BioPreservation Today®

BioLife Solutions®
BIOPRESERVATION TOOLS FOR CELLS TISSUES AND ORGANS

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FEATURE ARTICLE:

DEAD CELLS DON'T CURE CANCER: WHY EFFECTIVE BIOPRESERVATION IS CRITICAL FOR COMMERCIALIZATION OF CELL THERAPIES

ISCT 2018 ANNUAL MEETING, MONTREAL CANADA

WHAT'S INSIDE

ISCT 2018
Montréal · Canada
May 2-5

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WEB RESOURCES

alliancerm.org | Alliance for Regenerative Medicine

aabb.org | American Association of Blood Banks

bestcollaborative.org | BEST Collaborative

bioinformant.com | BioInformant

biolifesolutions.com/evidence | BioLife Solutions Cryopreservation and Hypothermic Storage Evidence

celltherapysociety.org | International Society for Cellular Therapy

fiercebiotech.com | FierceBiotech

insights.bio | Cell & Gene Therapy Insights

ishrs.org | International Society of Hair Restoration Surgery

lifesciences.knect365.com | KNect365 Life Sciences

phacilitate.co.uk | Phacilitate

regmedfoundation.org | Regenerative Medicine Foundation

regmednet.com | RegMedNet

UPCOMING EVENTS

International Cord Blood Symposium
June 14-16, 2018, San Diego, CA

11th Annual Business of Regenerative Medicine: Innovation, Clinical Translation, and Entrepreneurship Symposium
July 17-18, 2018, Philadelphia, University of Pennsylvania

10th Annual Hair Transplant 360 Workshop
August 3-5, 2018, Saint Louis University, Missouri

North American Veterinary Regenerative Medicine Association (NAVRMA) Conference 2018
Sept 6-9, 2018, Sacramento, CA

Cord Blood Connect (Hosted by the Cord Blood Association)
Sept 14-16, 2018 Miami Beach, FL

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EDITOR'S CORNER

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

Bonjour!

Greetings from the ISCT 2018 Annual Meeting in beautiful Montreal.

BOOTH #209

Customers, suppliers, partners, and friends of BioLife; welcome to Canada and the International Society for Cellular Therapy's 26th Anniversary Annual Meeting. We're pleased and proud to support this key industry event as a Silver sponsor in several ways, including continued industry leadership by providing biopreservation expertise and best-in-class CryoStor® and HypoThermosol® clinical grade biopreservation media products; being a best practices resource; and supporting the increasingly critical cold chain. We are especially excited about this year's cold chain developments. Our partner, SAVSU Technologies, continues to innovate; please visit SAVSU in **Booth #718** to see the new cloud-connected evo™ DV4 and DV10 Dry Vapor Shippers (DVS) with Smart Cap™ and QuickCore™ technologies, the evo™ Smart Shippers operating at CRT, 2-8°C and -80°C with dry ice, and the latest software innovations in the evo. is cold chain SaaS for time and temperature sensitive biologic materials. SAVSU recently formed a partnership with World Courier and anticipates announcing several additional key relationships to further expand the evo ecosystem.

Also, while at the conference, please browse our two poster sessions on Thursday, May 3rd from 6:00 pm – 7:30 pm (and afterwards) as we further expand best practices for the biopreservation of cells, tissues, and organs:

- Brian J. Hawkins, PhD, Scientific Director, will present "THE EFFECT OF CRYOMEDIA SELECTION AND TRANSIENT WARMING EVENTS ON POSTCRYOPRESERVATION HUMAN MSC FUNCTION." The poster illustrates how CryoStor yields improved post-thaw function in MSCs versus traditional homebrew at a lower cryopreservative concentration. Poster #101.
- Alireza Abazari, PhD, Scientific Director, will present "DELINEATING THE CRITICAL PROCESS PARAMETERS FOR CELL THERAPY CRYOPRESERVATION." This poster dissects the impact of processing steps on post-thaw quality of the final product, using a model T cell. Poster #164.

It's been a fast moving and exciting year since the last ISCT annual meeting, with the recent approvals of Kite's YESCARTA®, Novartis' Kymriah™, and TissueGene's Invossa's®, plus a myriad of pre-commercialization activity by companies. Read about the critical role biopreservation plays in the feature article *"Dead Cells Don't Cure Cancer: Why Effective Biopreservation is Critical for Commercialization of Cell Therapies."*

Along with biopreservation, the cold chain continues to advance and innovate as well. Todd Berard, our Vice President of Marketing, pens an article explaining the newest advances in cold chain management and live cell visibility software by our joint venture partner SAVSU and other partners in the space in *"Cold Chain Corner."*

In another article, Senior Vice President and Chief Technology Officer, Aby J. Mathew Ph.D., provides details of the growing body of posters, abstracts, and clinical papers citing our products and explains how we offer technical and consultative support to our customers in *"Ask the Scientists."*

Michael Weaver, Product Development Manager, then provides insight into BioLife's cGMP engineered media and how it compares to traditional "homebrew" media cocktails. Learn when and why companies make the switch as they continue along the path toward commercialization in his article *"BioLife Technology Versus Home-Brew Cryopreservation Media – Is Home Brew Dating You?"*

Finally, Dr. Brian J. Hawkins elaborates on the most recent scientific poster out of Dr. Mathew's scientific team in *"Transition to GMP, chemically-defined, xeno-free cryopreservation media increases post-thaw functionality of clinically-relevant cell banks."* This poster further adds to BioLife's 275+ customer Regenerative Medicine applications, 350+ scientific citations, and 2,000+ customers.

Thank you for your continued interest in BioLife Solutions. I hope you enjoy this issue of Biopreservation Today. We look forward to seeing you at our exhibit and during the conference.

Bienvenue à Montréal!

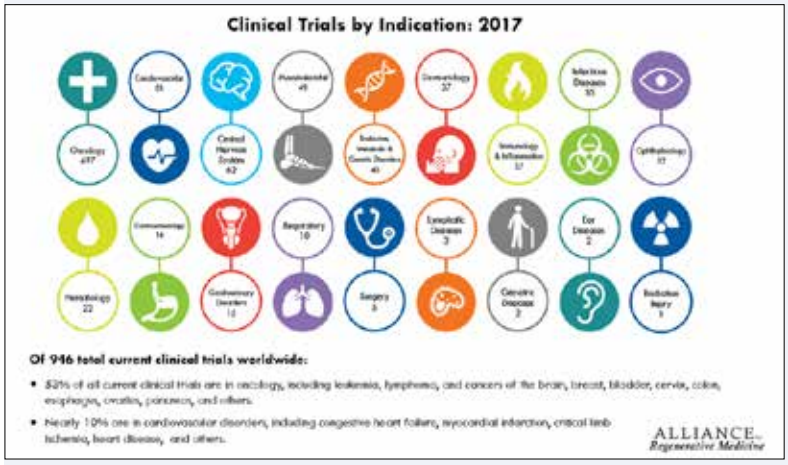
Mike

DEAD CELLS DON'T CURE CANCER: WHY EFFECTIVE BIOPRESERVATION IS CRITICAL FOR COMMERCIALIZATION OF CELL THERAPIES

Mike Rice, Chief Executive Officer, BioLife Solutions, Inc. (NASDAQ: BLFS)

It's 2018, and while only a small number of cell therapies have been approved for human use, the potential of personalized medicine approaches in treating and curing cancer and other leading causes of death is tremendous. The Alliance for Regenerative Medicine estimates that by the end of 2017, 946 clinical trials were underway, and funding for cell and gene therapies and tissue engineered products surpassed $22 billion during 2015 through 2017.

A key catalyst in driving investment in the space is the remarkable and durable therapeutic responses chimeric antigen receptor (CAR) T cells and other cell types have shown to elicit in very sick patients. As these novel engineered cellular therapies gain approval, and manufacturing scale-up commences to support distribution to worldwide patient populations, the critical role that effective biopreservation practices plays is gaining much wider appreciation.



This appreciation is being driven by several factors:

The Nature of Time and Temperature Sensitive Biologic Material

First off, apheresis collections (the starting material for CAR T-cell therapies) and manufactured cell products are both time and temperature sensitive. Removing any biologic material from the body starts a race against time. Hypothermic preservation is employed to reduce metabolism and the need for oxygen and nutrients. Frozen storage of manufactured cell products can extend the stability (shelf life) long enough to get the product to clinic, but there is catch; starting materials and cell therapies must be effectively preserved during storage and transport so they arrive intact; viable and functional. The use of non-optimized preservation media [Such as homebrew – see related article BioLife vs. Homebrew on page 9] and traditional shipping containers can result in cellular injuries from molecular stress responses and temperature cycling, rendering the cells useless. This is a tragic outcome; as dead cells don't cure cancer.

Personalized Medicine

In many cases the intended patient may be too sick to undergo a repeat apheresis collection procedure. This highlights the critical need for use of best practices in biopreservation of source material and manufactured cell products. In the case of solid tumor cancer therapy, a biopsy of the patient's tumor is excised and shipped to the cell therapy company, where a living dose of tumor-killing cells is manufactured. Again, both of these biologic materials are susceptible to injury and death during ex-vivo storage and transport, reinforcing the need for effective preservation so (a) the tumor biopsy arrives viable and can be used to make the treatment and (b) the manufactured cell product arrives back at the clinic in a healthy state, so the intended therapeutic effect can be achieved.

Reimbursement Requirements

Cell therapy manufacturers in the regenerative medicine space could experience significant pressure to produce documented compliance with best practices related to chain of custody, chain of identity, cold chain (maintenance of biologic material within a validated temperature range throughout the entire distribution spectrum) and use of time sensitive cell products before a validated stability period expires. With the positions taken by GSK, Novartis, and Gilead (Kite Pharma), where reimbursement amounts were negotiated based on a therapeutics response by the patient, this "pay for cure" paradigm is sure to drive more awareness of the need for effective biopreservation tools.

Freeze Media – From Home-Brewed Saline/ DMSO/FBS/HSA Cocktails to Engineered cGMP Formulas

Traditional cell freeze media consist of saline solution or culture media supplemented with any of DMSO, FBS, serum from animal or human sources and in some cases, a large molecular sugar such as Dextran. These home-brew formulas, still widely used today, provide reasonable preservation efficacy for many cell types. However, making freeze media on a recurring basis is clearly not best practice, considering the limitations, which include;

- Lot to lot inconsistencies
- Inclusion of serum components
- Research grade components
- Limited or no cGMP oversight
- Minimal release testing
- No FDA Master File support

Here at BioLife Solutions, our mission is to become the leading provider of biopreservation tools for cells, tissues and organs. We strive toward this goal so we can help our customers commercialize novel cell and tissue-based therapies. Our proprietary, engineered biopreservation media products are specifically formulated to protect human biologic material from low temperature stress by including components such as:

- pH buffers
- energy substrates
- apoptotic inhibitors
- oncotic/osmotic stabilizers
- free radical scavengers
- permeating & non-permeating cryoprotectants

Our quality management system is certified to ISO13485 and we also strive to comply with several relevant cGMP guidances related to sterile drug manufacturing. To support a robust quality and manufacturing footprint, we continually seek to source the highest available grade of components

and maintain a Type II master file with the US FDA. To date, our HypoThermosol® storage media and CryoStor® freeze media have been incorporated into more than 275 customer clinical applications, including two approved cell therapy products and 15 – 20 phase 3 clinical trials.

Shipping Containers – Moving into Cold Chain 2.0

Biopreservation is obviously a key component of the therapy chain, but chains are only as strong as their weakest links. Given BioLife's role as a key supporter and enabler of our customer's therapies and technologies, we've come to see a lot of these chains, of which the most critical, and often the most overlooked, is the cold chain.

SAVSU Technologies, our joint venture partner, has taken the lead in creating a suite of next-generation cold chain products and an information system to support cutting edge regenerative medicine therapies now, and into the future. Comprised of both hardware (the shippers themselves) and software (the cloud-based evo.is Software-as-a-Service (SaaS)) platform, together they provide high touch, high visibility to each and every lifesaving shipment. For more detailed information on each of these shippers, please visit www.savsu.com.

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Data is King! Live Cell Orchestration Platforms

As human health progresses from small molecule (drug) therapies to actual "living medicines" (live cells used in cell therapy), the importance of keeping these cells alive becomes paramount. However, the problem beyond biopreservation becomes twofold: - 1) making sure the cells stay in an optimal temperature range and 2) proving that this temperature range does indeed happen through documentation.

1) The first issue is maintaining the optimum temperature range. For example, when many people think "keeping something refrigerated" they usually think "make sure it doesn't get too hot". However, the inverse can be perversely true; cells that go below freezing, even for a few short minutes, can kill them, rendering potency or the entire therapy ineffective. Therapies usually have also been validated by companies to be within a certain temperature range; that range must be maintained. Savsu provides a suite of products with thermal diversity to maintain these cells and therapies within their own unique critical temperature ranges. Available in Liquid Nitrogen (LN2), -80° dry ice, 2-8°C, and Controlled Room temperature (CRT) ranges, these shippers maintain their thermal integrity throughout the entire shipping process.

2) The second issue is documenting the result, because the old adage "If you don't document it, it didn't happen" holds especially true when shipping cellular therapies. Sure, your CAR T Therapy apheresis starting material was shipped 2-8° C, and the final dose of cells was shipped on LN2 – now prove it. Well, thankfully the industry is now stepping up with the tools and technologies needed for this proof, and Savsu is leading the way. It all starts with the hardware itself, where the thermal performance of the shippers is enhanced by the "SMART" technology embedded within the shippers. A sophisticated suite of sensors monitor payload health, from current therapy temperature, ambient temperature, pressure, location, humidity, and security (has the shipper been opened or not?). All this data is then wirelessly ported up to the cloud, which can then be can be tracked, managed, and reported on from the evo.IS cloud based Software as a Service (SaaS) platform.

3) The third is documenting other areas of the manufacturing and supply chain process (vein to vein) above and beyond the cell preservation and cold chain shipping. This includes the actual withdrawal of the apheresis starting material from the patient at the clinic, the entire manufacturing process and scale up, QA/QC, and all the little things that need doing back at the hospital before infusion of the cells back into the patient. To support this ecosystem, several third-party companies have sprouted up to bridge the gap from disconnected data loggers and post administration QC to real-time, proactive live cell visibility platforms. These include TrakCel (https://trakcel.com/) which streamlines cell, gene, and immunotherapy workflows by electronically storing and managing records the journey of a cell therapy sample, including when, where and by whom it was handled. TrakCel's cell orchestration platform (COP) is a unique software solution that facilitates efficiency in cell and gene therapy development by providing a platform by which program-critical information can be collated, tracked, and documented. This means users can avoid the time, cost, and risks associated with outdated paper-based systems.

The COP software is a simple and efficient way of providing users with real-time information relating to every step of the cell and gene therapy process, from treatment development, manufacturing, to final delivery. This needle-to-needle approach enables faster decision making and process optimization and can provide personnel within the chain with actionable data – sending real-time updates to stakeholders. TrakCel provides integrated software that allows for the comprehensive management and tracking of the many stages of cell and gene therapy programs and allows users to prepare comprehensive regulatory audits with less stress. COP is helping customers to hugely reduce the time and costs associated with developing life-changing therapies. Vineti [https://vineti.com/] is also creating a platform to deliver on the promise of these next generation therapies. Built upon the philosophy that with life-saving cell and gene therapies, the patient is the product, the process, and the treatment, Vineti's proven digital platform for individualized medicine expands patient access to transformative therapies. Their goal is to provide digital innovation for manufacturing, scaling, and commercializing the newest breakthrough therapies, safely and efficiently, by providing a digital platform for ordering and scheduling, collection, transportation, manufacturing, and delivery of therapy. The vision is to connect patients, providers, pharmaceutical companies, and regulators with unprecedented visibility that simplifies the process, safely and efficiently. Align therapy data and workflows with a digital "platform of record" that provides for scale, traceability, and confidence.

BioLife Solutions, through our joint venture with SAVSU, the evo SMART shipper line, and the evo.is SaaS cloud-based platform, is proud to be a key financial supporter of this new and evolving ecosystem. Together, through gold standard biopreservation media products, next generation Cold Chain 2.0 shippers and shipment visibility, and ecosystem partners supporting the manufacturing side, we are delivering on the promise of life saving next generation regenerative medicine, gene, and cellular therapies now, and into the future.

COLD CHAIN CORNER SAVSU TECHNOLOGIES

Todd Berard, Vice President of Marketing, BioLife Solutions, Inc.

As we continue our mission of biopreservation best practices for the regenerative medicine, stem cell, gene therapy, and biobanking markets, it is important to note the importance we take of extending our vision to one of the most critical areas in the therapy chain – the cold chain. Through our joint venture with SAVSU Technologies, we bring an industry-leading suite of next-generation SMART shippers and the evo.is cloud-based SaaS application to provide high touch, high visibility to your therapy's health when they are in the "black box" known as the cold chain.

Along those lines, we'd like to announce some exciting developments:

In January 2018, our joint venture partner SAVSU Technologies ("SAVSU") announced that it will supply SAVSU smart precision shipping containers throughout the World Courier network. SAVSU designs and manufactures innovative high-performance cloud-connected passive storage and transport containers optimized for the cell and gene therapy supply chain. World Courier is the global leader in specialty logistics and a part of AmerisourceBergen (www.worldcourier.com). The combination of World Courier's global logistics network and SAVSU's patent pending, enhanced temperature-controlled evo DV4 & DV10 liquid nitrogen (LN2) shippers will enable cell and gene therapy developers to more efficiently and effectively move critical biologic shipments at cryogenic temperatures worldwide, with enhanced visibility and shipment monitoring capabilities.

The addition of these shippers to World Courier's packaging fleet connects therapy developers to the latest cold chain technologies; and offers a greater choice of options for cryogenic shipping. This extends World Courier's track record of developing and verifying the latest technology through its Climate Optimisation Research and Engineering (CORE) Labs, to provide its customers with access to a wide range of market-leading packaging solutions.

The combination of World Courier and evo LN2 dry vapor shippers offer significant value to cell and gene therapy developers by providing:

SAVSU's new next generation Dry Vapor Shippers (DVS)

- Short lead times, enabled by SAVSU's rapid charging and availability throughout World Courier's 140+ owned office global network
- High quality and extended temperature performance, underpinned by the transformative design of evo smart shippers and world-class specialty logistics capabilities of World Courier
- Low cost profile as a result of reduced shipper weight and dimensions
- Real time data visibility and traceability through the evo.is SaaS dashboard, amplified into actionable information by the global reach of World Courier to intervene and remediate if required
- Seamless integration of shipping container supply and logistics, coupled with full vein-to-vein traceability for customers through integration with cell orchestration platforms

Bruce McCormick, President and CTO of SAVSU, commented that "by partnering with World Courier, we are now able to provide customers with a new level of service and peace of mind for their personalized medicine and other biologic shipments, on a global scale."

World Courier is working to realize the full potential of cell and gene therapies for both patients and manufacturers. At the same time, the company aims to improve supply chain performance for the global healthcare market by providing more reliable and cost-effective clinical trial and commercial product transport. For more information please visit https://www.worldcourier.com/insights-events/market-insights/detail/savsu-dry-shippers.

Mike Rice, BioLife Solutions CEO, commented," The alliance between World Courier and SAVSU is an endorsement of the best in class technologies we have developed over the

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past three years. This is a critical inflection point in the cell therapy market, as awareness of the critical need for better cold chain technologies for personalized medicine has never been greater."

The partnership also has several other strategic relationships in the works to extend our network, and we look forward to providing greater details soon.

On March 26th, BioLife and Savsu were also notified that they were also Awarded a Second Patent for Next Generation Cold Chain Technologies Designed for Cell and Gene Therapies. The USPTO has issued a notice of allowance for a second patent application titled "Biologic Stability, Delivery Logistics and Administration of Time and/or Temperature Sensitive Biologic Based Materials". The inventors are Bruce McCormick, President of SAVSU Technologies, and Mike Rice, CEO of BioLife Solutions.

The patents were issued on April 10th, 2018, under patent numbers 9,939,422 and 9,939,423.

Since the formation of the BioLife Solutions – SAVSU Technologies joint venture in the fourth quarter of 2014, the companies have submitted 12 other patent applications related to novel innovations incorporated into current, or to be incorporated into future, precision thermal shipping containers under the evo brand, including models that precisely maintain biologic payload temperature stability across the entire shipping continuum including -196°C using liquid nitrogen dry vapor shippers, -80°C using dry ice, and 2° - 8°C and controlled room temperature (CRT) using conditioned phase change insulating materials. These patent applications also include claims related to the evo.is Cold Chain Software as a Service (SaaS) live cell visibility platform.

Bruce McCormick commented, "We are pleased to receive this second notice of allowance of what we believe will be a series of patent grants. We filed several applications to protect our growing intellectual property estate. We believe our hardware and software innovations, embodied in current and future evo models, will offer significant value to the growing regenerative and personalized medicine markets."

Mike Rice, BioLife CEO, remarked, "This notice of allowance is another validation of the vision SAVSU and BioLife formed several years ago, with a focused and intentional goal to improve cold chain logistics of cell and gene therapies. We believe the advanced and innovative evo hardware and software technologies will have tremendous impact on the preservation, delivery, and quality management of these precious, time and temperature sensitive biologic medicines."

For more information, please see our PRESS RELEASES. https://investors.biolifesolutions.com/news-releases

ASK THE SCIENTISTS

Aby J. Mathew, PhD – Senior Vice President & Chief Technology Officer, BioLife Solutions Inc.

Ask The Scientists, a key customer support initiative of BioLife Solutions, is intended to be a multi-channel resource that links avenues of print (BioPreservation Today®), direct website link (www.BioLifeSolutions.com/Evidence), and direct email. We welcome questions and feedback from our customers and prospective customers, and value the unparalleled direct communications and relationships that have led to HypoThermosol and CryoStor being incorporated into several hundred customer clinical applications, articles/abstracts citations, and poster/data references. For more information or if you have a question for the BioLife Solutions scientific team, please submit your questions at http://www.biolifesolutions.com/ask-the-scientists. Here is an example of a representative customer question, and our feedback:

"I need to submit regulatory documentation about HypoThermosol FRS and CryoStor, but your media is proprietary. How can you support our regulatory documentation needs for US FDA and outside the United States?"

HypoThermosol FRS and CryoStor have been qualified into 275+ customer clinical applications as an ancillary material or excipient. Biopreservation media for cell manufacturing are not classified as drugs or devices, and therefore do not carry specific therapeutic regulatory approvals. Within their classification as ancillary materials in a customer's process (or excipient if utilized in that manner by a customer), they are reviewed within the context of the customer's process. Each group would qualify the ancillary or excipient usage within their specific cell product application.

In order to assist customers with clinical applications requiring regulatory submissions, BioLife Solutions has registered Type II Master Files (for a Drug Substance, Drug Substance Intermediate, and Material Used in Their Preparation, or Drug Product) for HypoThermosol FRS and CryoStor with the United States Food and Drug Administration (US FDA) Center for Biologics Evaluation and Research (CBER). The Master Files may be cross-referenced during the submission process, allowing the US FDA to review the BioLife Solutions Master Files. The Master Files contain detailed information regarding Good Manufacturing Practices (GMP) compliance in use during the manufacture, testing, storage and distribution of HypoThermosol FRS and CryoStor. The contents of the Master Files are confidential, and disclosure is restricted to the US FDA.

Furthermore, we are able to provide customers with ~50 pieces of clinical supporting information, including publications and posters from the 350+ resources cited on our

search-enabled Evidence Page library (https://www.biolifesolutions.com/evidence/). Depending on the needs of each individual customer, the clinical supporting information may include customer clinical citations, GMP Quality questionnaires, onsite audits, and limited component information. For customers engaged in clinical applications outside of the US FDA, this detailed dossier of clinical supporting information serves as the primary resource for Quality/Regulatory support and risk assessment qualification. Raw materials, including biopreservation media, should be qualified to appropriate standards for each customer's process, product, and application. With being incorporated into 275+ customer clinical applications – including approved customer applications such as Kolon Life Sciences' Invossa™ and Kite Pharma's YESCARTA™ - and BioLife's extensive experience supporting customer clinical applications in North America, Europe, Asia, and Australia, our pathway for supplier and raw material qualification has been recognized as well-defined and efficient. We look forward to supporting the increasing number of partner customers through their clinical development, as well as their Biologics License Applications and Marketing Authorization Applications.

Dr. Mathew was part of the founding team of BioLife Solutions, Inc., and is a co-developer of BioLife's biopreservation media solutions. He is a co-inventor on multiple issued and pending patents related to methods, devices, and formulations for the preservation of cells, tissues, and organs. He holds a Ph.D. in Biological Sciences within the Biochemistry, Cell and Molecular Biology Program from Binghamton University and a B.S. in Microbiology from Cornell University. Dr. Mathew has been researching low temperature biopreservation since 1994, and his studies contributed to the development of BioLife's current commercial HypoThermosol® and CryoStor® product platforms and intellectual property foundation. Dr. Mathew was part of the scientific team that linked cell death via apoptosis (programmed cell death) to exposure to hypothermic and/or freezing temperatures. These discoveries were integral to the development of BioLife's intracellular-like biopreservation media, and also contributed to improvements in cryosurgical ablation of cancer. Dr. Mathew was BioLife's first Director of Manufacturing, established BioLife's initial Quality system, and has been Senior Vice President & Chief Technology Officer since February 2011. From January 2007 through February 2011, Dr. Mathew served as Senior Scientist, Director of Strategic Relations, and Senior Director of Strategic Relations. From June 2003 through January 2007, Dr. Mathew served as Director of Manufacturing. From September 2000 through June 2003, Dr. Mathew served as Clinical Accounts Manager and Director of Hypothermic Preservation for Cryomedical Sciences/BioLife Solutions. Dr. Mathew is currently active in, or previously a member of, AABB (formerly the American Association of Blood Banks), BEST (the Biomedical Excellence for Safer Transfusion collaborative), the International Society for Cell Therapy (ISCT), the Alliance for Regenerative Medicine (ARM), Tissue Engineering & Regenerative Medicine International Society (TERMIS), Society for Cryobiology, International Society for Biological and Environmental Repositories (ISBER), American Society for Cell Biology, and the Society for In Vitro Biology. Dr. Mathew is a member of, the Board of Directors and Advisory Panel of the Parent's Guide to Cord Blood Foundation, the Scientific Advisory Board of HemaCare Corporation, the founding Board of Directors of the Cord Blood Association, the NIST-AMTech National Cell Manufacturing Consortium, the California Institute for Regenerative Medicine (CIRM) Clinical Advisory Panel, and the Scientific Advisory Board of SAVSU Technologies. Dr. Mathew has obtained UCLA Corporate Governance Program Certification.

BIOLIFE TECHNOLOGY VS. HOMEBREW CRYOPRESERVATION MEDIA

Michael Weaver, Product Development Manager,
BioLife Solutions Inc.

Is Home-Brew Dating you?

Need drives technology which in turn drives innovation.

Amazing numbers of technological advances must be brought together to satisfy the need for particular therapeutic cells that can be used to treat a long list of afflictions that humans and animals are prone to. Given that multiple technologies create multiple paths toward a common goal, the ability to apply innovation can be a competitive advantage with the goal to translate research from the bench to cellular therapies at the bedside.

While cells are enormously fragile when removed from the mammalian body they represent a tremendous potential for host repair if cells can be maintained viable long enough. The need for long term preservation of cells outside the mammalian host drives the science and technology of cryopreservation. An examination of relatively recent milestones in cryopreservation is doubly illuminating taking into account both errors and advancements as they apply towards how much technology to apply on the path towards innovation.

Recent milestones in cryopreservation.

The accidental discovery in 1948 by Polge, Parks and Smith[1] that fowl spermatozoa could be frozen in a home-brew consisting of glycerol, albumin and water became a milestone in the history of cryopreservation. The discovery also points out major pitfalls of making home-brew, the potential for errors in formulation leading to mislabeled reagents! The trio intended to investigate the suitability of a solution of levulose for cryopreserving spermatoza. It took careful analysis by an analytical chemist to ascertain the true composition of the solution and additional experiments to determine glycerol was the active ingredient.[2] In 1950, Smith[3] published the use of glycerol for cryopreservation of human red blood cells. Certain cell types including bovine rbcs are not efficiently penetrated by glycerol. By 1954 Lovelock[4] evaluated additional cryopreservation candidate agents including mono-, di-, and poly-hydric

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alcohols, amides and sugars. In the article in The Biochemical Journal, Lovelock opined "In the practical preservation of human red blood cells other considerations than protection during freezing and thawing are important, e.g. the ease with which the protective agent can be removed from the cells, and the prolonged survival at low temperatures. In choosing a substance for the practical preservation of red blood cells these factors also must be taken into account." In 1959 Lovelock and Bishop[5] recognized the potential of dimethylsulfoxide, a small molecule capable of rapid cell penetration, and published data demonstrating enhanced permeability and protective action using DMSO for the cryopreservation of bovine rbcs against freeze damage and suggested its use as a cryoprotective agent. DMSO would go on to become the gold standard cryoprotectant added to a variety of solutions for freezing a wide range of cell types.

Home-brew.

Examples of home-brewed solutions often chosen for cryopreserving cells include complete cell culture medium containing 10 - 20% serum to which DMSO is added, usually at a "rule-of-thumb" concentration of 5 - 10%[6], or a 2x formulation of 20% DMSO in serum[7] added in a 1:1 ratio to a cell suspension to achieve a final DMSO concentration of 10%. Ingredients are readily available and relatively quick to assemble as needed. For cell lines shared within and between laboratories, home-brew can be a low tech solution as the requirements for use are low - "sufficient numbers" of viable cells recovered post thaw to be able to expand to "sufficient numbers" for use in a reasonable amount of time. Would you trust your cell therapy at this level of preservation?

In clinical and commercial use, home-brew can become a disadvantage when the goal is to deliver every viable and functional therapeutic cell possible. Disadvantages include:

- Home-brew is not an optimized cryopreservation solution and the number of viable, functional cells may not conform to expectations
- Home-brew as described is not manufactured according to cGMP
- Home brewing cryopreservation media lacks control over raw materials, performance, quality, and accountability
- Home-brew adds variability to the cell product
- Home-brew is a low tech solution
- Adapting your cell cryopreservation protocol to incorporate home-brew may be more complicated than incorporating formats designed to simplify use available with commercially manufactured, true off-the-shelf solutions
- Adapting (complicating) your cell preservation protocol early on makes the process more difficult to scale up later
- Serum may be immunogenic to the cell therapy recipient complicating future administrations
- The recipient may be reactive to serum proteins administered months or years previously.

BioLife's solution.

BioLife translates recent advances in mammalian cell cryopreservation described in scientific literature to develop and manufacture a state-of-the-art commercially available cryopreservation solution, BioLife's CryoStor® product family.

Advantages of CryoStor include:

- CryoStor is an intracellular-like optimized solution containing osmotic/oncotic agents, free radical scavengers, and energy sources to minimize apoptosis, minimize ischemia/reperfusion injury and maximize the post-thaw recovery of the greatest numbers of viable, functional cells
- CryoStor is cGMP-manufactured from raw materials of USP-grade or higher, has a three year stability profile, and is backed by BioLife's Quality Management System to deliver a consistent, quality, high performance product
- CryoStor is a true off-the-shelf family of solutions pre-formulated with 2%, 5% or 10% DMSO so the end user can adapt their cells to the DMSO concentration that results in optimal cell survival and function for a given cell type
- CryoStor is serum- and protein-free, non-immunogenic
- CryoStor is a high tech, innovative solution that is packaged in multiple volumes and in containers of multiple formats designed to provide the amount your process requires in a form factor amenable to scale up.

As important as the solution is, the science of cryopreservation is not only about the solution. BioLife Solutions advances a best practices approach to cryopreservation. BioLife scientists can help you optimize and scale your processes from growth and preparation of cells for freezing, cryopreservation solution selection, freezing protocol, thawing protocol, through to post-thaw recovery, viability and delivery. CryoStor is an ancillary reagent or an excipient depending on its role in the preparation/use of the final cellular product. BioLife scientists are experts in the regulatory requirements surrounding the use of CryoStor and will help you provide the necessary justifications and evidence required for your regulatory submissions.

Tremendous advances have taken place in the science of cell cryopreservation since 1959 which includes solution components, freeze and thaw protocols, equipment, software, containers, packaging and connectivity. Similarly, tremendous advances in immunology, proteomics and genomics, cell collection, isolation, genetic engineering, cell culture and expansion have enabled the manufacture of promising cell therapies and precision medicine to reach the forefront of clinical medicine. A tremendous effort has gone into manufacturing every cell in the therapy your patient deserves. Home-brew falls short of being able to deliver the promise of every viable, functional cell possible. BioLife's solutions and expertise convey competitive advantages over home-brew and deliver the innovations in cryopreservation you and your patients can count on to successfully translate your cellular therapies from concept to commercialization.

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TRANSITION TO GMP, CHEMICALLY DEFINED, XENO-FREE CRYOPRESERVATION MEDIA INCREASES POST-THAW FUNCTIONALITY OF CLINICALLY RELEVANT CELL BANKS

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Cryopreservation is an essential element for the generation of master and working banks for cell therapy and regenerative medicine applications. As these products are scaled-up for commercialization, cryopreservation protocols established early in preclinical development should be modified to comply with Good Manufacturing Practices (GMP) that allow for large-scale cell production for clinical use. Optimization of cryopreservation protocols for master and working cell banks prior to scale-up ensures GMP compliance, acceptable viability, and a return-to-function of frozen stocks post-thaw. At the International Society for Cellular Therapy (ISCT) 2017 Annual Meeting in London, BioLife Solutions scientists, in collaboration with the University of Washington Heart Regeneration Program (HRP), presented work detailing how the incorporation of clinical-grade cryomedia improved post-thaw cell viability and functionality over a traditional home-brew formulation.

In brief, BioLife scientists utilized a representative human Jurkat T cell line as a model for cryopreservation processes intended for clinical T cell applications in cancer treatments. Jurkat T cells were cryopreserved in either a traditional T cell cryomedia composed of Plasma-Lyte A supplemented with 10% human serum albumin and 5% DMSO (HSA/PLA) or GMP-manufactured CryoStor® media preformulated with 5% DMSO (CS5). Immediately post-thaw, and at increasing intervals up to 48 hr, viability and cell number was determined using the Via-1 cassette and visualized by an automated NucleoCounter NC-3000 imaging cytometer (ChemoMetec, Denmark). As evidenced in Figure 1, initial cell recovery and count were similar between CS5 and HSA/PLA but increased significantly in CS5 group at 48 hr (A). Further, Jurkat T cells cryopreserved in CS5 exhibited increased (B) viability at 24 hr and (C) viable recovery, and demonstrated increased cell growth at 48 hr post-thaw following cryopreservation in CS5. The observed loss in cell number over 24 hr may be attributed to cryopreservation-induced Delayed Onset Cell Death, which corresponded with DNA degradation and elevated activity of enzymes associated with programmed cell death (apoptosis). Membrane integrity is a common means of evaluating post-thaw cell viability and cryopreservation efficacy. From these data, BioLife and the HRP demonstrate that measures of immediate post-thaw membrane integrity can vary over time and may not accurately reflect long-term cell viability following cryostorage.

Due to the inaccuracy of membrane integrity measures, alternative measures of cellular function were evaluated to more accurately assess cell health following cryopreservation.

Glutathione (GSH) is the largest contributor to the cellular thiol pool and is essential for numerous oxidation/reduction reactions. An oxidative shift in the cellular redox balance results in the dimerization of two glutathione molecules and a general loss of cellular antioxidant capacity. Oxidative stress can be a trigger for apoptosis, secondary necrosis, and cellular dysfunction during environmental stress. To determine whether cryopreservation-induced Delayed Onset Cell Death was associated with oxidative stress, Jurkat T cells were evaluated immediately post-thaw and at increasing time intervals with the thiol-reactive fluorophore VB-48. In Figure 2, experimental data demonstrates that cryopreservation in HSA/PLA resulted in a large population of cells with low thiol (GSH) content versus non-frozen controls. Cryopreservation in HSA/PLA further required extended post-thaw culture to normalize cellular redox balance. In contrast, CS5 preserved cellular redox balance immediately post-thaw and throughout the 24 hr post-thaw culture. These data reveal that altered cellular redox balance can be detected rapidly post-thaw and suggest oxidative stress

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following cryopreservation. Oxidative stress and decreased GSH levels may contribute to cryopreservation-induced cell death/damage. These data stress the need for multiple measures of cell viability post-thaw, and suggest that optimized CryoStor media offers a more robust solution for the accurate evaluation of post-thaw viability from master and working cell banks using multiple measures of viability post-thaw.

Using the information gained from Jurkat T cell experiments, a clinically-relevant hESC line was transitioned from serum-containing home-brew cryomedia to CryoStor cryomedia at increasing DMSO concentrations. hESCs exhibited elevated cell viability following cryostorage in CryoStor media at all DMSO concentrations, compared to a PBS control, that was maximal using CS10 (10% DMSO). Due to the inaccuracy of immediate post-thaw membrane integrity as an indicator of long-term cell health, hESC were cultured and evaluated for up to 96 hr post-thaw. hESCs cryopreserved in CryoStor experienced a robust post-thaw expansion as measured by a greater than 15-fold increase in cell number. As such, hESCs cryopreserved in CryoStor cryomedia can be employed to establish a viable Working Cell Bank that can be rapidly expanded post-thaw for use in preclinical development.

These studies collectively reveal that, under suboptimal cryopreservation conditions, measures of post-thaw cell viability and functionality can be highly variable in the short term (<48 h), and do not match the long-term (>48 h) outcome. These data stress the need for multiple measures of cell viability post-thaw that can include indicators of apoptosis and cellular oxidative stress. Of note was the influence of the selected cryomedia on the accuracy of multiple cell viability measurements and long-term cell health. Specifically, CryoStor cryomedia improved viable recovery versus traditional 'home-brew' cryomedia, reduced cryopreservation-induced apoptosis and cellular oxidative stress, and conveyed more accuracy to membrane integrity as a long-term indicator of cell viability and expansion. As such, intracellular-like, chemically defined, and xeno-free CryoStor media manufactured under GMP can facilitate the rapid optimization of cryopreservation protocols for clinically-relevant master and working cell banks. The process outlined in these studies provides a roadmap that can be rapidly and easily implemented to cryopreserve valuable cell therapy samples, and ensure desired viability and functional recovery of master and working cell banks following long-term frozen storage. The poster presented at the ISCT 2017 Annual Meeting is available via this link: www.biolifesolutions.com/wp-content/uploads/2017/09/BioLife-UW-ISCT-2017-poster-v3_5-3-17-.pdf.

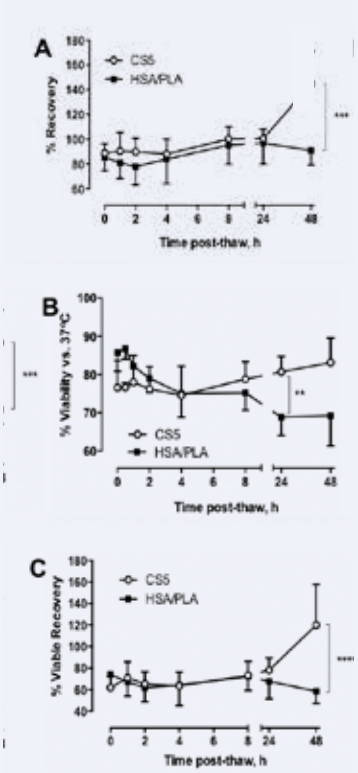

*Figure 1. CryoStor® reducespost-cryopreservation cell loss and speeds cell expansion. Jurkat cells cryopreserved in either a traditional 'home-brew' T-cell cryomedia or GMP-manufactured CryoStor® media were thawed and viability and cell number measured immediately post-thaw and at increasing intervals up to 48 hr, (A) Initial cell recovery/count was similar between CS5 and 'home-brew' (HSA/PLA) but increased significantly in CS5 at 48 hr (***p<0.001). Jurkat cells cryopreserved in CS5 exhibited increased (B) viability (**p<0.001) at 24 hr and (C) viable recovery (****p<0.0001). Jurkat expansion was evident at 48 hr post-thaw following cryopreservation in CS5.*

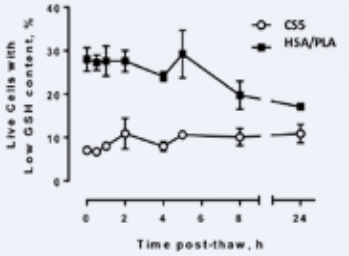

Figure 2. CryoStor® protects cellular redox balance. Jurkat T-cells were evaluated immediately post-thaw and at increasing time intervals with the thiol-reactive fluorophore VB-48. HSA/PLA. Fluorescence intensity was normalized to non-frozen controls as indicated. Cryopreservation in traditional 'home-brew' (HSA/PLA) resulted in a large population of cells with low thiol (GSH) content versus non-frozen controls. Cryopreservation in HSA/PLA further required extended post-thaw culture to normalize cellular redox balance. In contrast, CryoStor media preserved cellular redox balance immediately post-thaw and throughout the 24 hr post-thaw culture.