Cytotherapy, 2016; 18: 697–711

International Society for Cellular Therapy
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REVIEWS

Off the shelf cellular therapeutics: Factors to consider during cryopreservation and storage of human cells for clinical use

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Abstract

The field of cellular therapeutics has immense potential, affording an exciting array of applications in unmet medical needs. One of several key issues is an emphasis on getting these therapies from bench to bedside without compromising safety and efficacy. The successful commercialization of cellular therapeutics will require many to extend the shelf-life of these therapies beyond shipping "fresh" at ambient or chilled temperatures for "just in time" infusion. Cryopreservation is an attractive option and offers potential advantages, such as storing and retaining patient samples in case of a relapse, banking large quantities of allogeneic cells for broader distribution and use and retaining testing samples for leukocyte antigen typing and matching. However, cryopreservation is only useful if cells can be reanimated to physiological life with negligible loss of viability and functionality. Also critical is the logistics of storing, processing and transporting cells in clinically appropriate packaging systems and storage devices consistent with quality and regulatory standards. Rationalized approaches to develop commercial-scale cell therapies require an efficient cryopreservation system that provides the ability to inventory standardized products with maximized shelf life for later on-demand distribution and use, as well as a method that is scientifically sound and optimized for the cell of interest. The objective of this review is to bridge this gap between the basic science of cryobiology and its application in this context by identifying several key aspects of cryopreservation science in a format that may be easily integrated into mainstream cell therapy manufacture.

Key Words: *cell manufacturing, cellular shelf life, cellular stability, cellular therapeutics, cryopreservation, stem cell therapy*

Introduction

Cryopreservation is a process of preserving biological function by freezing and storing material below −80°C, typically at or near the temperature of liquid nitrogen (LN_2; −196°C). Other methods of biopreservation could include vitrification, which refers to avoiding ice all together through ultra-fast cooling rates and high solute concentrations to form a metastable, amorphous glassy state; lyophilization, in which cells may be "freeze dried" to sublimate water and allow for stability at higher temperatures; or even vacuum drying without the freeze step to achieve a similar result. Although much research is being done on these methods, cryopreservation via slow cooling methods remains the industry standard at this time and will be the focus of this review.

One of the most consistent findings from slow-cooling cryopreservation research on cells is the evidence-practice gap due to the failure to translate research into practice. As a result, cells are routinely cryopreserved using traditional, non-optimized methods while many scientific advances in basic cryopreservation science are not fully integrated into practice. As cellular therapy emerges, there are many challenges to its successful development and widespread use. Challenges in developing these products include product consistency, safety and potency as well as reliable

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(Received 4 January 2016; accepted 18 March 2016)

ISSN 1465-3249
http://dx.doi.org/10.1016/j.jcyt.2016.03.295

storage and transport with high efficacy at reasonable economic cost.

Fundamental cryobiological research focuses on revealing the underlying physical and biological mechanisms related to cell injury occurring during the processes of preparing cells for preservation, bringing them down to a freezing point where biologically stable mode is reached with cessation of enzymatic and metabolic activity, and finally bringing the cells back to physiologically relevant temperatures. This methodology was developed as a way to define the discrete challenges a cell faces during the process, with an understanding that the greatest challenge lies not within their ability to withstand storage at ultra-low temperatures but with the transitional phases (ice nucleation, ice propagation during cooling to −60°C or lower, thawing) during which they experience potentially lethal physicochemical events, specifically those associated with phase change of water in both the extra- and intracellular environments. However, the apparent discrepancy between the concept of cryopreservation and experimental findings suggests that there are other numerous lethal events (often poorly defined and not fully recognized) in a preservation cycle that can inflict damage to the cells. There exists injury to the cells due to osmotic intolerance [1] or due to the toxicity of the cryoprotectants [2] or chilling or cold-shock injury associated with the reduction of temperature from room temperature to nucleation temperature [3] and cooling injury associated with water-to-ice phase change until a glassy (i.e., ice crystal-free) state is achieved [4] (Figure 1). There also exists a hypothermic continuum and significant array of cellular stress events during warming/thawing or unexpected temperature excursions that may affect survival and function. A cumulative understanding of these injury mechanisms on cells immediate and delayed post-thaw survival and potency have become critical in development and implementation of optimized cryopreservation protocols for efficient manufacturing and banking of cellular therapeutics.

Another unavoidable fact is that not all therapeutic cells cryopreserve "equally." Cells from various tissue sources maintain varying physical and biological properties and could be expected to react differently to cryopreservation. Although not practical to deal with, there will be further differences in cryopreservation outcomes when the cells are from varying donors of the same species. Additionally the metabolic state of the cells (e.g., lag vs. log phase; end of passage-interval vs. early or mid-interval) cell culture density, highly metabolic, cytokine driven populations vs. non-stimulated cultures, and cell age in an expanded primary cell culture with limited doubling potential could also play roles in cryopreservation outcomes [5,6]. Consequently, to optimize survival after cryopreservation, the protocols used need to be designed specifically for the particular cell type to be stored, rather than simply borrowing protocols that have been successfully used for cells derived from

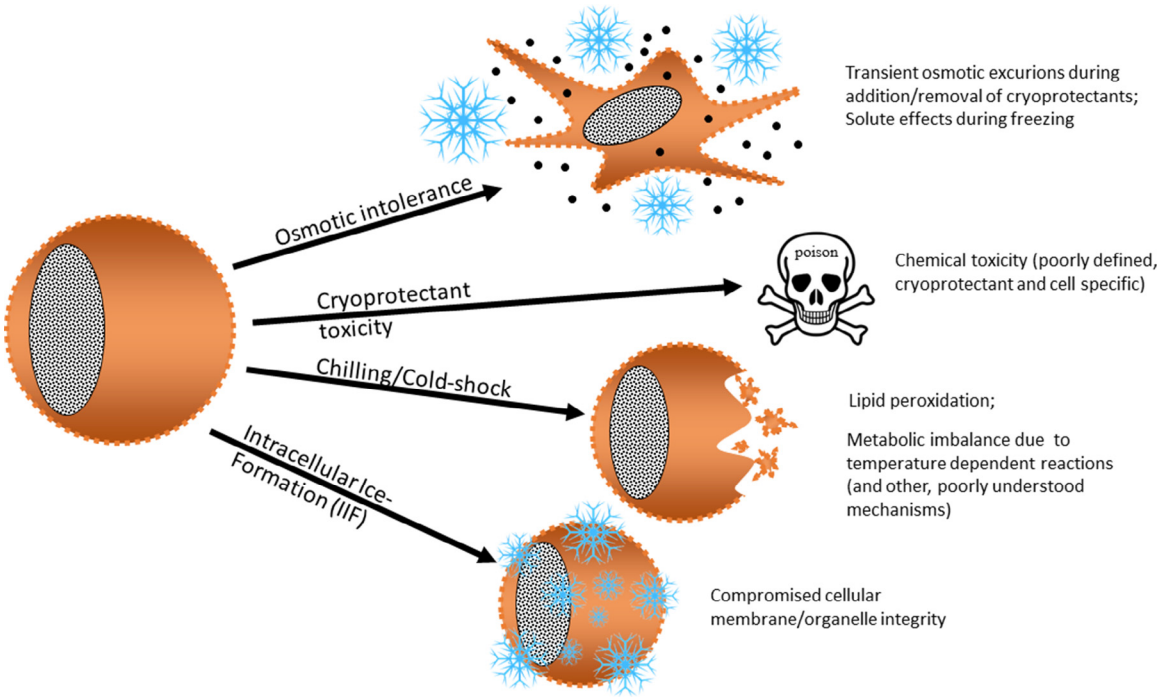

Figure 1. This representation depicts potential mechanisms of damage that can occur during cryopreservation processing.