

Preface

Preservation of cells is performed thousands of times every day by technicians across the world. For the vast majority of those people, the process is shrouded in mystery. The reasons for specific steps in the protocol are not clear. If there are problems with the protocol, the manner by which the problems occur is also unclear. There are numerous books that contain cryopreservation protocols for specific cell types or describe scientific understanding of the field or current research in the field. I could not find a book, which helped many, that uses preservation to develop new protocols or improve existing protocols. The objective of this book is to describe step by step the development of a preservation protocol and the scientific principles behind these steps. At the end of every chapter (with the exception of Chapter 8), specific links are made between scientific principles and the manner by which those principles are put into action.

Cells are being used for an increasing number of downstream uses. The applications of cells include the production of therapeutic proteins, viral vaccines, and antibodies. Cells are being used as biomarkers for health and disease and even for the treatment of disease. These applications are described in Chapter 1 and the role of preservation in the clinical and commercial applications of cells is also described. Different modes of preservation are also described. Different applications of cells may involve hypothermic storage of cells, cryopreservation, or vitrification.

Cells undergo a variety of processes prior to cryopreservation. These processes can include digestion from a tissue, selection of subpopulations, genetic modification, culture, and so forth. Chapter 2 describes these processes in more detail and the resulting nutrient deprivation, shear, or other sublethal stresses that can influence post-thaw recovery of cells. Strategies to minimize stress or cell losses from pre-freezing processes are described. Newly developed gene-editing technologies are described. The ability to edit cells may lead to both new challenges and opportunities for cell preservation. It is likely that insertion or deletion of specific genes may influence the ability of a cell to survive the stresses of freezing and thawing. Gene editing may also enable us to understand the role of specific genes in enhancing survival of certain cells.

Characterization of the cells being cryopreserved is also critical. Chapter 2 also describes standard testing of cells prior to cryopreservation, including identification of cells, testing for adventitious agents, and other types of testing such as genetic stability. Misidentification of cells is a serious concern in the area of life science research, and there is increasing emphasis on proper identification of both primary cells and cell lines being used.

Cryopreservation uses specialized solutions designed to help the cells survive the stresses of freezing and thawing. These solutions are not physiological. Chapter 3 describes formulation of a solution and development of methods to introduce the solution. A listing of molecules that have been used to stabilize cells during freezing is given in the chapter. The development of new solutions is an ongoing area of research, but these solutions will still, more than likely, need to be introduced and removed prior to downstream use.

The influence of cooling rate on the post-thaw survival of cells has been known for almost 50 years. Chapter 4 describes the cooling process and the manner by which cells are typically frozen (i.e., controlled-rate freezing, passive freezing, or vitrification). Designing a cooling protocol and methods of verifying the protocol are also described. The importance of temperature and its variation in time during freezing also suggests that the method of measuring temperature independently during freezing is valuable, in particular during the development of methods.

Cells that have been cryopreserved may be stored for weeks, months, or even decades. Chapter 5 describes the scientific basis for storage of cells in liquid nitrogen, fundamentals of repository design, safe operation of a repository, and shipping of samples from the site of storage to the site of use. The factors that influence stability of samples in storage are also discussed. Transient warming events (TWEs) are being documented for a wide variety of biospecimens in storage and our understanding of the influence of TWEs on sample quality continues to grow. It is likely that new technologies can be used to eliminate this issue and improve stability of samples in storage.

The purpose of preservation is to maintain the critical biological properties for downstream use of the cells; downstream use of the cells requires thawing of the sample. The thawing process and the manner by which you can characterize your average thawing rate and improve the thawing process are described in Chapter 6. Newly developed controlled-thawing technology will provide the opportunity to improve the consistency of thawing. In addition, new types of thawing protocols may be developed in the future, which will improve overall outcome.

It is common for cells to be washed post-thaw and prior to downstream applications. Methods and technologies for washing cells post-thaw are also described in Chapter 7. For vitrification solutions or cells that are sensitive to osmotic stress, strategies for improved methods of washing are described.

Effective methods of preserving cells cannot take place without effective methods of characterizing post-thaw recovery. Post-thaw assessment of cells is

a very common area for errors and poor practices. The need for post-thaw function of cells, in particular for cells used therapeutically, implies that post-thaw function is critical and methods of assessment must be meaningful. Different methods of post-thaw assessment are described. Specific recommendations are given to reduce bias and errors.

The traditional method of optimizing a preservation protocol typically involves empirical testing (i.e., varying composition and cooling rate and measuring post-thaw viability). Chapter 8 describes the use of a differential evolution algorithm to reduce the experimentation required to optimize composition, cooling rate, and other processing parameters for cells. As there is pressure to develop fit-for-purpose protocols, new methods to streamline the cost and time required for optimization are critical and this approach has the potential to be transformative.

The growth in clinical and commercial applications of preservation brings with it the need for consistency and reproducibility. Chapter 1 describes common errors in preservation practices that lead to poor reproducibility (both poor outcome and high variability). Subsequent chapters describe common pitfalls that can negatively affect reproducibility of the preservation process and strategies to avoid those pitfalls and improve reproducibility.

For all the reasons listed previously, conventional methods of cryopreservation may no longer be appropriate for a given cell type or a given application. Drift in preservation protocols is also common. As director of the Biopreservation Core Resource, I get phone calls and emails from organizations that start having problems with existing protocols. The overall goal of this book is to help both groups. The process of developing a new protocol or understanding problems with an existing protocol can be approached logically and systematically based on scientific principles. It is my hope that this book will enable more organization to achieve improved post-thaw recoveries and consistency.

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