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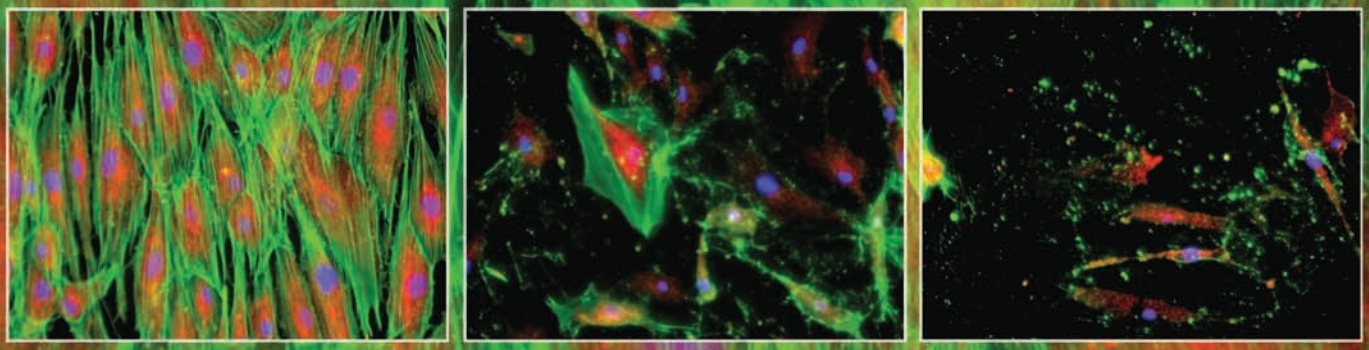
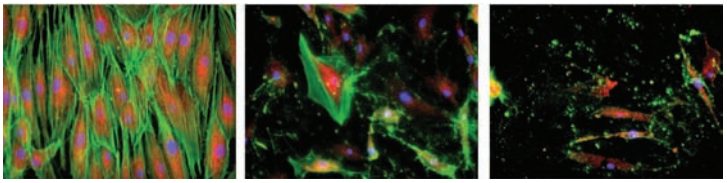


FEATURE ARTICLE
BIOPRESERVATION STABILITY CONSIDERATIONS FOR CELL THERAPY DEVELOPMENT AND COMMERCIALIZATION

What's Inside:

In This Issue



HypoThermosol®-FRS *Viaspan* *Celsior®*

ON THE COVER
Fluorescent Microscopy images of human fibroblasts after 72-hour storage at 2-8°C, enabled by three commercial hypothermic storage media: HypoThermosol®-FRS, Viaspan, and Celsior®.

UPCOMING EVENTS

ISCT 15th Annual Meeting *http://www.celltherapysociety.org/*	San Diego, CA	May 3-6, 2009
ISBER 2009 Annual Meeting *http://www.tri-conference.com/*	Portland, OR	May 12-15, 2009
Cord Blood Forum *http://www.cordbloodforum.org/*	Los Angeles, CA	June 6-7, 2009
ISSCR *http://www.isscr.org/meetings/index.cfm*	Barcelona, Spain	July 8-11, 2009
AABB Annual Meeting and TXPO *http://www.aabb.org/Content/Meetings_and_Events/Annual_Meeting_and_TXPO/*	New Orleans, LA	October 24-27, 2009

How Safe are Your 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 Stability Considerations for Cell Therapy Development and Commercialization

by Scott Burger, MD, Principal, Advanced Cell & Gene Therapy, LLC

I. IMPACT OF STABILITY

Raw material and finished product stability, or shelf-life, is a crucial factor in development and commercialization of biologic-based products. Stability impacts nearly all aspects of operations, from manufacturing and logistics to patient scheduling and ease of use (Table 1). Stability considerations drive a variety of critical decisions, from location and number of manufacturing sites to transportation methods.

Table 1
POINTS OF INFLUENCE OF STABILITY

Quality of Raw Materials and Product
Manufacturing: Centralized vs. Distributed; Sites, Locations
Manufacturing Capacity, Scheduling
Transportation Logistics: Raw Material, Product
Product Release
Product Storage
Final Product Manipulation, Administration
Patient Scheduling, Ease of Use

Without cryopreservation, cell therapy products prepared with conventional media have quite limited stability, often as little as three days [1, 2]. This necessitates releasing the product without final 14-day sterility testing results, overnight shipping, and administration shortly after arrival at the clinical site. Short shelf-life presents other problems as well, not the least that it is quite unforgiving of unexpected events, such as a patient whose treatment should be delayed due to illness on the planned treatment day, or severe weather delaying product transport.

Raw material stability also must be considered, particularly as living, metabolically active cells comprise the critical raw material used in cell therapy manufacturing. This often means stability of 1-3 days in conventional culture media, entailing overnight raw material transport, and with major consequences for manufacturing operations, capacity, and site location.

Cell-based products can be challenging to develop even under the best of circumstances. Lack of stability adds obstacles to commercialization, increasing operational complexity, cost, and risk of failure. For most cell-based products, increasing product stability is perhaps the single most effective way to reduce overall operating cost and risk.

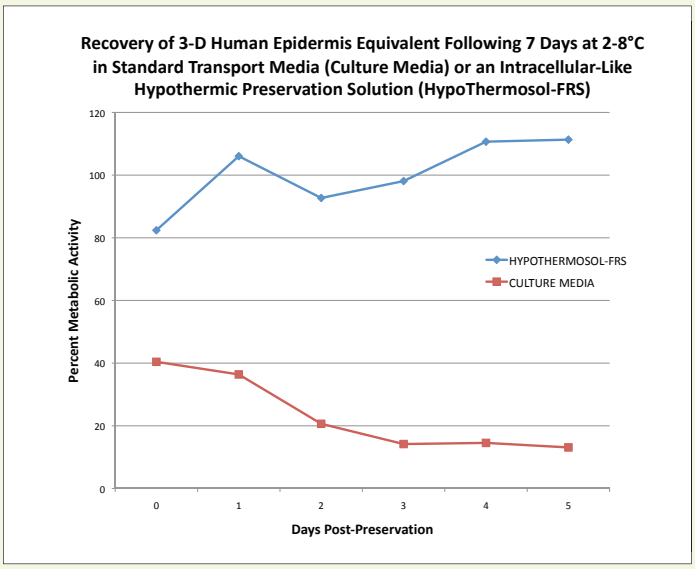
Optimizing storage conditions can improve stability significantly. Both cryopreservation and non-frozen (hypothermic) storage may be used.

II. ENHANCING STABILITY - CRYOPRESERVATION

Cryopreservation can greatly extend product stability, often for years, and simplify certain aspects of operations. Product release can be performed after all testing has been completed, with products stored until needed, and administration scheduled with maximum flexibility.

The benefits of cryopreservation do not come without difficulties of their own, however. Transportation of cryopreserved products can be costly and adds operational complexity. Depending on the composition of the cryopreservation cocktail, post-thaw processing may be necessary to remove cryoprotectant prior to administration. This is problematic, in that it adds a manufacturing step at the clinical site (a location that can be difficult to control), and triggers a requirement for additional safety testing on the thawed, washed cells [3]. Washing the thawed cells prior to administration also introduces questions about post-thaw/post-wash stability. In general, it is preferable to thaw and administer the product without additional processing, to more closely adhere to the risk mitigation approach of minimal manipulation prior to administration

It is essential to optimize cryopreservation and thaw, as these methods and materials affect post-thaw viable cell recovery and survival; and, if suboptimal, can eliminate the advantages of cryopreservation. Variables may include the cryoprotectant, cell concentration, cryopreservation container, cooling as well as thaw rates and methods. Administering the thawed product without washing eliminates the risks associated with processing at the clinical site. Because, however, the cryoprotectant is intended to be administered to the patient, it must be suitable for use as an excipient -- a higher



level of quality than needed for an ancillary material. Like any other process step, cryopreservation and thaw should be validated.

III. ENHANCING STABILITY - HYPOTHERMIC STORAGE

Hypothermic storage is a simpler and perhaps more broadly applicable method for enhancing stability. Cells maintained in the presence of an effective hypothermic preservation agent at 2-8°C can increase stability by days or weeks, with varying results obtained with different cell types [4]. Ideally, the hypothermic preservation solution will serve as an excipient as well, enabling administration of the product with no additional manipulation at the clinical site.

The potential for hypothermic storage to increase stability merits investigation for many cell-based products and associated cellular raw material. Extended shelf-life can eliminate several operational problems and make clinical and commercial success far more attainable. Hypothermic storage may be particularly valuable for cell/scaffold combination products, and other three-dimensional tissue-engineered products for which cryopreservation is not currently feasible.

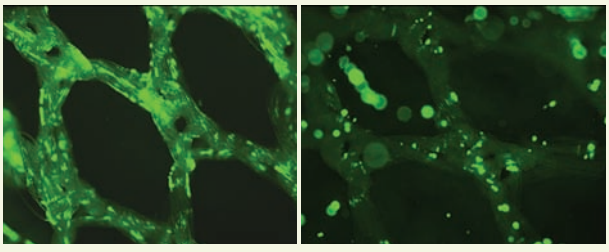
IV. NEW REAGENTS, NEW METHODS - EFFECTIVE PROCESS MODIFICATION

Changing to a new preservation solution, like any manufacturing process modification, raises questions about unintended effects on the product. These can be addressed by a validation study incorporating rigorous characterization testing to compare products manufactured using the original and modified processes. Testing should include product purity and identity characterization parameters, as well as the relevant biological function, i.e., potency.

It should be noted that introducing and validating a new reagent or process step does not invalidate the original process. In fact, the opposite is true. Successful validation indicates that the modified process is as capable of producing the desired product as the original method. A cell banking operation with an established, validated cryopreservation process could, for example, change to a different cryoprotectant, cryopreservation method, or product container, provided the new process could be validated. Cells banked using the original process would be no less valid than they were prior to introduction of the modified process.

During clinical development, it is preferable to introduce manufacturing process modifications, including new reagents, during the intervals between clinical trials. Changes made early in development are the simplest to implement. Regulatory requirements become increasingly stringent and rigorous as clinical development progresses, necessitating more extensive validation and documentation. As one would expect, barriers to change are greatest after product approval (BLA) has been attained. Even in Phase 4, however, manufacturing process and analytical modifications are possible, if difficult. Stability-enhancing modifications ideally would be introduced at the earliest opportunity in clinical development. The benefits of extended shelf-life may be realized at any stage, and the cost-benefit potential of improved biopreservation can be viewed as an aspect of the cell therapy process worthy of consideration throughout development and commercialization.

HUMAN KERATINOCYTES VISUALIZED WITH THE FLUORESCENT INDICATOR CALCEIN-AM



Mesh *Mesh-Hydrogel*

3-D cell systems, such as mesh constructs (left panel) and Hydrogels (including Hydrogel-mesh constructs, right panel), present new challenges for biopreservation of cells and tissues.

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Biopreservation Economics

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

The materials and methods used in the biopreservation of source material, intermediate derivatives, and finished cell therapy products create an economic system that greatly influences several critical success factors for the development and commercialization of the final product. The Cost Per Delivered Dose of a cell therapy product (arguably the most important metric once safety and efficacy are proven) is driven by a yield-cost relationship throughout the biopreservation system. Relationship components include the cost, yield, and stability of source material, as well as the concentration, volume, potency, toxicity, and stability of the finished product.

Stability in particular limits the geographic distribution and hence revenue potential for the product.

The biopreservation system used in the development and commercialization of a cell therapy product, from source material through manufactured dose, has a cumulative impact on the economics for the product.

The degree to which biopreservation system tuning and optimization has been achieved, and when/where such tuning has occurred or may occur along the processing spectrum, modulates the efficiency of the remaining downstream processing operations and the overall system yield-cost relationship

BIOPRESERVATION OPTIMIZATION MATRIX

	Stability/Holding Time	True Post Preservation Recoverable Yield	True Post Preservation "Functional" Yield	Acquisition & Processing Cost
Source Material	↑	↑	↑	↓
Intermediate Derivatives	↑	↑	↑	↓
Finished Product *(Fresh/Frozen)*	↑	↑	↑	↓

Table 1

Biopreservation system optimization may be viewed as a matrix (see Table 1 above) with the three key cell/tissue materials crossed against the critical success factors of shelf life/stability, true post-preservation recovery and functional yield, and acquisition/processing costs.

System tuning should focus on enabling the longest stability, maximum functional yield, and lowest cost for each biologic type.

The system yield-cost relationship impact on Cost Per Delivered Dose can be graphically represented as an ideal scenario where the functional yield of fresh material or final product and the related fixed but lowest cost point are the goals that optimization efforts should strive to achieve (see Figure 1 below).

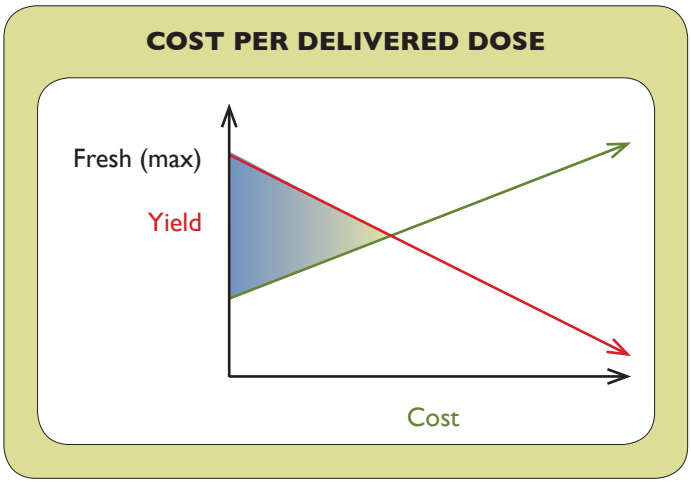


Figure 1

The blue shaded area between the yield and cost lines represents the optimization potential for the system, with the upper left corner representing the maximum optimized state.

Another critical success factor for a cell therapy product is the geographic distribution potential. Logistics around the transportation of source and intermediate material from origin to processing facility, and also for finished product from processing facility to the clinical markets, are greatly impacted by stability of the material. Figure 2 depicts source material or a finished product originating from the US or Europe with potential distribution constrained by poor stability. The red dashed circles represent potential limitations on intercontinental movement of source material and/or distribution of a finished product.

Figure 2 *Stability Constraints*

EVOLUTION OF BEST PRACTICES IN BIOPRESERVATION

by Ian B. Nicoud, Ph.D., Director of Technology & Business Development, BioLife Solutions, Inc.

PART 2: TISSUE COLLECTION AND STORAGE FOR PROCESSING

In the January issue of BioPreservation Today, the first article of this series briefly reviewed the best practices in the field of organ preservation for transplantation, with a specific focus on the evolution of optimized preservation solutions. The use of these solutions greatly advanced the field of organ transplantation and this success has prompted the development and use of optimized preservation solutions for collection, storage, and transport of other biologic materials, including tissues and cells. The need for extended storage times with improved recovery and viability of biologic source material and derived/isolated cells has been driven largely by the growth of the cell and gene therapy markets. Because specimens are often shipped among collection, processing, and clinical sites, maintaining therapeutic quality and quantity is paramount. The number of available doses, their potency, and efficacy of the delivered therapy can all be affected by the robustness of the biopreservation processes and media utilized.

For autologous applications, cell and tissue availability and preservation may not be of great concern if the patient's own cells or tissues are removed, manipulated, and returned within a short time frame in the same facility. On the other hand, cells and tissues required for delivery of autologous or allogeneic therapies that require transportation of source material and cell products among facilities may face commercialization limitations if sub-optimal biopreservation practices are employed.

Due to the infrastructure required to obtain diverse sample populations, tissue specimens are often sourced from a number of biorepositories (public and private), which serve to provide tissue specimens for research and commercial applications.

According to a 1999 study by the RAND Corporation, there were more than 307 million tissue specimens from over 178 million cases stored at facilities in the United States alone, and the estimated growth rate was greater than 20 million new specimens per year [1]. Many of these specimens are located at government facilities and academic medical centers nationwide. Unfortunately, due to the lack of national standard operating procedures for tissue collection, the utility of existing tissue specimens is questionable for many of today's research applications requiring highly standardized samples. In response to this lack of uniformity, the RAND study and others such as the International Society for Biological and Environmental Repositories (ISBER) and the National Cancer Institute (NCI) have compiled resources that outline Best Practices [1,2,3,4]. However, the majority of these guidelines focus on quality systems regulation (QSR) that ensure good laboratory practice (GLP) and not on standardizing the protocols for the physical collection. This leaves much to be considered with respect to the standardization and quality of the acquired biologic material itself.

The current best practices for tissue collection indicate that following appropriate institutional ethics review board and informed consent, a surgeon may remove tissues during a procedure specifically for research. In these cases, the tissue may be collected and processed by whatever means the researcher requests; however, in cases where the procedure is not performed specifically for the purpose of collecting a research sample, i.e., the tissue specimen may be required for diagnostic purposes, specimens for research may still be available following pathology review. Special care should be taken to ensure that the excised tissue is not compromised; the consensus opinion is that tissues should be collected fresh, not fixed, in a sterile container on wet ice and quickly transported to surgical pathology for examination.

Specimens collected or released by pathology for research may be processed in a variety of manners. The consensus is that tissues can be placed directly in sterile containers of saline or other liquid preservation medium with tissue processing taking place within an hour of surgical excision [1]. It should be noted that new optimized preservation solutions have the potential to extend the collection-to-processing time by maintaining cellular function and viability during intervals of hypothermia and hypoxia, and by preparing the specimen for long-term cryostorage. For vascularized tissues from which cells may be harvested, perfusion of the tissue ex-situ with biopreservation solution to remove native fluids (i.e., blood), followed by immersion in biopreservation solution, then storage and transport on wet ice, provides the most optimal recovery of viable cells. In general, the sooner the tissue is prepared in this manner, the better the recovery and viability.

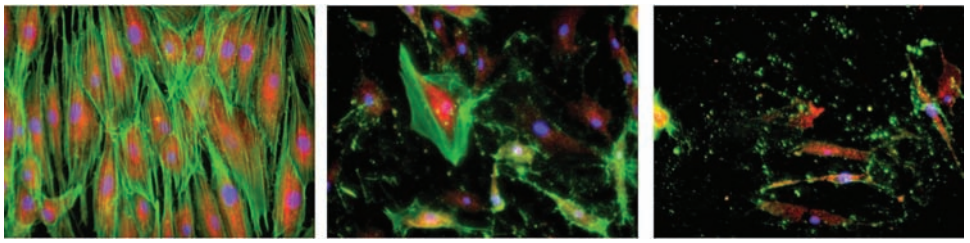
Cell therapy relies on collecting high-quality specimens from which to generate therapeutics. The quantity and quality of biologic source material as well as the shelf life of the finished product directly impacts the cost to produce, which in turn, impacts consumers both from a quality and cost perspective. Utilization of optimized preservation media solutions for the

Continued on page 8.

BIOPRESERVATION ECONOMICS, *Continued from page 6.*

Specific biopreservation system optimization initiatives may include the evaluation of various commercial hypothermic storage and cryopreservation media products in comparison to in-house formulated cocktails. Particular attention should be paid to the true preservation efficacy of the products. This can be measured in split sample comparisons using assays capable of accurately indicating post-preservation viability and functional recovery, as well as the duration of stability of the biologic enabled by the preservation media. Also, the quality and regulatory footprint of the biopreservation media products and in-house formulations should be compared for robustness. Quality of components (USP grade or multi-compendial, serum-free, protein-free), cGMP manufacturing, FDA/regulatory agency familiarity (i.e., Master File, technical file), and finally, a robust set of release criteria (sterility, endotoxin, and cell-based assays) should be evaluated when selecting or changing biopreservation media used in cell therapy product development and commercialization.

Figure 3 illustrates the 72-hour stability footprint of human fibroblasts enabled by three commercial hypothermic storage media. Fluorescent staining for cytoskeletal integrity, mitochondrial activation, and cell nuclei is clearly visible in the micrographs.



HypoThermosol® FRS *Viaspan* *Celsior®*

Figure 3

System optimization via utilization of best-in-class preservation media has a flow-through effect and impact on the yield-cost relationship. The ability of optimized preservation media products to highly leverage source material and finished product cost, yield, and stability, impacts final product distribution and profit potential.

EVOLUTION OF BEST PRACTICES IN BIOPRESERVATION, *Continued from page 7.*

acquisition of biologic source material as well as transportation and storage of finished therapeutics offers the potential for cost savings, improved quality, and expanded delivery geography.

More information on optimized preservation solutions and a protocol for the collection of vascularized tissues can be viewed at: http://www.biolifesolutions.com/products/protocols.htm.

In the next issue of BPT, Part 3 of this article series will discuss historical and emerging best practices for blood collection and storage.

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4 *National Cancer Institute Best Practices for Biospecimen Resources. NCI, NIH, USDHHS. June 2007*

BioLife Solutions develops and markets patented hypothermic storage/transport and cryopreservation media products for cells, tissues, and organs. BioLife's proprietary HypoThermosol® and CryoStor™ 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.