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FEATURE ARTICLE

BIOPRESERVATION ECONOMICS IN REGENERATIVE MEDICINE

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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

Int'l Cord Blood Symposium *http://www.cordbloodsymposium.org/index.php*	San Francisco, CA	June 3-5, 2010
ISSCR 8th Annual Meeting *http://www.isscr.org/*	San Francisco, CA	June 16-19, 2010
World Stem Cell Summit *http://www.worldstemcellsummit.com/index.html*	Detroit, MI	Oct 4-6, 2010
AABB Annual Meeting & CTTXPO 2010 *http://www.aabb.org/events/annualmeeting/Pages/default.aspx*	Baltimore, MD	Oct 9-12, 2010

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EDITOR'S CORNER

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

Readers of our Spring 2010 issue of BioPreservation Today®,

Greetings from Philadelphia, and the 16th Annual Meeting of the International Society for Cellular Therapy. We're pleased to again support the ISCT and to exhibit and present at the annual meeting for the 5th consecutive year. Over the last twelve months, there has been significant progress in the field of regenerative medicine, with the commencement of numerous additional clinical trials, increased investment by independent and corporate venture partners, and the approval of Dendreon's Provenge® prostate cancer vaccine.

Furthering our goal to raise sensitivity about the critical role biopreservation systems play in the development and commercialization of new regenerative medicine products, in this issue, I authored our feature article on biopreservation economics. I discuss topics such as best practice-based biopreservation system objectives, yield-cost trade-offs related to end-product release criteria, and stability extension opportunities enabled by optimized biopreservation media products. These topics will also be presented in a corporate tutorial during the ISCT annual meeting.

Also in this issue, we provide corporate profiles of American Fluoroseal and OriGen Biomedical, two well-known biologic packaging suppliers who have been active for many years in the regenerative medicine market. We also included an overview of an animal safety study completed on our clinical grade biopreservation media products at the Fred Hutchinson Cancer Research Center, as well as the republishing of an earlier BPT article addressing considerations for qualifying ancillary and excipient reagents used in the manufacturing, shipment, storage, and clinical delivery of regenerative medicine products and therapies.

Thank you for your interest and support of our products and services. I hope you find this issue of BioPreservation Today informative and a resource to optimize your biopreservation systems and results.

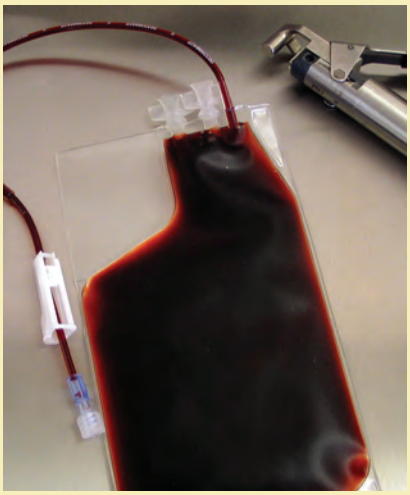
Best regards,

Mike

ORIGEN BIOMEDICAL, INC.

OriGen Biomedical is currently the world's leading supplier of bags for cryopreservation and cell culture, with more than 500,000 bags in cryopreservation. Founded in 1990, OriGen supplies medical devices in two distinct business units: cell culture and cryopreservation and cardiopulmonary products. All OriGen products have worldwide regulatory approvals and distribution.
Our cell culture and cryopreservation products include:

PermaLife: *FEP (Teflon) bags; Unmatched biocompatibility and gas transfer*
PermaLife cell culture bags are used for very sensitive stem cell expansion and immunotherapy treatments. They exhibit superior biocompatibility, high gas transfer and low water loss, making them ideally suited for delicate cell cultures. And, as a closed system, the risk of contamination and loss of sterility is greatly reduced. Origen has been selected as a supplier to NASA for Cell Culture Bags used in microgravity.



Cryostore EVA bags: *Unsurpassed, affordable cell freezing performance*
Cryostore cryopreservation products are drop-in replacements for many existing freezing bags, but with distinct improvements over those products. Hermetically-sealed spike ports and a rugged, more durable base polymer make the bags less susceptible to damage while retaining verifiable sterility.

Overwraps: *Easy to use, reliable overwraps for cryogenic applications*
Th Origen O-Wrap is easy to seal, and offers outstanding performance in Liquid Nitrogen environments.

Cryoprotectants: *DMSO compounds for freezing*
Origen offers a range of DMSO-based cryoprotectants and custom formulations, in vials, ampules and syringes.

Evolve Cell culture bags: *Inexpensive, large-scale breathable bioreactors*
The Evolve bag is cell culture bag with amazing performance for adherent cell cultures with an equally amazing price.

Our Cardiopulmonary products include highly specialized ECMO catheters in pediatric through adult sizes, and sterile perfluorocarbon lung lavage solutions (approval pending in the US).

All of our products have received premarketing clearance from FDA, are CE marked and generally approved for worldwide use. Since 2003, the company has maintained EU and Canadian certification to ISO 13485 (Medical Devices) and a Certification of Compliance with the European 92/43 Medical Device Directive. Copies of the certificates are available on our website.

OriGen currently has twenty-five employees. Richard Martin is the President and CEO, and Bo Johnson is the Vice President of Quality and Regulatory affairs. For more information about the company and our products, please contact us directly.

OriGen
BIOMEDICAL

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ANCILLARY & EXCIPIENT REAGENTS

by Mark Sandifer, Director of Quality, BioLife Solutions, Inc.

As the complexity of cell and tissue therapy products has increased, so have the regulatory issues surrounding the ancillary materials, excipients, and other reagents used in the manufacture of these products. It is the expectation of the US Food and Drug Administration (FDA), the European Agency for the Evaluation of Medicinal Products (EMEA), and regulatory agencies in other countries that reagents are produced using Good Manufacturing Practice (GMP) standards. For cell and tissue-based products, the level of compliance with GMP standards is expected to increase as the company progresses from pre-clinical to Phase III clinical trials. Because each product is unique, regulatory bodies do not directly control the manufacture of reagents used in the process, but rather the final cell and tissue therapy products that use the reagents. Therefore, it is the responsibility of the manufacturer of human cells, tissues, and cellular and tissue-based products (HCT/Ps) and other regulated drug, device, and biologic products to evaluate the safety of their reagents within their overall development and manufacturing systems for the final product.

Regulatory authorities detail the level of safety expected for reagents, but provide little guidance on how to comply. To fill this gap, the US Pharmacopeia has published a guide on how to qualify reagents. This advisory guide is entitled, "Ancillary Materials for Cell, Gene, and Tissue-Engineered Products." The guide describes a robust qualification program that addresses the issues of identification, selection and suitability for use, characterization, vendor qualification, as well as quality assurance and control. The guidance recommends that reagents manufactured according to GMP principles should be used whenever possible and that the risk associated with each reagent be carefully considered.

As many manufacturers do not have sufficient resources to produce their own GMP-compliant reagents, they must depend upon vendors to supply reagents of sufficient quality and quantity. In order to qualify a vendor, a paper-based audit is normally adequate during the early stages of development. As the company moves into Phase II/III trials, an on-site audit is recommended. The decision as to the scope of the audit is determined by a risk analysis. Reagents that remain in the cell or tissue therapy product (excipients) represent the highest risk, while ancillary reagents that are solely used for processing are a lower risk.

BioLife Solutions' products are uniquely positioned to minimize risk and decrease regulatory burden. All products are manufactured under strict GMP standards, and independent studies have demonstrated the biocompatibility and safety of BioLife products. FDA Type II Master Files allow the manufacturer of cell and tissue therapy products to easily cross-reference the quality attributes of BioLife Solutions' products in regulatory applications. To request a cross-reference, please visit biolifesolutions.com/regulatory/mfrequest.htm.

Table 1
KEY ASPECTS OF A SUCCESSFUL QUALIFICATION PROGRAM

Identification
List reagents, their source, and how used in the manufacturing process.

Selection and Suitability for Use
Establish criteria for purity, identity, and biologic activity.

Characterization Conduct
Quality control tests to assess key quality attributes, including sterility, identity, purity and functionality.

Vendor Qualification
Conduct vendor audits (paper-based for early stage development; on-site audits for Phase II/III clinical trials).

Quality Assurance and Control
Oversee qualification program and entire quality system.

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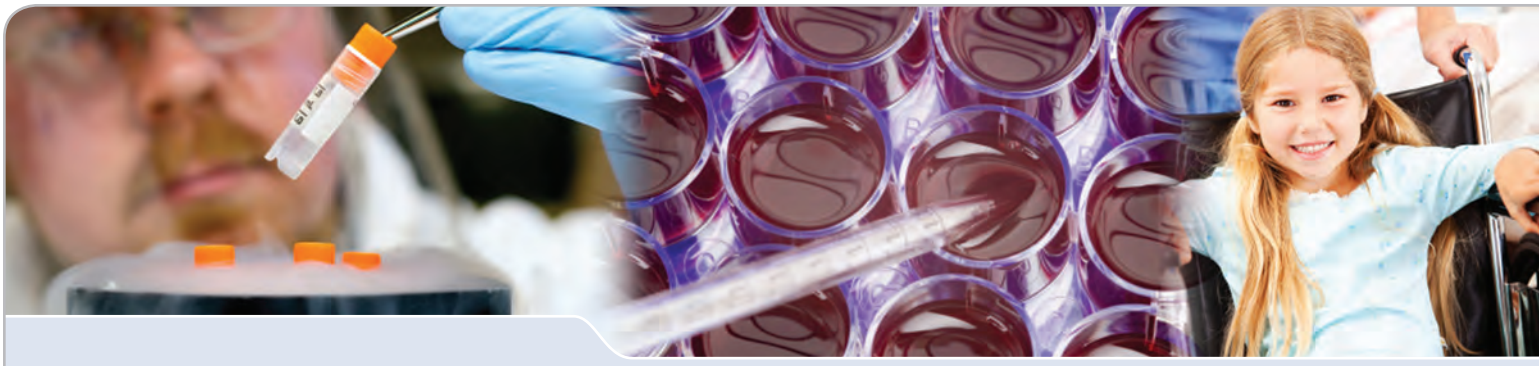

BIOPRESERVATION ECONOMICS IN REGENERATIVE MEDICINE

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

Biopreservation (2-8°C hypothermic storage and shipment, freezing) of biologic source material, intermediate products, and final isolated, manipulated, cultured, packaged cells or tissues as a clinical regenerative medicine therapy is often a non-optimized set of processes that can have a significant impact on process and product development and commercial success. Once removed from a supportive normothermic environment, biologics begin to degrade and unless returned to a nutrient rich host system at culture temperatures or a reduced-metabolism hypothermic environment, cell damage and death occurs over varying time periods. Shipping and maintaining biologics at normothermic temperatures is cost prohibitive, fraught with logistical challenges, and not practical due to a number of factors. Hence, biopreservation at hypothermic and frozen temperatures has emerged as the desired mode of stability maintenance throughout product development and commercialization workflow.

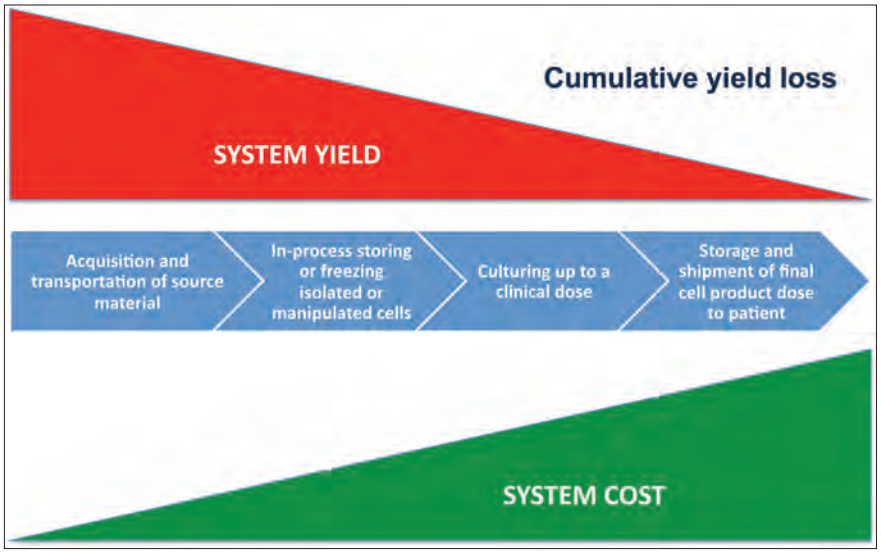

Figure 1 *Yield and cost impacts in a non-optimized system*

However, biopreservation workflow tuning (or lack of) affects yield, cost, quality, and clinical efficacy in a cumulative manner. Many considerations must be addressed early in product development, including process validation and GMP scalability, component selection, quality, and supply continuity, labor and material costs, and release criteria including pre-infusion cell viability, stability, and potency.

Best practices for optimizing a biopreservation system should include:

1. Optimizing commercial scale manufacturing and the broadest geographic distribution by maximizing: (a) source material stability (transport time and recoverable yield of cells of interest), (b) manufacturing time for flexibility in receiving source material, cell isolation, manipulation, culturing, and packaging, and (c) final dose stability (longest transport time that provides the highest cell viability, functional recovery, and engraftment).

2. Minimizing system costs of direct labor, internal facility overhead, contract lab time, components, materials, and supplies.

3. Minimizing system risks in process variability, component quality, supply continuity, and bioburden exposure through the workflow process.

Many alternatives should be considered in determining the optimal components and conditions in a biopreservation system for source material and the final clinical dose including biologic packaging (syringe, vial, bottle, bag), intermediate and exterior packaging, transport temperature (ambient, 2-8°C, frozen), biopreservation storage/transport media (home-brew, commercial, isotonic, extracellular, intracellular-like), and freeze/cryo media (home-brew, commercial, serum/serum-free, protein/protein-free, DMSO concentration).

Optimally, the system should enable maintenance of stability and maximum isolation yield of source material after a two to three day shipment interval, and preservation efficacy that meets the release criteria and stability of the final dose over at least a two to three day end-process shipment interval to the clinic. Clinical dose volume and cell concentration also affect the stability profile and may require splitting the clinical dose into more than one final package.

The premise of this article is that biopreservation media selection is critical and offers the greatest negative or positive impact on overall system results.

The following four hypothetical graphs illustrate the potential critical impact of biopreservation system design decisions.

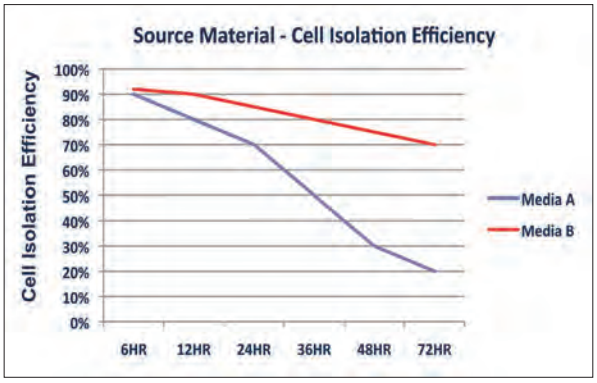

Figure 2 *Source material storage/shipping media impact on viable cell isolation yield efficiency*

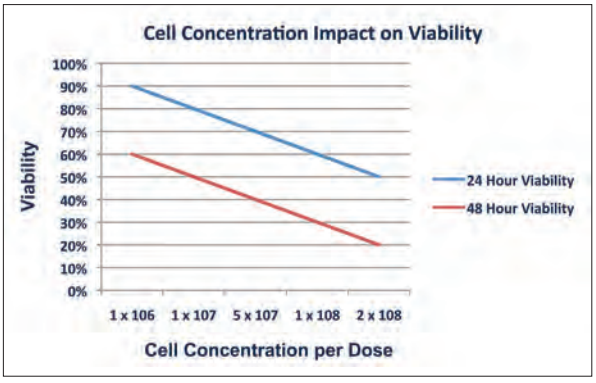

Figure 3 *Viability impact of clinical dose cell concentration*

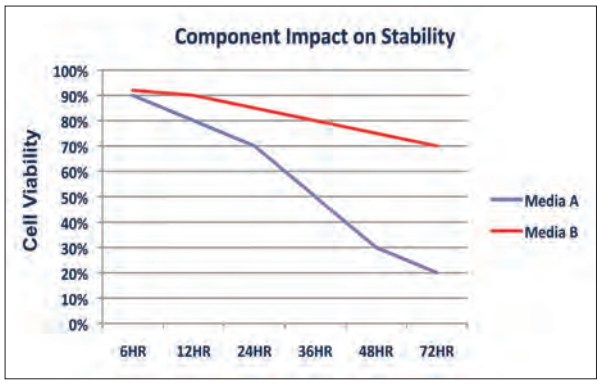

Figure 4 *Shipping media impact on clinical dose stability*

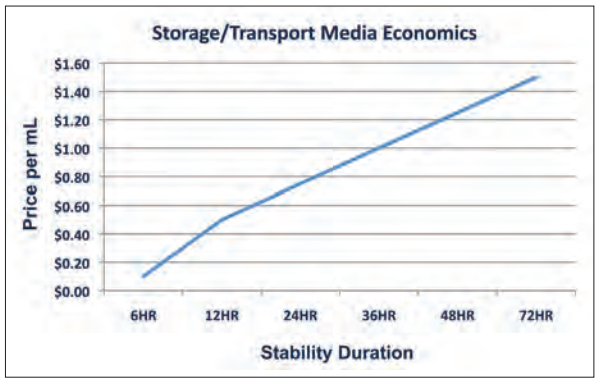

Figure 5 *Price/performance trade-off of shipping media*

The potential negative, cumulative cost and yield impacts of a non-optimized system design could result in stability-constrained geographic clinical distribution that requires very expensive redundant production facilities.

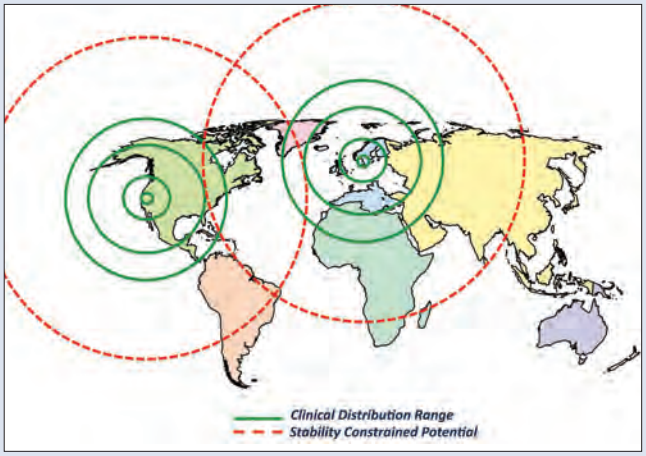

Figure 6 *Clinical distribution constrained by limited stability*

Fortunately, a growing body of evidence supports significantly improved preservation efficacy (viability/other assay results at or above clinical dose minimum release criteria, for greater than traditional stability profiles of one to two days), enabled by a class of optimized, intracellular-like, cGMP grade biopreservation media products. Compared to "home-brew" or in-house formulated preservation cocktails, which are often performance limited due to non-optimized isotonic or extracellular ionic concentrations, and quality compromised to various degrees due to component quality, process variability, limited release criteria, and inclusion of serum and or protein, HypoThermosol® storage/shipping media and CryoStor™ freeze/cryo media have established a superior preservation efficacy and quality profile.

The foundational science and intellectual property of HypoThermosol and its complementary CryoStor freeze/cryo media product are centered on specific discoveries related to preservation-induced molecular cell damage and death. These discoveries enabled the reformulation of traditional organ preservation cocktails for use in cell and tissue models by reducing the impact of cell stress pathways initiated by exposure to hypothermic environments. Formulation optimization included tuning ionic concentrations to balance the intracellular state at low temperatures, and the addition of energy substrates and components that provide free radical scavenging, inhibition of apoptosis, osmotic stabilization, and pH buffering, in the absence of serum and protein.

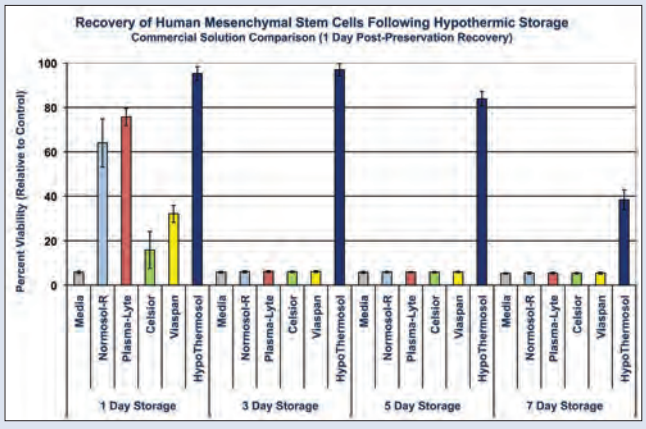

Figure 7 *1-7 day hypothermic storage of hMSC*

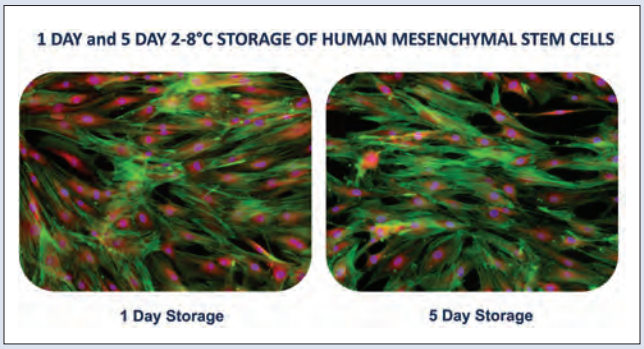

Figure 8 *hMSC cytoskeleton, mitochondria, and nuclear stains*

Performance & Quality Attributes	Commercial Product	In-House Formulation (Home-Brew)	Risk Assessment
Pre-Formulated			
Serum-Free			
Protein-Free			
Component Quality (USP, R & D), water quality (WFI)			
cGMP Manufactured			
FDA Master File			
Release Criteria (sterility, endo, pH, appearance, etc.)			
Preservation Efficacy (stability, viability, etc.)			

Figure 10 *Biopreservation media selection criteria*

Fluorescent micrographs further illustrate the maintenance of stability over an extended period of hypothermic storage enabled by HypoThermosol.

Perhaps surprising to some readers, traditional serum-containing freeze/cryo media cocktails do not provide improved post-thaw viability compared to optimized

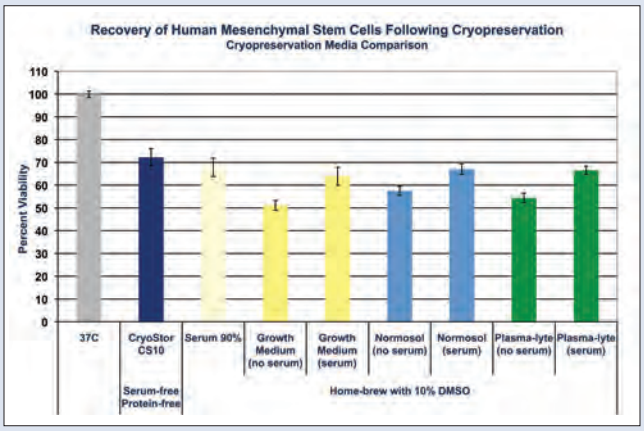

Figure 9 *Comparison of post-thaw viability of hMSC*

serum-free alternatives.

Organizations engaged in research and potential commercialization of new regenerative medicine therapies are encouraged to critically evaluate biopreservation media alternatives early in the product development process. While low costs and a perception of acceptable efficacy, quality, and risk of traditional non-optimized commercial and home-brew cocktails tend to make these formulations the default choice, it is clear that overall system performance can be highly compromised from this weak link in the preservation chain.

An additional, often unrecognized or under appreciated preservation-related phenomena can lead to a potential system design pitfall; preservation-induced, delayed-onset cell death, which manifests itself over several hours post-thaw, and coupled with traditionally performed immediate post-thaw viability and other assays, may promote incorrect clinical dose calculations and measurements, since the full extent of preservation related cell injury and death is not apparent immediately post-thaw.

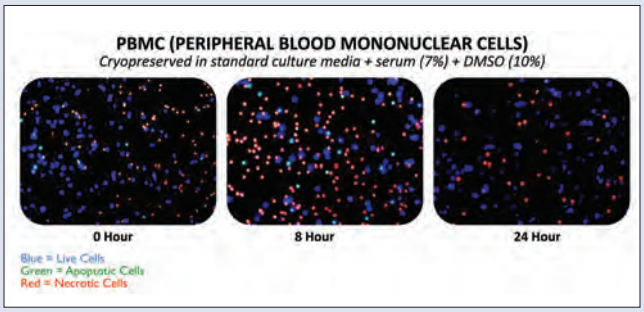

Figure 11 *Delayed onset, preservation induced cell death*

In summary, the often overlooked and non-optimized biopreservation system, as part of overall clinical product development and commercialization, has shown to play a critical role in enabling the execution of a best practice-based approach. Biopreservation media product selection has tremendous potential to impact overall system cost, yield, stability, distribution, and clinical efficacy. For more information about the topics discussed in this article, the products mentioned, or a no cost consultation to optimize your biopreservation system, please visit www.biolifesolutions.com.

AMERICAN FLUOROSEAL CORP.

Afc invented and patented a proprietary laser sealing, contact-free, manufacturing process for joining fluorocarbon plastic films together to make unique blood component containers over 25 years ago. The process was specifically engineered and ingeniously designed through use of a computer controlled laser sealing technique. This technique made it possible to develop a new, inert, and very durable type of container. This technology resulted in the further advancement of a unique bag system to permit high quality closed system cell culture and transfer of blood components. Afc is proud to be taking part in today's fourth generation of cancer therapy called immunotherapy. Ultimately, new and promising therapeutic techniques could be advanced further by these specialized containers for stem cell and immune cell cancer therapy.

Products

Afc's primary products are made from fluorinated ethylene propylene (FEP). The products are principally comprised of various bags for cell culture and cryopreservation. The product lines are comprised of three trademarks: 1.) the VueLife™ line of cell culture containers, 2.) the KryoSure™ line of cryopreservation bags, and 3.) the KryoVue™ line of cryopreservation pouches and over wraps. Additional products include tubing sets and accessories such as bar sealers, separation clamps, and centrifuge cassettes.

Technical Features

Because FEP is inert, transparent, tough, and flexible at extreme temperatures (+200C° and -200C°) Afc products are used extensively in aerospace, biotechnology, chemical manufacturing, forensics, medical products, cryopreservation, tissue and organ banking, and other applications. The best material found for cryopreservation is FEP. It stays flexible in liquid nitrogen, therefore eliminating the fear of breakage like other materials that become brittle, crack, and break when exposed to stress below their glass transition temperature. No other Class IV container material is known to remain flexible in liquid nitrogen making it well suited for cryo-storage of blood components.

The bags are permeable to oxygen and carbon dioxide but impermeable to water; they remain flexible in liquid nitrogen, and have no known solvents. They contain no plasticizers, leachable, or extractable materials, and they do not stimulate any cellular or biologic activity. FEP transmits ultraviolet and infrared light making it ideal in light activation protocols. FEP does not dissolve or soften in DMSO, DMF, or any other solvent. Cells or other biologics do not bind to the bags. Covered ports alleviate the need for cumbersome over wraps. With proper support the bags may be used for centrifugation.

AFC bags are not wettable. Therefore stem cells and all other cells can grow without experiencing the unnatural effects of contact with a foreign surface. Naïve cells do not differentiate. Platelets do not stick. Surface receptors are not activated by Afc bags. However it is possible for the material to be made adherent, so for some cell therapy applications this advantage is possible. This is simply called an adherent cell bag, AC bag for short.

Specialty use

Many specialties incorporate the use of Afc bags. They include those involved in medical trials, clinical applications, NASA and the aerospace industry, and the chemical processing industry. Pathology clients use DNA-free Afc bags for forensic research. Afc overwraps are used for holding and protecting cord blood and tissues while in liquid nitrogen.

Afc offers both standard and custom shapes and sizes of bags and tubing sets for different types of research, shipping, and storage. Afc FEP bags are offered in sterile, non-sterile, adherent, and non-adherent versions. The products are sold through referrals and internet exposure.

Credentials

Afc maintains NASA manufacturing certifications, FDA registration, Device Master Files, and 510(k) approvals. Afc manufactures under 21CFR regulations for GMP.

American Fluoroseal Corporation (Afc) was incorporated in 1986 and Mr. Herb Cullis has been its President since 1997. Its administrative offices and manufacturing facilities are located at:

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SAFETY STUDY OF INTRAVENOUS INJECTION OF BIOPRESERVATION SOLUTIONS

by Mark Sandifer, Director of Quality, BioLife Solutions, Inc.

The FDA or other agencies do not regulate biopreservation media products as drugs, devices, biologics, or combination products. These products are considered either ancillary material when used in the manufacturing of cell, gene, or tissue-engineered products, but are not intended to be in final product (washed or removed), or as an excipient component intended to be part of the final product and in contact with the patient. Good Tissue Practices (GTP – 1271.210) require that highest quality reagents must be utilized in the process of developing Human Cellular, Tissue, and Tissue-Based Products (HCT/Ps) and that "all reagents must be verified to meet specifications designed to prevent circumstances that increase the risk of the introduction, transmission, or spread of the introduction, transmission, or spread of communicable diseases".

BioLife Solutions' HypoThermosol® and CryoStor™ products have been cited in numerous IND's where minimal manipulation techniques and the quality and safety of the cellular product vehicle/carrier solution are of the utmost importance, especially in situations where infusion occurs with no wash step to remove the vehicle. The highest safety concerns surround intravenous injection. Because of this, BioLife conducted a murine pre-clinical safety study in collaboration with the Fred Hutchinson Cancer Research Center (FHCRC).

The goal of the study was to collect quantitative data to better characterize the safety profile of the products.

week old male C57Bl/6 mice in groups of 5.

Stage I was intended to assess the effects of volume and solution composition; 250µL and 500µL of HTS, CryoStor or controls were injected. Controls consisted of PBS or FHCRC standard freezing medium. Stage II assessed the safety of injecting 20 million fresh human umbilical cord blood (UCB) cells suspended in the test solutions. In stage III, UCB cells were cryopreserved in the test solutions, thawed, and injected with no wash.

To assess potential toxicities, complete blood counts were performed on samples collected 1 day prior to injection (baseline), and

TABLE 1
SUMMARY OF EXPERIMENTAL GROUPS

	Solution	Injection Volume (µL)	Cells	N
Stage I	CSB	250	NA	5
	CS5			5
	CS10			5
	PBS	500		5
	CSB			5
	CS5			5
	CS10			5
	HTS-FRS			5
Stage II	PBS	500	20×10^6 (Fresh)	5
	FHCRC			5
	CSB			5
	CS5			5
	CS10			5
	HTS			5
Stage III	FHCRC	500	20×10^6 (Freeze-Thaw)	5
	CSB			5
	CS5			5
	CS10			5
	HTS			5

Study Stages and Results

The study was divided into three stages to evaluate the safety of intravenous injection of HypoThermosol®-FRS (HTS-FRS) and CryoStor™ (CS), containing 0, 5, or 10% DMSO as CSB, CS5, and CS10, respectively, into 6-8

30 minutes, 24 hours, and 7 days after injection during necropsy. Organ specimens were collected and processed for histopathology with no abnormalities observed.

In Stage I, injection of solutions alone resulted in blood counts

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Continued from **SAFETY STUDY OF INTRAVENOUS INJECTION OF BIOPRESERVATION SOLUTIONS**, *page 11.*

that were similar among groups within each time point. In all groups, an immediate spike in neutrophil counts was observed at 30 minutes following injection; however, these normalized by 24 hours. Platelets, neutrophils, and reticulocytes were slightly elevated after 7 days. One mortality was observed in the 500µl CS5 group at the time of infusion and was deemed unrelated to the solution composition.

In Stage II, the addition of fresh UBC produced cell counts similar to vehicle solutions alone. There were no mortalities and all animals gained weight during 7-day follow-up. Again, in all groups an immediate spike in neutrophil counts was observed at 30 minutes following injection that normalized by 24 hours. Platelets, neutrophils, and reticulocytes were slightly elevated after 7 days.

Stage III evaluated the safety of no wash infusion of frozen and thawed UBC. 20×10^6 cells were frozen in 500µl, or in the case of HypoThermosol (HTS), the same density of cells were stored overnight at 4°C. All solutions were infused cold (4°C). Again, blood counts were similar between groups for each post-infusion time, there were no mortalities, and all animals gained weight during 7-day follow-up. Similarly, in all groups an immediate spike in neutrophil counts was observed at 30 minutes following injection that normalized by 24 hours. Only reticulocytes were slightly elevated after 7 days.

Additional conclusions

Statistical differences in the data were observed. In particular, there was an induction of neutrophil and reticulocyte counts within 30 minutes that normalized by 24 hours then became elevated over the next 6 days; these data are difficult to interpret as there was no negative clinical manifestation, therefore they were deemed to be of minimal safety risk at this time.

The findings of the study support further consideration of the use of cGMP, pre-formulated, fully defined, serum-free and protein-free HypoThermosol and CryoStor biopreservation media products as a vehicle for direct intravenous injection of cellular therapeutics.

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