

# Preservation of Cell-based Products for Human Therapeutic Applications

By Allison Hubel, PhD and Marilyn S. Waxberg

Cell-based products for the treatment of human disease continue to grow in prevalence. Hematopoietic stem cell transplantation is used to treat tens of thousands of patients every year, with the majority of growth in this field fueled by nontraditional hematopoietic stem cell products.<sup>1</sup> Hundreds of thousands of patients have been treated with tissue-engineered skin substitutes.<sup>2</sup> More than 10,000 patients also have been treated with cell-based therapies to treat defects in articular cartilage. An increasing number of patients with Diabetes Mellitus (Type I) are being treated with islets of Langerhans transplants.<sup>3</sup> Clinical studies are examining the performance of hematopoietic, non-hematopoietic and muscle-derived cells for the treatment of myocardial infarctions.<sup>4</sup> The large number of cell-based products in development insures that the number of patients receiving this type of treatment will continue to grow.

The production and distribution of these and many other cell-based therapies in development rely upon or are limited by the ability to effectively preserve these products. Short-term preservation frequently is needed to permit transportation of the product from the site of harvest to a processing or manufacturing facility. For example, babies are delivered at a variety of locations and at different times; umbilical cord blood is collected at the delivery site and shipped to a central processing facility where it is routinely red blood cell depleted and cryopreserved. The cord blood must be shipped under conditions that permit

subsequent processing and result in minimal changes in post thaw viability. Cell preservation is also used during the manufacture of certain cell-based products. Dermal fibroblasts and keratinocytes harvested from neonatal foreskins are cultured and cryopreserved to create a master cell bank from which tissue engineered skin is produced.

The most common need for preservation of cell-based therapies comes after the manufacturing process is completed. Cell preservation is needed to permit product safety and quality control test completion prior to release. These therapies are used to treat very ill patients who may not be able to receive the therapy when the product is ready.

The ability to preserve a cell-based product facilitates coordinating therapy with patient care requirements. Finally, preservation of cell-based products permits product transport from the manufacturing site to patient locations (hospital, doctor's clinic, or battlefield). Thus, the ability to effectively preserve a cell-based therapy is critical for its clinical and commercial application.

The preservation of cell-based products for human therapeutic applications has its own unique challenges. For example, specialized solutions are used to improve post-thaw cell survival. Conventional cryopreservation solutions contain tissue culture media, a cryoprotective agent (typically dimethylsulfoxide) and fetal calf serum. None of these components are approved for human use. Both dimethylsulfoxide (DMSO) and fetal calf serum are associated with adverse reactions upon reinfusion into humans.<sup>5,6</sup> Substitutes for tissue culture medium and fetal calf serum have been found for clinical hematopoietic stem cell cryopreservation.<sup>7-9</sup> Reducing and/or eliminating DMSO in cell products is an ongoing research subject; several different approaches are being pursued including validating reduced-DMSO protocols, increasing the concentration of cells in cryopreserved products (and thereby the total dose of DMSO infused), using naturally occurring sugars to provide cryoprotection and post-thaw cell processing to remove DMSO.<sup>10</sup>

Blood cell transfusion and tissue/organ transplantation have been used for decades to treat trauma and disease. Cell-based products in development differ significantly from native tissues and minimally manipulated cell products. Cells may be cultured *ex vivo*, depleted or enriched for specific subpopulations, or even seeded into an extracellular matrix construct to reconstitute a tissue equivalent. The biological properties of these products differ from their native equivalents.<sup>11</sup> For example, skin equivalents used to treat such dermal injuries as ulcers or burns are avascular. The density of cells and extracellular matrix differs greatly from that of native tissue. These distinct differences may imply that protocols developed for cadaveric skin may not be appropriate for use with a tissue-engineered skin equivalent.

Cell-based products are also subject to regulatory oversight (21 *CFR* Parts 16, 1270 and 1271).



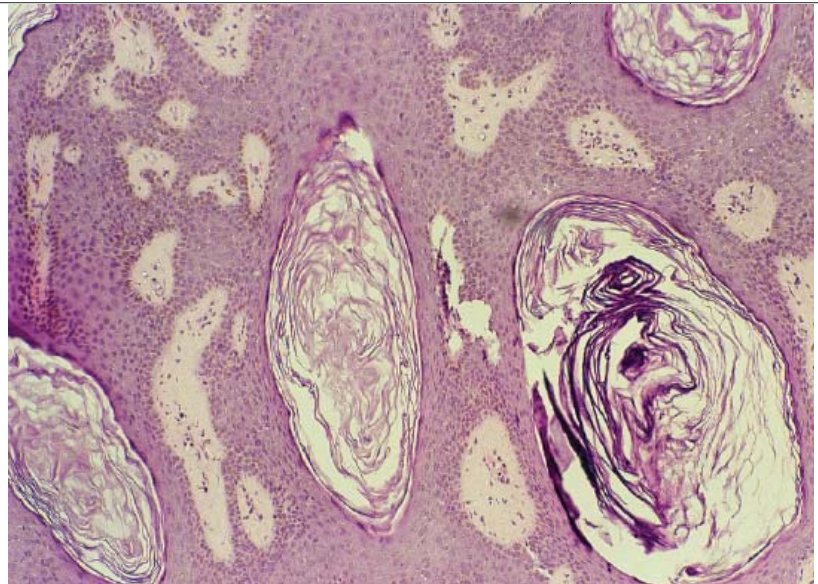
These products must be manufactured in compliance with current Good Tissue Practices (cGTPs) with additional requirements for Good Manufacturing Practice (GMP) banking, manufacturing and quality control, labeling, recordkeeping and reporting. Regulatory concerns include preventing improper processing that may result in damage to the cells and ensuring that highly processed cells are both safe and effective. Improper preservation protocols can damage the cells and thereby diminish the therapy's effectiveness.

Regulatory authorities look to industry to assist in furthering the technology for commercialization. However, it is academic institutions that focus on the basic research and innovative approaches to move the technology toward clinical application. It is important that these potential therapeutic clinical applications comply with regulatory requirements in order to become commercial products, i.e., academic institutions conducting research and development must provide adequate science-based regulatory documentation, meeting the regulatory requirements to support a submission subject to review by FDA or other agencies to evaluate safety and effectiveness. For example, a cell's full lineage and characterization, specific to a cell-based therapeutic would be required.

Resources to assist the development of cell-based therapy preservation protocols continue to increase. Standards for repositories (storage facilities for cryopreserved products), temperature monitoring during shipping and inventory control for low temperature storage have been developed by a variety of organizations, including the American Association of Tissue Banks, the American Association of Blood Banks and ASTM International. Recent guidelines for preserving cell-based products also provide guidance on the development of preservation protocols for tissue-engineered medical products (ASTM F2386-04).<sup>12</sup> FDA and the European Medicines Agency (EMA) have developed guidances for Human Somatic Cell Therapy and Gene Therapy. In addition, professional short courses are available to provide training for those interested in gaining basic or advanced scientific, technical and regulatory expertise in this field.<sup>13</sup> These resources will be important as the variety of cell-based products for human therapeutic applications continues to grow and effective preservation methods are essential for their widespread application.

#### REFERENCES

1. Read EJ, Sullivan MT. Cellular therapy services provided by blood centers and hospitals in the United States, 1999: an analysis from the Nationwide Blood Collection and Utilization Survey. *Transfusion* 2004;44:539-46.



2. Horch RE, Kopp J, Kneser U, Beier J, Bach AD. Tissue engineering of cultured skin substitutes. *J Cell Mol Med* 2005;9:592-608.
3. Nath DS, Hering BJ. Islet cells replacement therapy. *Clin Lab Med* 2005;25:541-56.
4. Zammaretti P, Jaconi M. Cardiac tissue engineering: regeneration of the wounded heart. *Curr Opin Biotechnol* 2004;15:430-4.
5. Davis J, Rowley SD, Santos GW. Toxicity of autologous bone marrow graft infusion. *Prog Clin Biol Res* 1990;333:531-40.
6. Selvaggi TA, Walker RE, Fleisher TA. Development of antibodies to fetal calf serum with arthus-like reactions in human immunodeficiency virus-infected patients given syngeneic lymphocyte infusions. *Blood* 1997;89:776-9.
7. Areman EM, Dickerson SA, Bender JG, Cullis H, Sacher RA. Use of licensed electrolyte solutions and anti-coagulant citrate dextrose for bone marrow collection. *Progress in Clinical and Biological Research* 1992;377:353-9.
8. Areman EM, Dickerson SA, Kotula PL, Spitzer TR, Sacher RA. Use of a licensed electrolyte solution as an alternative to tissue culture medium for bone marrow collection. *Transfusion* 1993;33:562-6.
9. Killian D, Wright P, Bentley SA, Brecher ME. A cost-effective and Food and Drug Administration-approved alternative to tissue culture media in cryopreservation. *Transfusion* 1996;36:476.
10. Fleming KK, Hubel A. Cryopreservation of hematopoietic and non-hematopoietic stem cells. *Transfus Apheresis Sci* 2005;in press.
11. Hubel A. Cellular Preservation - Gene Therapy, Cellular Metabolic Engineering. In: Baust JG, editor. *Advances in Biopreservation*. Boca Raton, FL: CRC Press, 2006.
12. ASTM F2386-04, "Standard Test Guide for Preservation of Tissue Engineered Medical Products (TEMPs)," ASTM International. For referenced ASTM standards, visit the ASTM website, [www.astm.org](http://www.astm.org), or contact ASTM Customer Service at [service@astm.org](mailto:service@astm.org). For Annual Book of ASTM Standards volume information, refer to the standard's Document Summary page on the ASTM website.
13. Preservation of Cells, Tissue, and Gametes, University of Minnesota, Biomedical Engineering Institute, information at <http://www.bmei.umn.edu/Events/ShortCourse.htm>

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