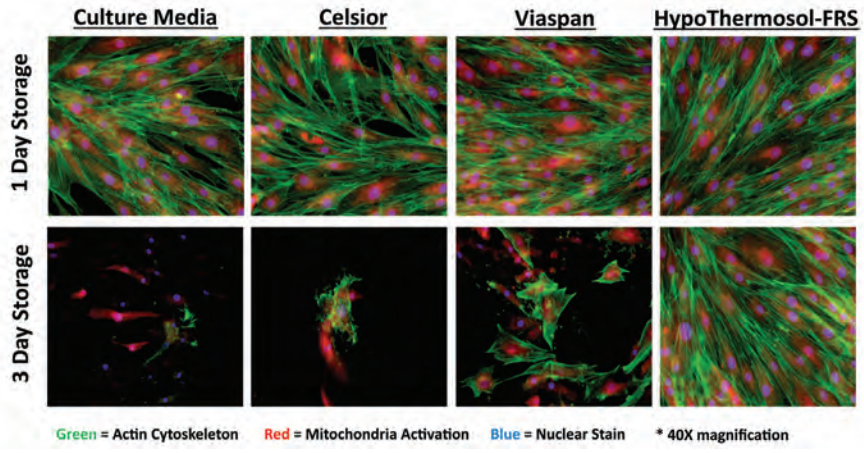
Example 1B
Cryopreservation of Human Mesenchymal Stem Cells (hMSC)

days, little to no protection was afforded to NHDF cells stored in culture media (with serum), Celsior, or Viaspan; while a complete recovery of cells was observed in cells stored in HypoThermosol-FRS. In comparison, no viable hMSC cells were recovered following 1 day storage in culture media or Celsior. Viaspan did provide a minimal improvement in recovery, while HypoThermosol-FRS enabled a complete recovery and a significant extension of stability, compared to each of the other tested solutions (Example 2B).

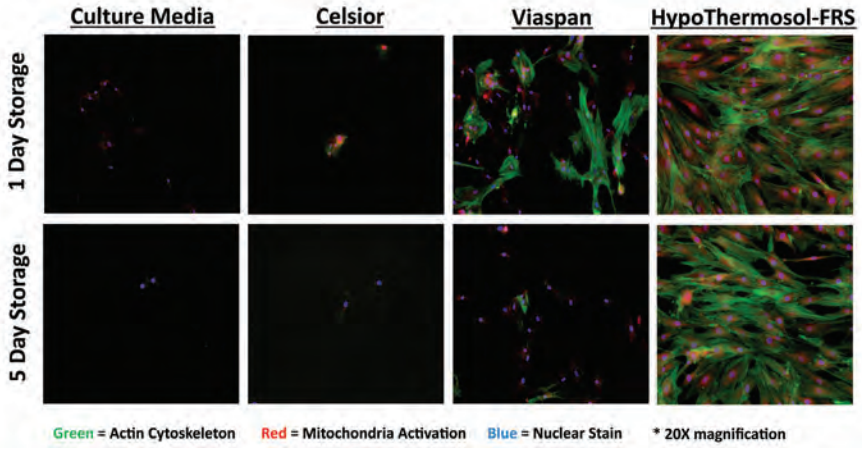
Viability, function, overall recovery, and the stability footprint of the preserved biologic are of critical importance, but the following aspects should also be considered when preservation solutions are evaluated and considered: component quality (USP, serum, proteins, WFI quality water), manufacturing environment (GMP, sterile filtered), release testing (sterility, endotoxin, particulates). The estimated cost of home-brew solutions is usually based only on the cost of components used, and typically excludes labor costs.

Home-brew solutions appear to offer a quick, simple, and relatively inexpensive alternative for short-term and long-term storage of cells and tissues. Unfortunately, home-brew preservation media formulations may expose the practitioner to several compromises and areas of risk related to lower than expected cell viability and recovery, limited stability, and quality concerns. Compared to home-brew solutions, many commercially available solutions offer improvements in specific quality aspects (i.e. sterility, grade of components), but extended stability options are limited and post-preservation viability and recovery remain sub-optimal.

While a number of biopreservation solution options are available, it's clear that not all preservation solutions are created equally. HypoThermosol and CryoStor provide broad improvement in preservation efficacy and stability of biologics, while offering a very robust quality and regulatory footprint.



Example 2A



Example 2B

CRYOPRESERVATION STORAGE STUDY USING THE CELLSEAL™ CRYOGENIC CONTAINER SYSTEM AND SERUM-FREE CRYOSTOR™ PRESERVATION SOLUTION

by Erik J. Woods, Ph.D., President & CEO General BioTechnology LLC

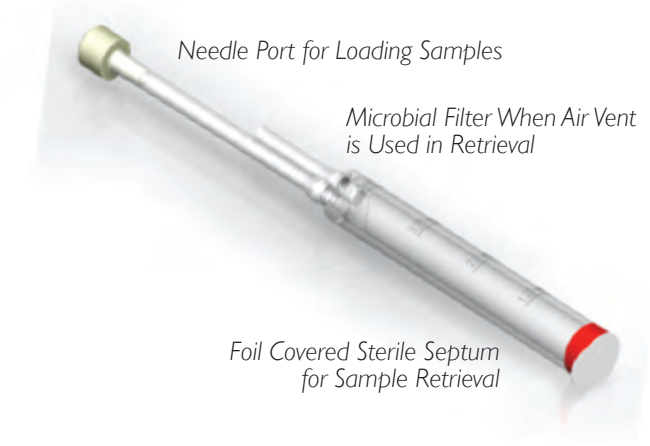
A major constituent of the traditional home-brew freeze media used for the cryopreservation of cells is serum derived from animals. It is common practice to include serum in solutions used for frozen stock as a source of nutrients and other perceived benefits during post-thaw cell culture. However, current good manufacturing practices (cGMP) recommend the elimination of animal serum in freezing solutions to eliminate an undesired source of xenogenic antigens and the risk of transmitting animal viral and prion contaminants. The proposed criteria for cGMP also recommend the cryopreservation storage of cell therapy products in an aseptic, closed system to prevent any possible contamination or infection during freezing, storing, thawing and shipping.

Here we demonstrate the benefits of using the novel CellSeal™ Cryogenic Container (CSCC) system (General Biotechology, Indianapolis, IN) in combination with preformulated sterile, serum-free, protein-free GMP-grade CryoStor™ CS10 freeze media (BioLife Solutions, Bothell, WA) to improve the post-thaw viability of mesenchymal-like stem cells derived from human endometrium (Endometrial Regenerative Cells, ERCs). The results were compared with that of traditional home-brew cryopreservation media containing 10% DMSO in fetal bovine serum (FBS) and cryopreserved using Corning™ cryovials.

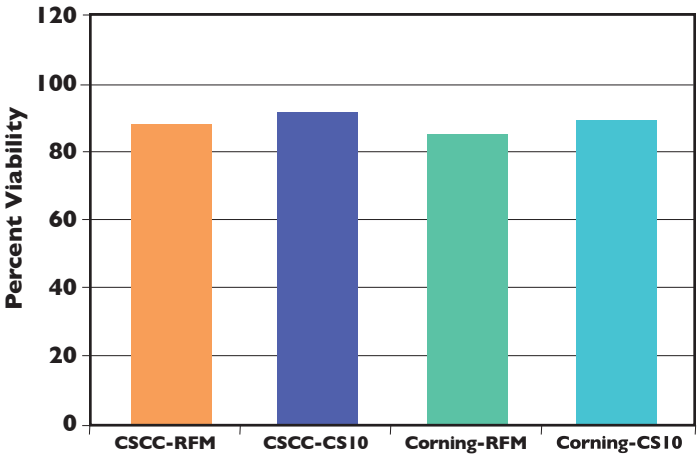
The CellSeal cryovial has been developed and evaluated as a closed, sterile cell cryopreservation device for the robust prevention of microorganism ingress, and secure control over sterility during routine cryopreservation and storage of clinically relevant cellular products. The design of the CSCC has three ports. One port is a foil covered fixed needle septum at the distal end of the vial body for fluid extraction. The second port is barbed to allow the attachment of a flexible tube with another needle septum at the end to be used for filling the CSCC. This is sealed using an RF welder after introduction of the specimen. The third port is fitted with a microbial barrier followed by flexible tubing for venting. This third port has tubing that is sealed using an RF weld pre-freeze, then cut open post-thaw to aide in fluid extraction.

The results of this study demonstrated that the combination of the CSCC and CryoStor™ CS10 forms an effective cryopreservation storage system for ERCs intended for clinical applications.

CONCEPT AND VARIOUS COMPONENTS OF CELLSEAL™ CRYOGENIC CONTAINER (CSCC)



Immediate Post-Thaw Viability of ERCs in CSCC and Control Vials (Corning™) and CryoStor™ (CS-10)



All samples were exposed to slow cooling rate (~1°C/min) using dump freezing at -85°C and stored in LN2 for more than four weeks.

BIOLIFE SOLUTIONS IS ISO 13485 CERTIFIED!

BioLife Solutions recently announced that it has successfully completed audits of its quality systems and GMP production facility in Bothell, Washington by BSI Group and has been issued a certification to ISO 13485:2003, an international standard for quality systems supporting the design, development, and manufacture of medical devices.

Mike Rice, BioLife's chairman and CEO, noted, "This achievement is a strategic quality milestone for BioLife. Our growing clinical customer base expects us to set a high bar for the production of our GMP biopreservation media products, since many customer applications include the use of our products as a combined preservative/injection delivery solution for cell-based therapies used to treat cancer, heart failure, and a host of other diseases. We're continuing to enhance the quality footprint of our proprietary, best-in-class HypoThermosol® and CryoStor™ biopreservation media products with this certification and updates to our FDA Master Files. Furthermore, we expect to achieve CE Mark conformity for our products in 2010."

In addition to certification to ISO 13485, BioLife's manufacturing facility and quality systems adhere to 21 CFR Part 820 - Quality System Regulation for Good Manufacturing Practices (GMP) of medical devices, 21 CFR Parts 210 and 211 covering GMP for Aseptic Production, Volume 4, EU Guidelines, Annex 1 for the Manufacture of Sterile Medicinal Products, ISO 13408 for aseptic processing of healthcare products, and ISO 14644 for Clean Rooms and Associated Controlled Environments.

BIOLIFE ANNOUNCES CUSTOM cGMP MANUFACTURING AND LICENSE AGREEMENT WITH CENTOCOR

BioLife Solutions also announced that it has executed a license and custom cGMP manufacturing agreement with Centocor Research & Development, Inc. The agreement includes the production of a custom variant of BioLife's proprietary serum-free and protein-free CryoStor biopreservation media product, which is formulated with a reduced concentration of 2% DMSO.

Mike Rice, BioLife's chairman and CEO, noted, "We are pleased with this request for a custom variant of CryoStor DLite, which will be manufactured in our Bothell cGMP production facility, which offers robust quality systems, manufacturing capacity, and flexibility in providing customer-specific biopreservation media products critical to the successful commercialization of new life-saving cellular therapy products."

SUGGESTED READING

1. Alginate Encapsulation as a Novel Strategy for the Cryopreservation of Neurospheres

Rita Malpique, Luísa M Osório, Daniela S Ferreira, Friederike Ehrhart, Catarina Brito, Heiko Zimmermann, Paula M Alves. Tissue Engineering Part C: Methods. -Not available-, ahead of print. doi:10.1089/ten.TEC.2009.0660.

2. Improved Post-Thaw Recovery of Peripheral Blood Stem/Progenitor Cells Using a Novel Intracellular-Like Cryopreservation Solution.

Clarke DM, Yadock DJ, Nicoud IB, Mathew AJ, Heimfeld S. Cytotherapy. 2009;11(4):472-9.

3. Cryopreservation of Adherent Cells: Strategies to Improve Cell Viability and Function after Thawing.

Malpique R, Ehrhart F, Katsen-Globa A, Zimmermann H, Alves PM. Tissue Eng Part C Methods. 2009 Sep;15(3):373-86.

4. Hypothermic Storage of Isolated Human Hepatocytes: A Comparison Between University of Wisconsin Solution and a Hypothermosol Platform.

Ostrowska A, Gu K, Bode DC, Van Buskirk RG. Arch Toxicol. 2009 May;83(5):493-502. Epub 2009 Mar 19.

BioLife Solutions develops and markets patented hypothermic storage/transport and cryopreservation media products for cells, tissues, and organs. BioLife's proprietary HypoThermosol®, CryoStor™ and BloodStor™ platform of biopreservation media products are marketed to academic research institutions, hospitals, and commercial companies involved in cell therapy, tissue engineering, cord blood banking, drug discovery, and toxicology testing. BioLife products are serum-free and protein-free, fully defined, and formulated to reduce preservation-induced, delayed-onset cell damage and death. BioLife's enabling technology provides research and clinical organizations significant improvement in post-preservation cell and tissue and viability and function.

BioLife Solutions
POWERING THE PRESERVATION SCIENCES