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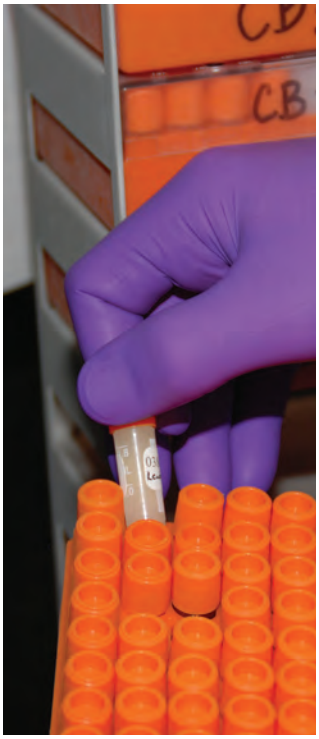
FEATURE ARTICLE
THE EVIDENCE-BASED BIOBANKING MOVEMENT

What's Inside:

In This Issue



RESOURCE CENTER

www.isber.org | *International Society for Biological and Environmental Repositories*

www.celltherapyblog.com | *For Executives in the Cell Therapy and Regenerative Medicine Industry*

www.celltherapynews.com | *Promoting the Field and Facilitating the Exchange of Information and Resources within the International Cell Therapy Community*

UPCOMING EVENTS

ISCT Somatic Cell Therapy Symposium *www.celltherapysociety.org/ISCT_Meetings/Somatic_Cell_Therapy_Symposium*	Bethesda, MD	Sept 14-15, 2009
IIR Biorepositories Conference *www.iirusa.com/biorepositories*	Philadelphia, PA	Sept 21-23, 2009
BEST Collaborative Meeting *www.bestcollaborative.org/meetings/upcomingmeetings.html*	New Orleans, LA	Oct 21-23, 2009
AABB Annual Meeting & TXPO *www.aabb.org/Content/Meetings_and_Events/Annual_Meeting_and_TXPO*	New Orleans, LA	Oct 24-27, 2009
CHI Science of Biobanking *http://www.healthtech.com/bnk*	Philadelphia, PA	Nov 16-17, 2009

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EDITOR'S CORNER

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

Thank you for reading our Summer 2009 issue of BioPreservation Today. Our feature article focuses on current unmet needs and the push for adoption of evidence-based practice and best practices in the biobanking field. Well known industry consultant and 2009 ISBER Councilor Lisa Miranda conducted interviews with two leaders in the biorepository field and authored our feature article. I'd like to thank Doug Fugman from the Rutgers Cell & DNA Repository, and Fay Betsou from Biobank de Picardie for participating in the drafting of this article and for recognizing how our pre-formulated, serum-free, and protein-free HypoThermosol® and CryoStor™ biopreservation media products can support the implementation of evidenced-based practice and biopreservation best practices.

I'd also like to thank our multitude of customers for your business. We take much satisfaction from our opportunities to consult with you to optimize your biopreservation processes to improve yield, lower costs, and improve outcomes. To our prospective customers, we stand ready to assist you in extending the stability and improving the post-preservation yield of your source material and isolated/finished cell products.

We take our mission very seriously and have built a corporate culture and quality system to provide best-in-class biopreservation media products.

Best regards,

BioLife Solutions®
POWERING THE PRESERVATION SCIENCES

BIOLIFE SOLUTIONS, INC. MISSION STATEMENT

We strive to be the leading provider of biopreservation tools for cells, tissues, and organs; to facilitate basic and applied research and commercialization of new therapies by maintaining the health and function of biologic source material and finished products during the preservation process.

EVALUATIONS BY LEADING RESEARCH CENTERS INDICATE SIGNIFICANT ADVANTAGES OF CRYOSTOR™

Reprinted from the March 10, 2009 BioLife Solutions Press Release

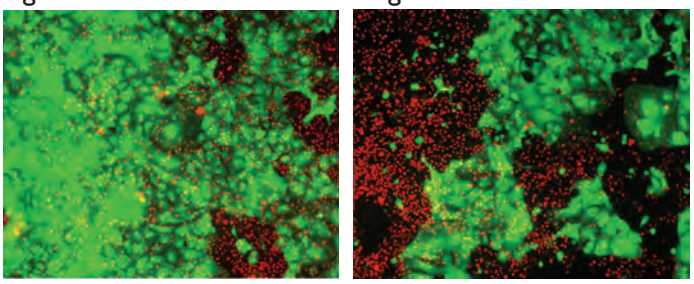
An independent European comparison of BioLife's CryoStor™ pre-formulated serum-free and protein-free biopreservation media against traditional in-house formulated culture media/serum/DMSO showed CryoStor offers a significant cryopreservation process improvement and better cellular outcomes. Compared to media/serum/DMSO, CryoStor enabled enhanced post-thaw cell membrane integrity and a full recovery of metabolic activity and differentiation capacity within 24 hours after thawing.

The study, titled "Cryopreservation of Adherent Cells: Strategies to Improve Cell Viability and Function after Thawing," is to be published in the September 2009 issue of Tissue Engineering Part C. This reports the results of experiments conducted by research teams at the Fraunhofer IBMT in St. Ingbert/Sulzbach, Germany and IBET/ITQB-UNL in Oeiras, Portugal.

The study findings also confirm that despite improved cell recovery immediately after thawing for media/serum/DMSO cryopreserved cells beneath alginate, up to 50 percent cell death still occurred within 24 hours post-thawing. The authors agreed with other published data describing post-thaw survival overestimates, and suggest that the decrease in cell

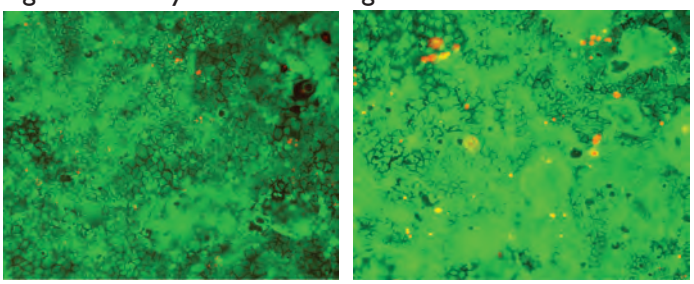
EFFECT OF CRYOPRESERVATION MEDIUM AND ALGINATE ENTRAPMENT ON THE MEMBRANE INTEGRITY OF CACO-2 CELLS AFTER CRYOPRESERVATION.

Figure 1a - Culture Media and Alginate



Immediate Post-Thaw *Day 1 Post-Thaw*

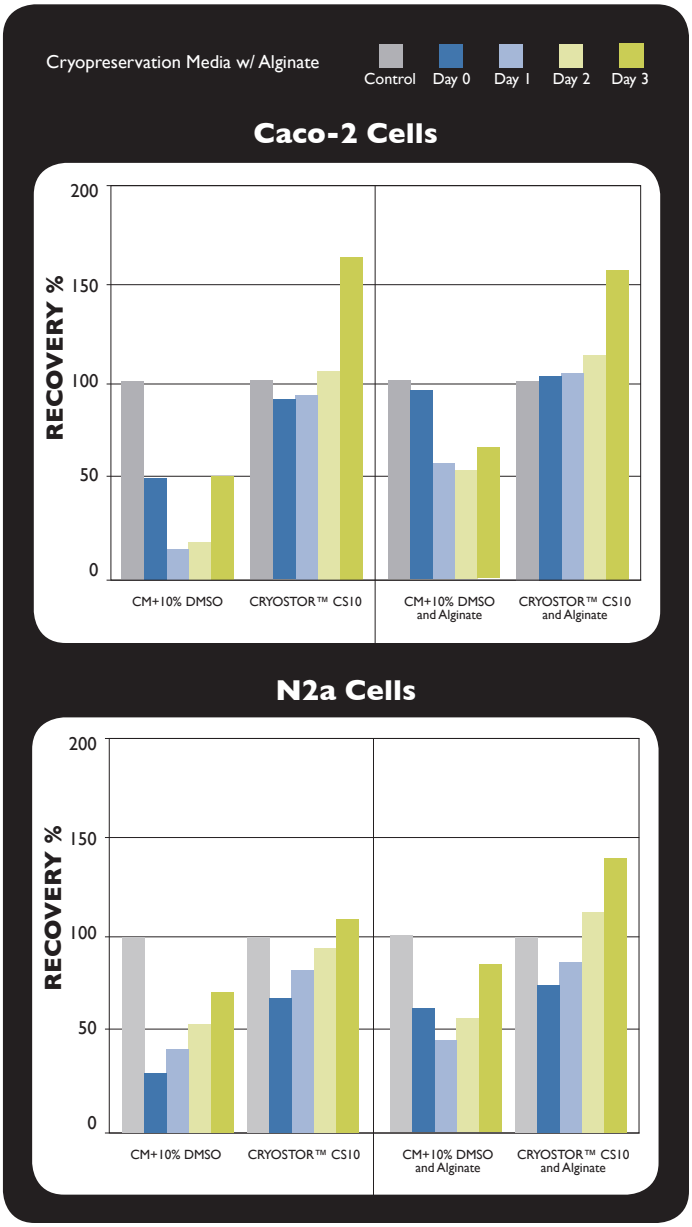
Figure 1b - CryoStor™ and Alginate



Immediate Post-Thaw *Day 1 Post-Thaw*

Green - Fluorescein Diacetate
Red - Ethidium Bromide
Red Cells - Damaged Membranes
Green Cells - Undamaged Membranes

Figure 2



viability might be related to sequential apoptotic and necrotic processes not evident immediately subsequent to thawing. This supports very early BioLife research and discoveries of the phenomena of preservation-induced, delayed onset cell death.

Mike Rice, BioLife's chairman and CEO, noted "The tissue engineering market is highly strategic for BioLife. Our products have great potential to resolve the current cell yield and shelf-life issues that often limit large scale commercialization and clinical distribution of new biologic-based therapies. We currently supply CryoStor™ and HypoThermosol® to several start-up customers and support product evaluations at other key companies in the tissue engineering market."

According to a 2009 analysis and report by Life Science Intelligence, the largely untapped global market potential for tissue engineering and regenerative medicine products will exceed $118 billion by 2013. The actual current market, which represents only a fraction of the potential market, was estimated at $1.5 billion in 2008. The report forecasts rapid growth driven by various factors including increased adoption in various clinical areas and international market trends.

Figure 1 and data for Figure 2 are original, unpublished, and provided by ITQB. Used with permission.

BIOLIFE EXPANDS CAPABILITIES WITH UNVEILING OF NEW MANUFACTURING FACILITY

BioLife Solutions announced that it has completed the construction, validation, and startup of its internal GMP manufacturing facility and has released several production lots of its biopreservation media products for sale.

In the third quarter of 2008, BioLife announced that it was transitioning from using a contract manufacturer to internal production in order to reduce production costs and enable custom packaging and formulation offerings to the growing market for biopreservation media products.

BioLife's Quality System and nearly 6,000-square-foot GMP production facility incorporates a uni-directional workflow design that was finalized with the input of several clean room consultants, BioLife's Quality and Scientific teams, and members of its Regulatory Advisory Board. The facility consists of ISO14644 classified airlocks and rooms for product formulation, filling, final inspection, and cold storage; as well as other mixed–and dedicated–use space including research and development and quality control laboratories, and order fulfillment space. All critical systems are supported by auto-switched generator power. Annual capacity is estimated at up to 12,000 liters.

THE EVIDENCED-BASED BIOBANKING MOVEMENT

by Lisa Miranda, President, Biobusiness Consulting, Inc.

One of the biggest risks for biospecimen collections today is suboptimal biopreservation. Biobanking, considered by many to be a bridge science between basic and applied research, requires meticulous foresight and arduous dedication. The work of the biobanker is never-ending–a high maintenance activity, one would say. In an effort to fulfill their research mission, biobankers strive for excellence in all things. The germane term is "best practices": a continuous quality improvement approach intended to ensure provision of a framework of daily biobanking practice that promotes high value biomedical research. For the biobanker, every sample is precious, a potential "missing link" to the "cure", highlighting mechanisms of infinitely abounding disease. Now with the global advent of personalized medicine, the charge for best-practices has never been higher.

Despite current advances in biobanking, the reality is that the true individual quality of many collections, both historical and prospective, remains unknown. Lack of universal standards further compound ambiguity as to the measurable aggregate value of existing local as well as global biosample collections. Emerging efforts in biobanking best-practices have attempted to address this issue, via harmonization of standard processing approaches and integration of method validation of current biopreservation techniques. Still, disparity remains in regard to both prevalence and formal implementation of evidence-based practice. Evidence-based practice is the timely process of implementing validated scientific discoveries into daily techniques and procedures to support definition of relevant research gold standards, also referred to as benchmarks, with the aim of enabling scientific discoveries that foster refinements in patient care and overall treatment outcomes.

Biobanking evidence-based practice involves collection, interpretation and integration of research derived evidence from biospecimen analyses, otherwise known as biospecimen science. In fact, the biobanks' tremendous potential to support biospecimen science and advance evidence based practice has been recognized worldwide as common priority by nations and associated institutions alike. While the race for evidence-based practice is underway, many believe that standardizing current biobanking practice and performing method validation are the initial common sense approaches to "bridge the gaps". In an attempt to elucidate the rationale, requirements, current applications and potential benefits of the "Evidence-Based Practice Movement", I interviewed two of its prominent pioneers, Dr. Doug Fugman, Managing Director of the Rutgers University Cell and DNA Repository (RUCDR), based in Piscataway, New Jersey, USA, and Dr. Fay Betsou, R & D Lab Manager at Biobanque de Picardie, based in Amiens, France.

DIRECT IMPACT OF SUBOPTIMAL BIOPRESERVATION ON BIOMEDICAL RESEARCH

At the commencement of each of our discussions, I asked both Dr. Fugman and Dr. Betsou to quantify the direct impact of suboptimal biopreservation on biomedical research.

Dr. Fugman stated that "Suboptimal biopreservation has a direct impact on biomedical research resulting in the loss of valuable research materials, which drives up the cost and extends the time frame of scientific discovery and medical advances. From a historical perspective our laboratories have been asked to take on

externally-produced cell lines and recover them to provide biomaterials for research investigators. These materials came from reputable institutions; however, it is unfortunate that in many cases these biomaterials were of suboptimal quality. Poor cryopreservation and cryostorage techniques resulted in suboptimal post-thaw viability and recovery with anywhere from 5% to 50% sample loss. In many cases samples from these subjects were not able to be acquired again. In our case, to establish a cell line and DNA sample from a fresh blood sample is about 7% to 10% of the cost of acquiring the samples. The other 90% to 93% of the cost is in the clinical setting working with the families and collecting the annotation data. The loss of a single sample can cost literally thousands of dollars, but more costly is the loss of the materials for scientific discovery."

Dr. Betsou concurred with Dr. Fugman that suboptimal biopreservation can dramatically increase the risk of loss of historical collections, possibly with catastrophic effects. She relayed her past expertise of evaluating the quality of a small collection stored in low-quality media for 10 to 15 years in which the serum and fluids were completely evaporated. "If the quality control testing demonstrates zero viability, the media may not be okay. Therefore, not only is the choice of preservation medium significant; but biobankers should also aim to quantify and manage pre-analytical variables in order to diminish risk of potential bias."

CRITICAL FACTORS FOR OPTIMIZING POST-PRESERVATION VIABILITY

I asked Dr. Betsou if she could clarify some of the critical factors related to optimizing post-preservation viability. She noted that critical factors in post-preservation cell viability include but are not limited to pre-analytical factors such as lag time in collection and processing, deficits in traceability, and in the case of cell culture, maintaining appropriate transport temperatures of the primary samples (e.g. whole blood) under 20 to 25°C. She stressed that it is absolutely crucial to remember that cell death can manifest at a prolonged temperature of 4°C. Dr. Betsou also noted that ironically, it is commonplace that one focuses so intensely on preventing unnecessary cell exposure to high ambient temperature, that they can fail to regularly exercise caution in regards to transport temperatures being too cold. Furthermore, one can forget to focus their preventative efforts on prevalent cryopreservation issues such as ice crystallization and "freezer burn". Often, the laboratory workload further complicates such issues. For example, unduly heavy workloads second to limited staff can contribute difficulties in preventing overexposure to DMSO. This tends to be a shared problem in most biobanks.

Dr. Fay Betsou, R & D Lab Manager, Biobanque de Picardie

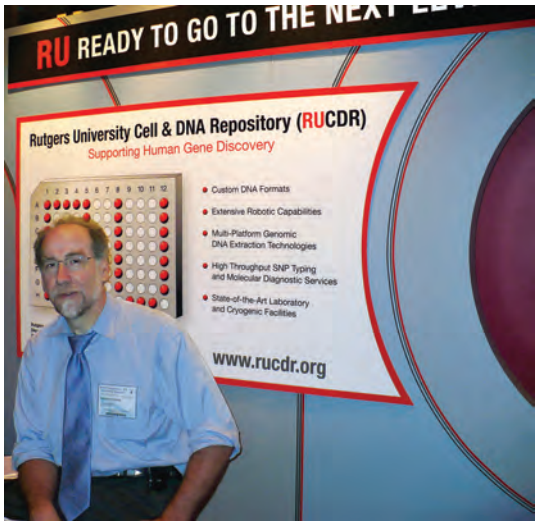
Quality Lab at Rutgers University Cell and DNA Repository.

MANAGING LOGISTICS OF CRYOPRESERVATION OF CELL LINES

It appears that the largest challenge in regards to promoting quality preservation is managing logistics in processing. At RUCDR where they typically cryopreserve 300 to 400 cell line cultures per day, three times a week, coordination, teamwork and validated protocols are essential. The process described by Dr. Fugman is quite complex: "Samples have to be screened, processed, re-suspended in cryopreservation solution, accurately loaded into cryovials, cryopreserved in a controlled rate freezer, and placed in assigned liquid nitrogen storage locations. Every step is critical and sample processing needs to flow smoothly, efficiently, and accurately from beginning to end. Every cryopreservation of a cell line must have an extra cryovial for post-thaw viability testing. We do not consider a cell line successfully cryopreserved unless it demonstrates post-thaw growth. Utilizing a high-quality controlled and validated cryopreservation solution is just one of the factors in this equation." In addition, Dr. Betsou noted that temperature fluctuation traceability of manual "cherry picking" i.e., how many times one removes a single tube from the cryogenic tank, is almost impossible, further complicating quality assessment and interpretation of artifact.

LESSONS LEARNED FROM BIOPRESERVATION

I asked Dr. Fugman and Dr. Betsou about what "lessons learned" they could convey to colleagues in regards to biopreservation. Both were in consensus that one protocol does not work for all cell/tissue types, and that vigilance with quality control and testing is essential to prevent low sample integrity and reduce the risk of sample loss. Dr. Betsou quoted a French expression, "Oui-dire" which literally translates to mean "rumors" or "on hearsay" to make a point that while current theories exist, there is no conclusive proof. In essence, many in biobanking feel that researchers are basing crucial decisions "on hearsay" – hence the current endeavor to acquire further scientific evidence to support biobanking protocols. For example, DMSO has historically been known to be toxic, but it would be beneficial to possess documented proof regarding range of toxicity and its effect on the biospecimen. It is also difficult to discern the best method of controlled rate freezing. There are some in the scientific field that believe biological material such as tissue may be as robust as serum. For now, researchers must consider these all interesting theories and aim to validate and/or rule out such beliefs with documented proof. Too often biopreservation and biobanking/theory and practice are not congruent. Actions need to be dramatically improved. For example, best practice theory highly advocates rigor in implementing quality control at all points in the process chain. This is often known as "QC in and out" and can be nearly impossible for many biobanks.



Dr. Doug Fugman, Managing Director of the Rutgers University Cell and DNA Repository

DOMINANT ISSUES IMPEDING OPTIMAL PROCESSING AND ANALYSIS

For Dr. Fugman, whose high-throughput laboratory typically cryopreserves an average of 900-1,200 newly established lymphoblastoid cell lines a week, the chief concern with cryo and biopreservation is maximization of bio-sample integrity and viability while limiting minimal cytotoxic or degradative effects. He believes that it is critical to maintain high quality control standards at all times and to have a thoroughly validated cryopreservation solution that maximizes post-thaw viability and recovery. It has been his experience that sub-optimal post-thaw viability and recovery impedes sample processing, staging, and analysis. In harmony across the ocean, Dr. Betsou's team takes the same approach. Her lab focuses on method validation, particularly if the downstream analysis is undefined, and it is the only surefire parameter of analyses. Both Dr. Fugman and Dr. Betsou's labs have implemented CryoStor™ in their current operations as a solution to support standardization of their protocols for specimen processing for national research projects.

IMPETUS FOR CRYOSTOR™

Both Dr. Fugman and Dr. Betsou's laboratories spent significant time performing pilot studies to investigate the performance and quality of CryoStor, and both noted positive results. Dr. Betsou found that CryoStor demonstrated superior metabolic activity measured via ATP production. Dr. Fugman experienced improved overall success with cell recovery.

RUCDR implemented CryoStor into mainstream operations to support a majority of its work for four United States NIH Divisions: National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institute on Drug Abuse (NIDA), National Institute on Alcohol Abuse and Alcoholism (NIAAA) and National Institute of Mental Health (NIMH). For RUCDR, "the decision to transition from an in-house formulated media containing culture media and fetal bovine serum to CryoStor was due to the need to optimize cell processing protocols to improve the quality of cell lines"… and was based on "improved recovery and elimination of serum in cryopreservation in cell lines."

I Asked Dr. Fugman to explain in further detail the impetus for RUCDR's decision: "The RUCDR had been utilizing a cryopreservation solution that contained fetal bovine serum (FBS) and DMSO for many years. Because FBS is a biological reagent, it may potentially carry rare pathogens that could contaminate cells or present immune cytotoxicity (i.e., cytotoxic antibodies). By minimizing exposure to biological reagents, the risk of contamination or cytotoxic effects would be reduced. Also because the current cryopreservation medium is made in the lab in smaller batches, there is a greater possibility of human error that could have detrimental effects. In addition FBS cost fluctuates widely with market economy making it difficult to predict cost from a purchase of one batch to another. Our goal was to evaluate serum-

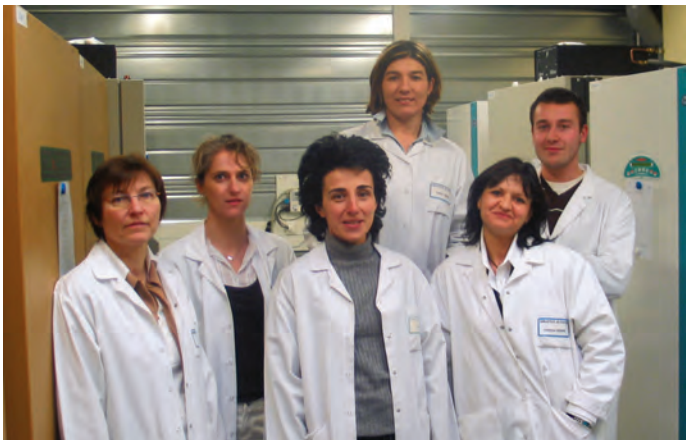
free reagent-grade products that were manufactured under stringent quality controlled conditions. BioLife's CryoStor CS5™ was one of these. We performed validation studies in our laboratories to compare the effectiveness of CS5 with our standard cryopreservation solution. CS5 demonstrated similar post-thaw viability to our standard solution, however cell lines cryopreserved in CS5 showed better (i.e., shorter) doubling times over a five day period post thaw. This indicated that there were less cytotoxic effects of the cryopreservation process with CS5. These validation studies were presented as an abstract and poster at the ISBER 2008 annual meeting in Bethesda, MD."

Biobanque de Picardie uses CryoStor on a project for the Institut National de Veille Sanitaire (INVS) to support processing of the INVS's Hematology collection. For this project, it was necessary to adopt a medium devoid of animal proteins and serums i.e., CryoStor to support processing of L'INSERM's Hématologique collection. The rationale was that use of a protein- and serum-free medium would hopefully reduce bias and any potential confounders in the analysis. Dr. Betsou's interest was also peaked by reading some of BioLife's earlier studies on cryopreservation.

CHOOSING BIOLIFE SOLUTIONS® AS A PRESERVATION PARTNER

In Dr. Fugman's words: "Our validation studies demonstrated that CryoStor was as effective or better than our standard in-house cryopreservation solution, and it could be obtained as a standardized high-quality controlled reagent-grade product. This did not necessarily make it a certainty. There were other factors to consider such as availability in the format and quantities the RUCDR required as well as cost considerations."

Dr. Betsou was equally impressed by the quality of the product, its scientific value; and the benefits of a standard formulation. They both felt that BioLife's open minded, collaborative approach and range of scientific expertise was rare and very welcome.



Dr. Fay Betsou with her fellow staff members at the Biobanque de Picardie, France.

CRYOSTOR™ AS A BIOPRESERVATION BEST PRACTICE SOLUTION AND ADDED VALUE FOR BIOSPECIMEN RESOURCES

I asked Dr. Betsou and Dr. Fugman what generalizable value they thought CryoStor might offer to assist biobanking best practice techniques. Dr. Fugman noted that "utilizing a high-quality controlled reagent-grade product that has been thoroughly validated for a particular process provides enhanced consistency, reproducibility, and quality assurance." Dr. Betsou noted that it "may offer help with advancing evidence-based practice, and decrease some of the variance second to disparity in technician training…" she went on to say that it was "reasonable to suspect that it may increase the frequency of observation of meaningful, reproducible data" and "perhaps diminish some of the artifact and/or "noise" in regards to analysis, specifically regarding differing variability in gene expression. CryoStor's ability to promote standardization may be compatible for implementation into proficiency programs. Further exploration of such biopreservation media could increase awareness and scientific understanding of the apoptosis pathway. Those involved in the Evidence-Based Practice Movement have much to look forward to in the standardization and validation of bioprocessing techniques.

Lisa B. Miranda, President, Biobusiness Consulting, Inc, works as a Biospecimen Resource Consultant for government (NCI OBBR), industrial, nonprofit and academia sectors worldwide. Ms Miranda also serves as a global Biobanking professional on the ISBER Board of Directors, ISBER Education/Training and Long Range Planning Committees, and Marble Arch International Working Group on Biobanking for Biomedical Research, including membership in related scientific societies such as P3G, LRIG and HUPO. Fourteen years of her 19 year career were spent working in research at the University of Pennsylvania, located in Philadelphia, Pennsylvania, USA in which her most recent role was as the Technical Director and Creator of the TTAB Core Facility.

EVOLUTION OF BEST PRACTICES IN BIOPRESERVATION

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

PART 3: CONSIDERATIONS IN REPRODUCTIVE CELL CRYOPRESERVATION – EVIDENCE-BASED PRACTICES

The pioneering discovery in 1948 that glycerol enabled survival of spermatozoa after freezing to minus 70°C[1] represents the foundation of our present understanding of reproductive cryobiology. Sperm, oocytes, and embryos from a number of mammalian species, including humans, have since been frozen and recovered with mixed success. The reasons for differential success between oocytes and embryos are not entirely certain; however, variability between species and individuals within a species make it extremely difficult to apply a single cryopreservation method for reproductive cells. As with other cell types, factors that must be considered in order to maximize survival include the following: the cryopreservation medium (CM) composition, equilibration time with cryoprotectant, method and extent of cellular dehydration, and the freezing and thawing rates.

The CM composition is quite simply a buffered medium that serves as a carrier for one or more cryoprotective agents (CPAs). Two broad categories of CPAs exist: "permeating", which can cross the plasma membrane (e.g., glycerol, dimethylsulfoxide, propanediol, ethylene glycol, etc.) and "non-permeating" (macromolecules, sugars, etc). The functions of a CPA are to increase the extracellular osmolality to promote dehydration during cooling and to bind residual water thereby preventing the deleterious effects of intracellular ice formation (IIF). Though necessary under current practices, CPAs have their own toxicity that is associated with the temperature, concentration, and time of exposure.

In addition to the CPA, the buffering mechanism of the cryopreservation medium is especially important with regard to reproductive cells, which are sensitive to changes in pH. Compositions based on phosphate buffering systems are not recommended due to poor pH buffering at lower temperatures; instead, zwitterionic buffers should preferentially be used.[2] Commonly used zwitterionic buffers are HEPES, TES, and TRIS. In combination, TES and TRIS are often referred to as TEST; when combined with egg yolk, citrate, and glycerol (as the CPA) it produces TEST-yolk-glycerol (TYG), which has become the standard for semen preservation.[3]

The freezing process is a complex orchestration of interrelated events (reviewed in 4,5). Briefly, when a CM containing a permeating CPA is added to a mixture of cells, an osmotic gradient is generated that causes rapid efflux of water and shrinking of the cells. Because the CPA enters the cells more slowly than water can leave, the cells gradually return to their isotonic volume. If the temperature is reduced too quickly or too slowly during the equilibrium phase, the cells may not have completely dehydrated or returned to isotonic volume and consequently will not be properly prepared for freezing. Removal or dilution of the CPA upon thawing causes a similar reverse phenomenon. These rapid changes in cell volume can lead to irreversible damage of the cytoskeleton and even rupture of the membrane. Reproductive cells are especially sensitive to osmotic stress; therefore, common practice is to utilize a drop-wise addition of the CM to the cells under continuous mixing. Similarly, if the post-thaw product is not to be directly administered, dilution of the specimen should also be performed drop-wise, preferably with a non-phosphate buffered solution to control for pH shifts.

THE FREEZING PROCESS IS A COMPLEX ORCHESTRATION OF INTERRELATED EVENTS.

Two primary schools of thought exist with respect to freezing and thawing rates – vitrification and slow freezing. Vitrification involves the exposure of a biologic to high concentrations of CPAs for short periods of time in order to quickly dehydrate the cell, followed by rapid cooling such that any residual water proceeds immediately to the glass-transition state and becomes solid without the possibility of ice crystallization.[6] Though crystallization does not occur during freezing; unless the cells are rapidly rewarmed, ice crystallization can occur during thawing while the solution is near the

glass-transition temperature. The high concentrations of CPA and the difficulty of homogenous rapid rewarming have favored the use of slow freezing.

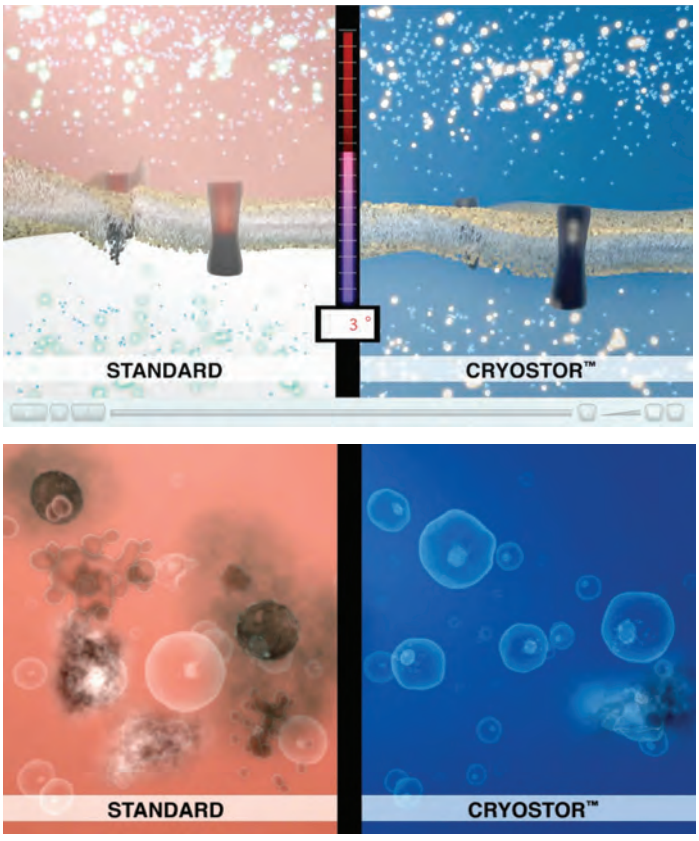
Evidence-based practices point to the following conclusions with respect to preservation of reproductive cells: No single cryopreservation media or method has proven successful among all reproductive cells, primarily due to extraordinary variability among gametes and embryos from different species and among individuals within a species. The inherent differences between sperm, oocytes, and embryos demand individualized optimization of cryopreservation conditions.

Future improvements in this field will likely be directed to identifying less toxic CPAs and further delineating the molecular events that contribute to non-functional reproductive cells and tissues after thawing with the hope of improving fertility preservation.

An additional consideration is what the consequences are of using biopreserved reproductive cells/embryos at the level of a produced organism (i.e., is there any increased risk of disease, shortened lifespan from accelerated aging, etc.). Humans conceived from frozen in vitro reproductive cells and tissues are still relatively young – the first successful birth from cryopreserved embryo was in 1983, oocytes in June 2007[7], and ovarian tissue in July 2009.[8] Therefore, any effects of biopreservation injury may not become apparent for some time.

1 Polge C et.al. 1949. Nature 164: 666-76
2 van den Berg L and Rose D. 1959. Arch Biochem Biophys 81; 319-29
3 Stanic P et.al. 2000. Eur J Obst Gyn Reprod Biol 91; 65-70
4 Life in the Frozen State. 2004. CRC Press LLC chs 1, 11-14, and 18.
5 Gao D et.al. 1997. Reproductive Tissue Banking. Academic Press, New York.
6 Rall WF and Fahy GM. 1985. Nature 313; 573-5
7 European Society of Human Reproduction & Embryology's 23rd annual meeting, July 1-4, 2007
8 European Society of Human Reproduction & Embryology's 25th annual meeting, June 28-July 1, 2009

BIOLIFE ANIMATION DRAWS ATTENTION



Recently BioLife Solutions teamed with Cosmocyte, a leading developer of medical and scientific visualization solutions, to create a Flash animation video illustrating preservation-induced cellular stress responses from exposure to very low temperatures and cryoprotectants such as DMSO. The one minute animation, hosted on BioLife's website at **www.biolifesolutions.com/flash/cryostormovie.htm**, includes voice-over narration which describes the effects on cells with the introduction of DMSO and reduced temperatures, including cellular dehydration, reduced metabolism and ion pump activity, and changes in cell membrane permeability.

In side-by-side video panes, the animation illustrates a comparison of cells undergoing cryopreservation in a non-optimized process, as well as a model using BioLife's CryoStor™. CryoStor is an intracellular-like preservation media formulated to protect cells from preservation induced injury by balancing cellular ionic concentrations at low temperatures, and enabling a balanced osmotic hydration upon thawing. This protection reduces swelling and membrane rupture. Other components in serum-free and protein-free CryoStor minimize necrotic and apoptotic cell death.

IMPROVEMENT IN RECOVERY AND FUNCTION OF PERIPHERAL BLOOD STEM/PROGENITOR CELLS

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

Peripheral blood stem cells (PBSC's) are a preferred stem cell source for hematopoietic transplantation therapy. Cryopreservation of PBSC's is a common and important step in clinical practice but is still fraught with therapeutic efficacy constraints due to preservation induced cell damage that may limit engraftment and also patient exposure to high concentrations of DMSO if no wash step is performed. In a recent collaborative study, the CryoStor® family of novel, intracellular-like, fully defined, serum- and protein-free biopreservation solutions were compared to standard methods and an in-house formulated freeze media used by the Fred Hutchinson Cancer Research Center (FHCRC) for cryopreserving PBSC's.

In the study, human PBSC samples were collected via apheresis and prepared for cryopreservation in the following solutions:

1) FHCRC standard – Normosol, HSA, 20%, final DMSO concentration of **10%***
2) CryoStor CS5 - no serum or protein, final DMSO concentration of **2.5%***
3) CryoStor CS10 – no serum or protein, final DMSO concentration of **5%***

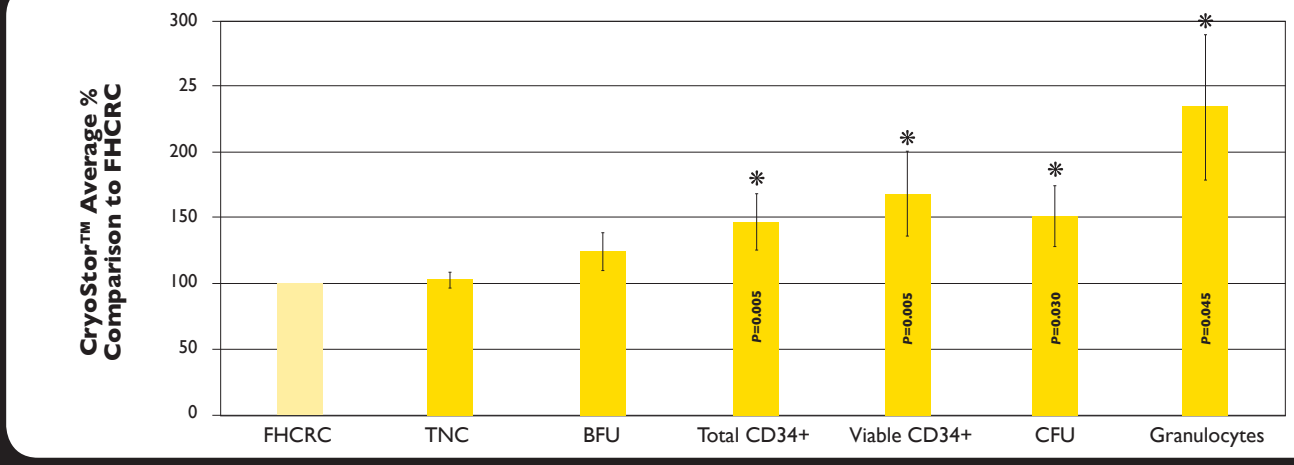
Each solution was diluted 1:1 with Normosol. * Final DMSO concentration.

Following standard controlled-rate freezing and storage in a liquid nitrogen freezer, samples were thawed and evaluated for TNC, BFU-e, CFU, CD34, and granulocytes. The CS5 (2.5% DMSO) results were comparable to FHCRC (10% DMSO), while CS10 (5% DMSO) resulted in significantly improved recovery. Specifically, preservation with CS10 yielded a significant increase in total CD34+ (47%), viable CD34+ (68%), CFU (51%) and recovered granulocytes (234%).

The results of the collaborative study demonstrate that use of CryoStor can provide significantly improved recovery of stem/progenitor cells in PBSC samples when compared to standard cryopreservation methods. In addition to eliminating serum and serum-derived proteins, CryoStor enabled improved overall viability and cell function with half the DMSO concentration. These findings suggest incorporation of CryoStor into standard protocols for cryopreserved storage of PBSC's could ultimately lead to improved engraftment, reduce the need for additional apheresis collections, and decrease the risk of adverse infusion reactions for patients undergoing autologous transplantation.

Clarke DM, Yadock DJ, Nicoud IB, Mathew AJ, Heimfeld S. Improved post-thaw recovery of peripheral blood stem/progenitor cells using a novel intracellular-like cryopreservation solution. Cytotherapy. 2009; 11(4):472-479.

CRYOSTOR™ CS10 IMPROVES POST-THAW OUTCOMES OF PBSC'S



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.

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