CELL & GENE THERAPY INSIGHTS

SUPPLY CHAIN FOCUS: BIOPRESERVATION & COLD CHAIN MANAGEMENT

INNOVATOR INSIGHT

Biopreservation and cold chain biologistics risk points in the cell and gene therapy workflow

Todd CJ Berard & Aby J Mathew

Cell & Gene Therapy Insights 2020; 6(8), 1363–1380

DOI: 10.18609/cgti.2020.150

INTRODUCTION

The current state of regenerative medicine is a transformational period for cell and gene therapies. In addition to Novartis' Kymriah®, Kite Pharma's Yescarta® and Tecartus™, Spark's Luxturna®, AveXis' Zolgensma®, and bluebird bio's Zynteglo® blazing the commercialization trail, there are over one thousand Phase 1, 2, and 3 cell and gene therapies (CGT) in pipeline development [1]. Although this bodes well for patients, clinicians, industry, and investors, some unique aspects of cell- and gene-based therapies versus traditional pharmaceuticals or biopharma has highlighted the myriad of "new" manufacturing, clinical, and commercialization, challenges our industry now faces [2,3]. Independently, each one of these challenges presents its own unique set of risks. Furthermore, when lined up in sequence and aggregated together in the manufacturing chain, if each portion is not optimized and risk-mitigated, the subsequent impact to the CGT product may be a compounding of the risks; and the sum total of

all parts of the workflow will suffer. These beginning-to-end manufacturing risk points warrant appropriate assessment, and they are recommended to be addressed with the same diligence and priority as the therapies themselves, if the promise of Regenerative Medicine is to be fully realized. Fortunately, much has been learned regarding optimization of a number of key critical process parameters (CPP), and those looking to improve these parameters can leverage what has already been learned. This overview represents targeted lessons learned based on numerous experiences with CGT partners. Although intended to share feedback from experiences that may not always be detailed in the literature, it is not intended to address every aspect of the CGT workflow.

REPRESENTATIVE CELL IMMUNOTHERAPY WORKFLOW

Figure 1 is one representative CGT manufacturing workflow. Similar workflow representations, and related points of risk, have been outlined within a number of publications [4–8].

In common CGT manufacturing workflows, starting source material is obtained; and then is processed, selected, and/or isolated. Often, the material undergoes a biopreservation step (cryopreservation or hypothermic preservation), and transported to a manufacturing facility; where activation, transduction, expansion, and/or final formation take place, before additional transport/storage for clinical application. This workflow highlights several biopreservation and biologistics areas where CGT may be challenged:

1. Ensuring high quality starting material;
2. Optimizing viable functional recovery, and minimizing variability and risk, in process development throughout the workflow chain;
3. Determining appropriate conditions for source material, intermediates, and final product – non-frozen or frozen (and, optimizing the biopreservation steps by utilizing Biopreservation Best Practices [5]); and
4. Exploring and implementing enabling tools and technologies throughout the workflow.

Such tools might consist of: novel CGT processing and packaging technologies; next generation closed systems for fill, finish, and packaging; class-defining biopreservation media; high capacity-controlled rate freezers; cryogenic storage systems; 'smart' cold chain management systems (shipping containers, tracking, and reporting); and automated, water-free thawing equipment technologies. [The normothermic culture state of the cells is also a variable that can impact the quality of the cell product, however that is not a focus of this overview.]

ENSURING HIGH QUALITY STARTING MATERIAL

The importance of obtaining high quality starting material has been previously highlighted [4,5,7]. An early challenge in the CGT manufacturing workflow is ensuring high quality, and consistent, starting material. Cell-based manufacturing and therapies present a unique challenge that does not exist to the same complexity or criticality as with non-cell-based therapies – that difference being the needs, the vulnerabilities, and variability of, living cells. Cells embody an intrinsic variability of normal conditions, response, and function, that can influence the therapeutic efficacy. As such, CGT manufacturing should take into account the inherent variability of starting cell-based materials, as well as the processing methods for these living cells, that will eventually impact the quality of the therapeutic product.

The potential variability and quality of CGT starting materials have been an increasing focus of CGT concern, and has been discussion points of Cell & Gene Therapy Insights experts [4,7]. Those discussions have

DOI: 10.18609/cgti.2020.150

also presented evidence-based pathways for increasing the non-frozen or frozen stability, and/or minimizing variability, of cell/tissue starting materials [4–9].

NON-FROZEN OR FROZEN? CELLULAR RESPONSES TO COLD

It is important to ask a basic question: How can cell viable recovery and function be preserved throughout the manufacturing workflow, in order to facilitate efficacy? It is recognized that low temperatures can slow metabolic activity, reduce oxygen demand, and decrease degradation; but it may be beneficial to understand the benefits and limitations, in order to support biopreservation optimization and risk management of the process/product.

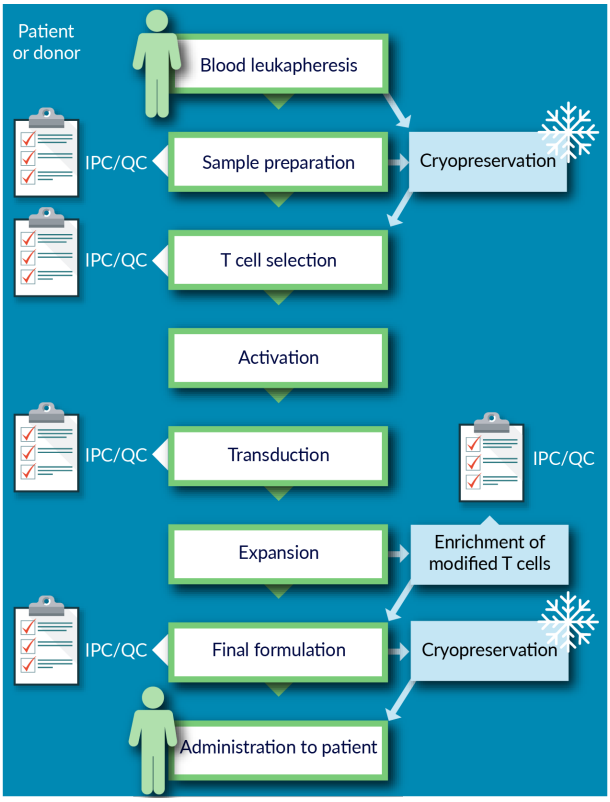
Figure 2 shows three states of cell/tissue application temperature (as primarily utilized in CGT manufacturing and biopreservation), and the relationship between temperature and cellular metabolic activity. At normothermic temperatures and conditions, the cell metabolic function should operate as designed to support activity at the cellular, tissue, organ, and organism levels. Under normothermic conditions, cells maintain homeostasis through a multitude of mechanisms, including ion pumps on the cell membrane and intracellular organelles. Ion pumps tightly regulate vital intracellular and extracellular ionic balance, which also impact osmotic balance, cell volume, etc. [5].

As temperatures decrease to hypothermic temperatures (below 37°C normothermic), lipid membranes undergo phase transitions: a type of structural change that results in loss of fluidity and continuity. Hypothermia induces phase transitions in the lipid membrane that lead to pore formation and loss of integrity. This leads to an influx and outflux of ions and small molecules due to the cross-membrane concentration gradients [9]. Under hypothermic conditions, there is deceleration of ion pumps and reduced ATP synthesis by mitochondria. Ion pumps then have a reduced capacity to regulate intracellular ions, leading to a myriad of issues. This further impedes restoration of ionic balance in the intracellular milieu. This disrupts the overall ionic balance, resulting in dysfunctions in intracellular cell signaling, salinity, osmolality pathways, osmosis, and cell volume, that previously relied on a tightly regulated cell balance. Osmolality and ionic distortions can induce mitochondrial stresses, which can initiate a cascade of adverse events within the cell by increased reactive oxygen species (ROS) and free radicals generation, and lipid peroxidation. When combined with membrane phase transitions, these phenomena can lead to membrane blebbing and other irreversible membrane injuries, among other mechanisms of cell damage and cell death [5,9,10].

Furthermore, in the absence of oxygen and normothermic conditions, glycolysis becomes the main source of limited ATP generation instead of oxidative phosphorylation, resulting in acidification of the intracellular

FIGURE 1

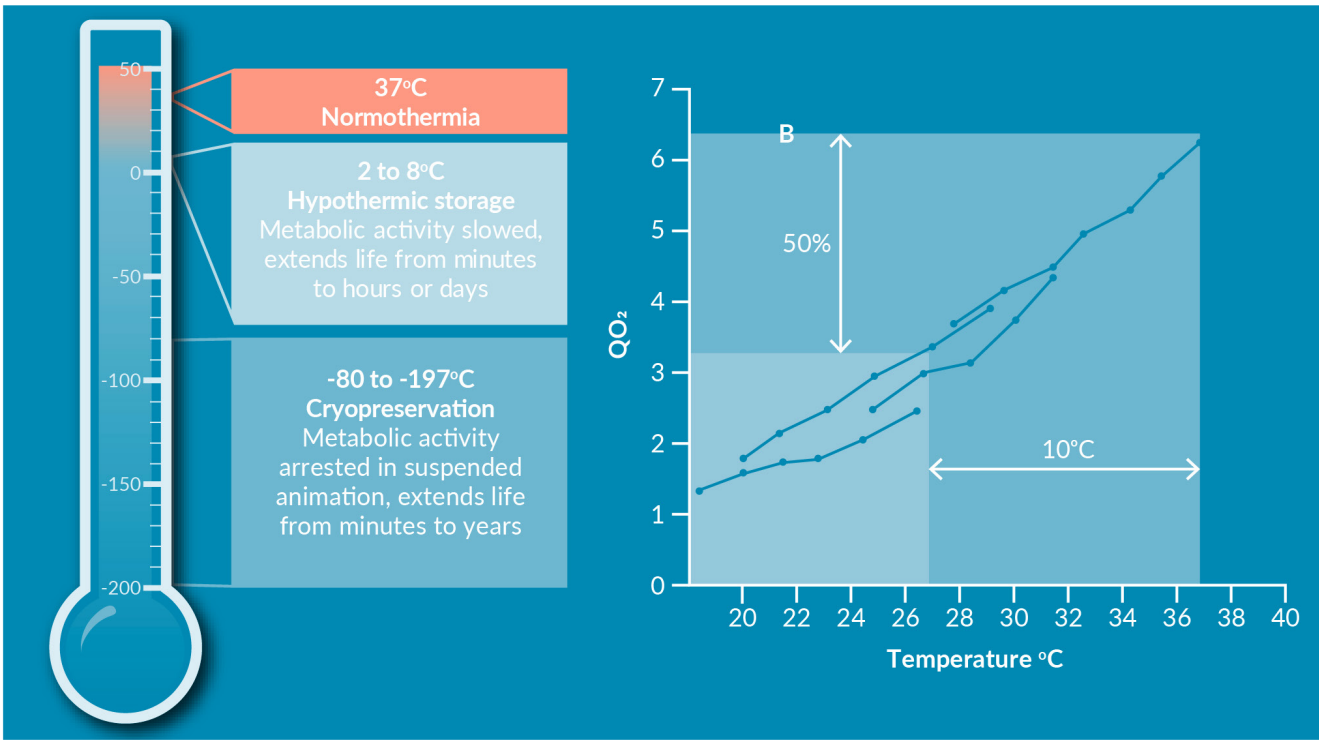
CGT manufacturing workflow.



Adapted from [8].

FIGURE 2

The relationship between temperature and cellular metabolic activity.



Graph modified from Fuhrman and Fuhrman 1959.

milieu. Changes in pH and salinity may irreversibly impact protein solubility and its functional structures, which are necessary for protein-protein interactions and trans-membrane positioning.

Temporal accumulation of these damages during hypothermic intervals and storage may eventually overflow beyond the tolerable limits for the cell, leading to irreversible activation of apoptosis, necrosis, and secondary necrosis cascades; at which point, the cell is lost. In addition, the actual onset of cell damage and cell death may not translate until post-preservation and re-warming, and may subsequently manifest as Delayed Onset Cell Death [5,10].

To alleviate some of these issues, an intracellular-like designed biopreservation media may be incorporated to replace traditional saline/culture media (or other formulations that mimic the normothermic isotonic ionic balance). By reducing the cross-membrane concentration gradient of ions during cold exposure, intracellular ionic balance and salinity would be less altered, even if membrane permeability is impacted. Biopreservation Critical Quality Attributes (BCQA) incorporate intracellular-like design, including impermeant (non-permeating) molecules such as large sugars, which exert membrane-stabilizing and osmotic-supporting effects, in order to mitigate cell swelling and membrane damage during storage. Free radical scavengers can decrease the burden of ROS. Also, buffers that are effective specifically at low temperatures, in contrast to traditional buffers for normothermic conditions, may be more effective at controlling toxic pH changes [5]. This intracellular-like approach to Biopreservation Best Practices is applicable to non-frozen hypothermic preservation and cryopreservation.

THE PHYSICS OF FREEZING

Another mode of cell and tissue biopreservation is cryopreservation. Hypothermia-induced

DOI: 10.18609/cgti.2020.150

acute stresses occur slowly and accumulate during the storage period. The accumulation of such adverse effects on cells usually trigger cell damage and cell death after hours to days in cold storage. On the other hand, acute cellular stresses during freezing conditions and cryopreservation occur within a relatively short period of freeze-thaw. For both modes of biopreservation, many cell damage and cell death effectors may only fully manifest over 24–72 hours post-preservation via Delayed Onset Cell Death [5,10]. To better understand the physical and chemical stresses during freezing conditions, consider a cell suspension in a simple salt solution such as physiological saline. In Figure 3, a typical phase diagram of a saline-like representative solution is shown. The phase diagram describes the state of the solution – liquid, solid, or both – at any given temperature and salt concentration.

The freezing process starts with cooling the solution to below its freezing point (Figure 3A). Once the first ice nuclei form at subzero temperatures, ice crystals grow until they reach an equilibrium with the remaining unfrozen fraction. As ice crystals form from pure water, the unfrozen fraction now contains a higher salt concentration and a lower freezing point. The cells remain in the channels of the unfrozen fraction [11,12].

As freezing continues by reducing the temperature, more water solidifies out of the solution in the form of ice, resulting in increased salinity, solute toxicity, and increasingly lower freezing temperature of the remaining unfrozen fraction (Figure 3B & C).

The cells in the unfrozen fraction are then exposed to increasing salinity (and solute toxicity) as the temperature plunges (Figure 3D). At temperatures in the range below -20°C, the salinity of the unfrozen fraction may be up to 10–20 times the normothermic initial salinity. Recall that cell membranes become more permeable at lower temperatures. This increased salinity, and solute toxicity, impacts the intracellular milieu during freezing. Therefore, the magnitude of freezing-related stresses due to physical effectors (ice formation), and biochemical effectors (salinity, solute toxicity, protein structural damages, intracellular signals, etc.) is not insignificant. Furthermore, the cells respond osmotically to increased extracellular solute concentration by shrinking in size due to water efflux. Cells that are sensitive to these mechanical and biochemical changes are more likely to experience cell injury and cell death during freezing, including as freezing continues toward the glass transition temperature (Tg) of the cell-solution mixture, and then as vitrification into a glassy state occurs, under appropriate conditions [11].

THE CELL RESPONSE TO FREEZING

Now consider how a cell is affected by this freezing process, in the context of manufacturing a cell-based product: A slow freezing rate will allow the cells to respond osmotically to the ever-increasing osmolality of the extracellular milieu by losing water and shrinking in size (Figure 4A). This process reduces the potential for intracellular ice formation; which is a major factor in damaging the cells beyond repair during cryopreservation [5,8,9].

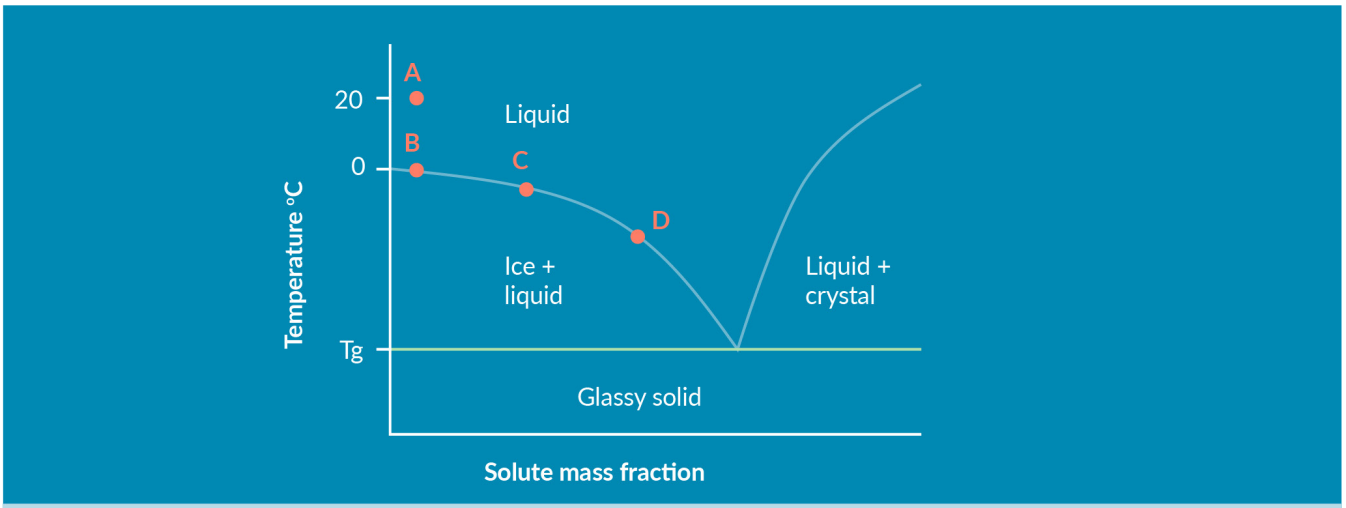
Osmotic shrinking, as a result of low temperatures and the cellular environment, is a dynamic process. As such, a fast freezing rate may not allow sufficient time for the cell to dehydrate enough water, and therefore increases the probability of intracellular ice formation (Figure 4B) [5,8,9].

Growth of intracellular ice can physically rupture membranes. In the case of fast freezing rates, the cell may be lysed if the amount of ice is excessive, or may be damaged beyond repair even with lesser amounts of intracellular ice (Figure 4B) [5,8,9].

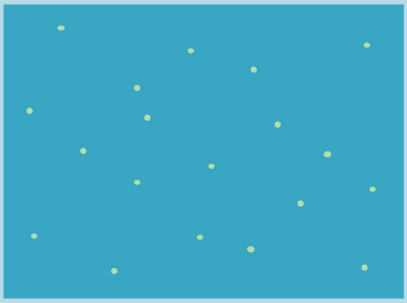
In general, freezing rates around -1°C/min or so are observed to allow water-membrane dynamics to dehydrate CGT-relevant cell types sufficiently to reduce intracellular ice formation (Figure 5A). However, the level of osmotically-induced volume shrinkage may reach as low as 30% of the original

FIGURE 3

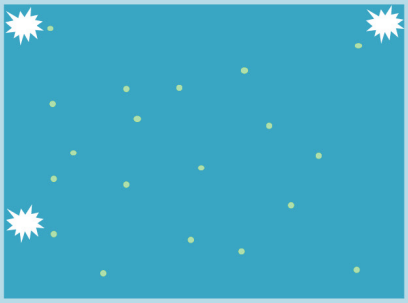
A typical phase diagram of a saline-like representative solution.



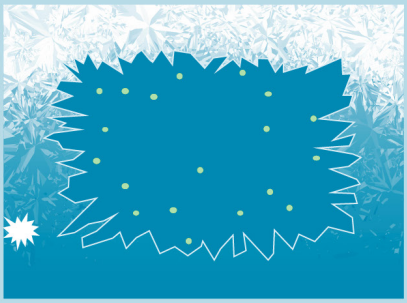
A. Room temperature
Salt solution, i.e., physiological saline at ambient room temperature



B. Freezing point T = -1.5°C
Freezing process starts with random nucleation of ice at subzero temperatures



C. Freezing point T = -10°C
A significant portion of water has turned into ice, leaving behind a 10x concentrated salt solution



D. Freezing point T = -23°C
At -23°C and below, further cooling result in formation of salt crystals, and the remaining liquid portion variably transitions into stages of a vitrified glassy state

cell volume. This may result in other forms of physical damage – including membrane folding and fusion, which is generally observed in the form of lower average cell volume, and an increase in the number of small non-cell vesicles post-thaw. The toxicity due to orders-of-magnitude increase in salinity, combined with mechanical cues from excessive osmotic shrinkage, induce adverse events in cells. These forms of cell damage and cell death include acute necrosis; and later Delayed Onset Cell Death (that becomes apparent as loss of viable recovery and function over hours to days post-thaw) [5].

DOI: 10.18609/cgti.2020.150

To reduce the osmotic shrinkage, and the toxicity due to increased solute concentration, cryoprotective agents (CPA) are added to the solution (membrane-permeable and/or non-permeating). One of the most well-known and most studied cryoprotective agents is dimethyl sulfoxide, or DMSO (Figure 5B) [5].

While referred to by some as an "anti-freeze" agent, DMSO offers protection against freezing in rather complex ways. In the unfrozen fraction, DMSO reduces salinity-induced toxicity and mechanical osmotic shrinkage by engaging water molecules and preventing ice crystal growth. As such, the cells are exposed to less salinity at any given temperature with the presence of DMSO. Furthermore, by permeating the cell, DMSO reduces the cell volumetric changes during freezing and minimizes intracellular ice growth [9]. This particular set of actions of DMSO may not be readily replicated by other non-permeating cryoprotective agents and sugars, or other permeating cryoprotective agents with similar efficacy.

WHY CRYOPRESERVE CELL-BASED PRODUCTS?

Clinical and commercial manufacturing models drive several critical aspects about

FIGURE 4

Cell responses to freezing.

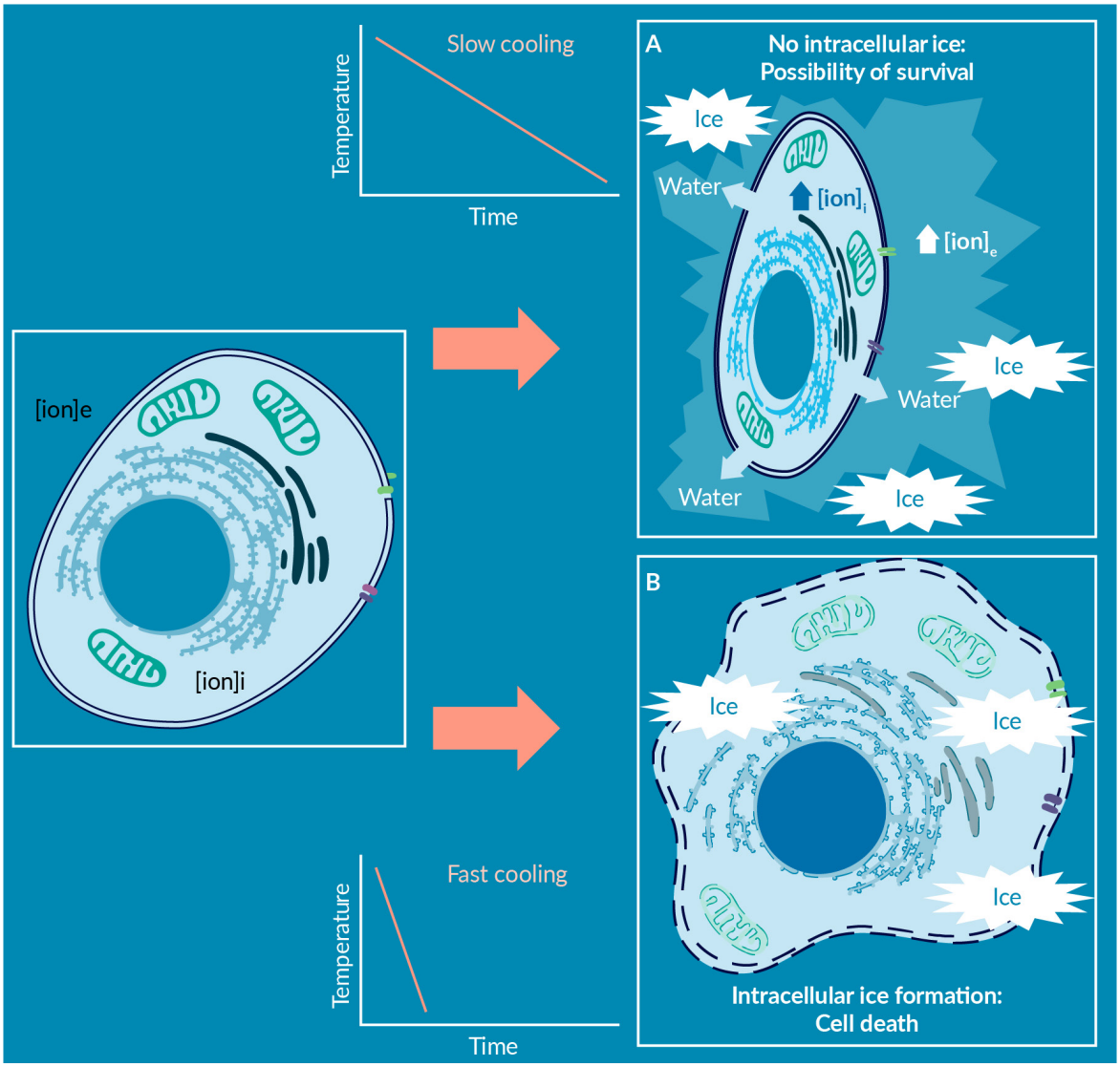
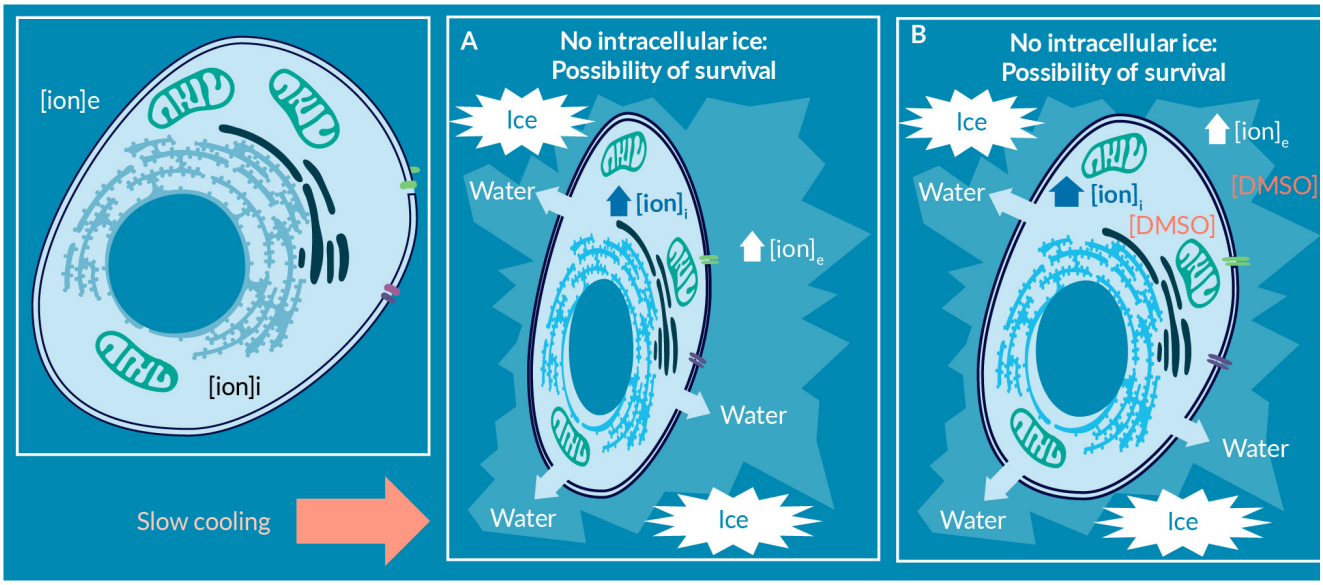


FIGURE 5

Addition of the cryoprotective agent, DMSO can offer protection against freezing.



the CGT process and workflow. While, in theory, "fresh" non-frozen materials may be preferred by some (if even possible/feasible) due to simplicity (no cryopreservation step, no LN2 dewar shipping step, no thawing, no documentation for cryo-related procedures, etc.), the spatial separation biologistics of source starting materials/manufacturing activities/patients, and the globalization of supply chain management, are ameliorated by the temporal time management benefits of cryopreservation.

Living cells age, differentiate, and/or degrade over time, even under normothermic conditions. A reduction in temperature at strategic points in the CGT workflow reduces the biological activity and metabolic demands of cells, and slows down degradation. As temperatures decrease, metabolic and enzymatic activity slows, and at or below a glass transition temperature (Tg) of approximately -120°C to -130°C, molecular motion in water-based systems is virtually arrested [9]. This vitrified state allows potential storage of the cell-based material for many years, and is a key temporal storage component of cell therapy manufacturing. An "investment" in cryopreservation buys time, provides flexibility, pays dividends through additional options, and is the most feasible current modality for long-term storage of CGT-related cell-based products.

PROCESS DEVELOPMENT CONSIDERATIONS FOR CRYOPRESERVATION OF CELL-BASED THERAPIES

Given the physics of freezing, and its effects on cells discussed above, it is important to determine if cryopreservation is appropriate and achievable for each CGT process/product. As developers of CGT therapies designed for successful commercial viability have looked to achieve a functional cryopreserved product, it is of value to understand that optimal cryopreservation of cells is not simply a matter of lowering the temperature below freezing. Some may think that cryopreservation consists of just freeze and thaw. However, the steps within a cryopreservation (and thaw) optimized method consists of multiple steps, with each step within the overall method potentially as a point of Risk and point of potential Optimization (Figure 6). Cryopreservation is one of the most critical, and often underdeveloped, critical process parameters (CPP) of the manufacturing

DOI: 10.18609/cgti.2020.150

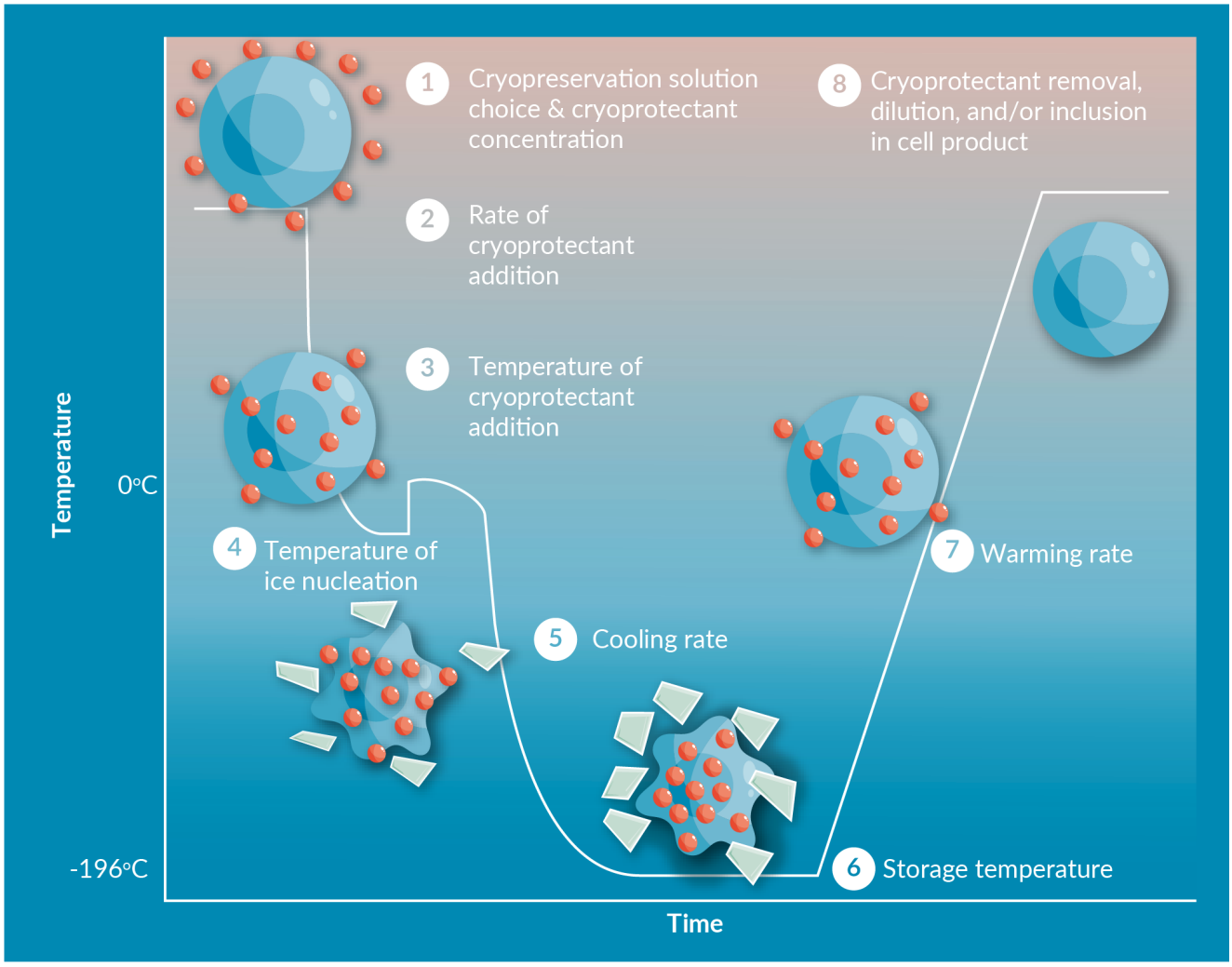
model. It may be helpful to look at the process in greater detail:

As illustrated in Figure 6, there are a number of steps within the cryopreservation method/protocol, that would be recommended to qualify/optimize from a Biopreservation Best Practices approach.

Consideration 1: Cryopreservation solution of choice. The traditional approach to the freeze media has been to formulate a home-brew cocktail of cryoprotectant (such as DMSO or glycerol), with serum (human or animal) or protein (albumin). These would be added to an isotonic (extracellular-like) vehicle solution such as culture media or saline-like solution, that had not been designed for low temperature biopreservation, but rather had been designed for normothermic ionic conditions. This formulation approach has been the traditional clinical center in-house home-brew cocktail, "grandfathered" into historical hematopoietic stem cell (HSC) transplant cryopreservation protocols [13], designed into some initial CGT cell therapies [14], and even incorporated into some guiding standards (USP <1044> Cryopreservation of Cells) [15]. In contrast, another more recent approach to the cryopreservation media has been to utilize a serum-free and protein-free intracellular-like formulation design, as discussed above [5,6,10]. This more recent methodology has been incorporated into many developing CGT, including ones that have obtained

FIGURE 6

The biopreservation best practices approach to cryopreservation.



Regulatory clearances and Marketing Authorisations [16–19].

Consideration 2: Rate of Cryoprotectant addition. Many research and clinical cryopreservation protocols proscribe slow/gradual/dropwise rates of addition of the cryoprotectant, in consideration to potential osmotic fluctuations and membrane permeability rates for the CPA. This consideration may, or may not, be impactful depending on the cell product/process. This consideration may also be less impactful with cryopreservation media that incorporate osmotic buffering components [20–22].

Consideration 3: Temperature of Cryoprotectant addition. Similar to the considerations related to the rate of CPA addition, some protocols proscribe a temperature for application of the freeze media. The choice of temperature may be related to facilitating more rapid permeability of the CPA, or related to reducing potential toxicity of the CPA [20–22].

Consideration 4: Temperature and consistency of ice nucleation. Some protocols may not speak to the point of ice nucleation within the cryopreservation procedure. Even with recognition of the ice nucleation, and related latent heat release, noted on freezing curves/graphs, there is often a passive approach to controlling ice nucleation within a method, let alone optimizing a method for consistent nucleation points from batch-to-batch of cell products. Lack of appropriate ice nucleation within a cryopreservation method may result in undercooling/supercooling of the sample, which may in turn be linked to deleterious intracellular ice formation and batch-to-batch variability. There are various approaches to the ice nucleation consideration [23], and even approaches for method consistency with passive freezing devices [24]. Programmable controlled rate freezers (CRF) are often utilized to provide consistent freezing rates and nucleation, however abnormal freezing curves and variable nucleation events may still occur and require troubleshooting [25].

Consideration 5: Cooling rate. Although most CGT cell products might find cooling/freezing rates of approximately -1°C/min (averaged, or focused on the initial stage around nucleation) to be adequate, if not optimal [8,9,11,26], it would be recommended (and often expected) to verify, and perhaps optimize, the freezing rates as appropriate for each manufactured cell product as an evidence-based Biopreservation Best Practice. Even with use of a programmable CRF, the stages within the CRF program may be optimized for various cell product parameters (cell type, cell volume, membrane permeability, cell concentration, product volume, product packaging, number of product units, etc.). CRF abnormal freezing curves may still occur and require troubleshooting [25].

Consideration 6: Storage temperature. Cryopreserved CGT products are generally stored in liquid nitrogen (LN2), to facilitate ultra-low cryogenic temperatures below their glass transition (Tg) temperature, and to enable many years of stability [27]. Alternatively, there may be potential for further consideration of shorter-term stability (weeks to months) at temperatures in the range of -80°C. The feasibility of varying storage temperatures (and the related pros and cons) may be worth exploring, and may be able to support short-term storage aligned with less burdensome storage/transport needs, with more robust cryopreservation methods and cold chain management [28,29].

Consideration 7: Warming/Thawing rate. In alignment with most CGT slow-freeze cryopreservation protocols, the most common thawing methods for those cryopreserved cell products involve fast-thaw methods with traditional 37°C waterbaths. At a superficial level, the process mirrors that of freezing: warming of the sample from cryogenic temperatures toward the solid-to-liquid phase transition, melting of ice to form liquid water, and rehydration of the cells. Similar to historical cryopreservation methods, this method of fast thawing has been largely adequate. The criticality of thawing rates is a noted point of discussion [26], and thaw methods (including rate of thawing) would be a worthwhile process parameter to investigate

DOI: 10.18609/cgti.2020.150

and verify for each cell product/process with an evidence-based approach to asses Risk and potential Optimization [8,9].

Consideration 8: Post-thaw wash, dilution, or direct application. There are a variety of approaches (and dogma) regarding the post-thaw status of the cryopreservation medium. One school of thought is that the cryoprotectant(s) must be removed post-thaw. The CPA removal might be via a single step wash/centrifugation, or via stepwise dilution and wash in consideration to osmotic fluctuations. There has also been development and application of various washing devices. Another approach would be to dilute post-thaw, but not wash/remove the CPA in entirety. And then there is the approach of avoiding wash or dilution with direct post-thaw application. Each of those approaches has potential benefits and drawbacks, that might range from extensive cell damage/loss (wash and removal methods) to potential (or perceived) cryoprotectant toxicity (direct application). Each approach also entails a different level of post-thaw manipulation, and potential variability at the point of post-thaw application [5,8,10,30].

BIOPRESERVATION BEST PRACTICES CONSIDERATIONS

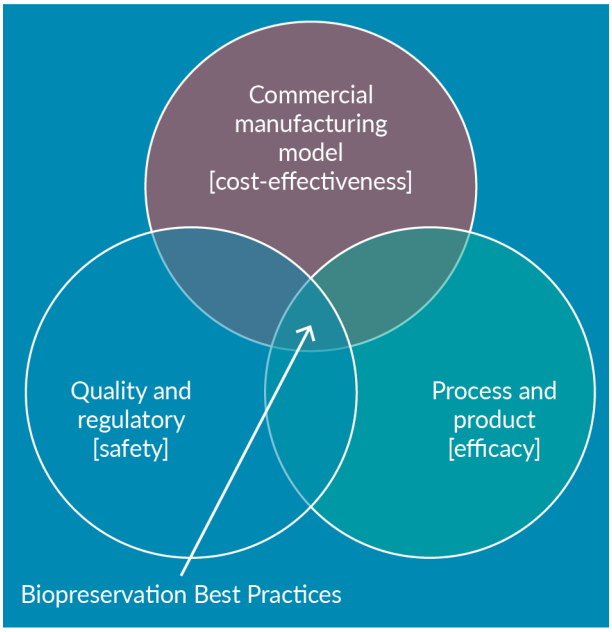
Most evidence-based best practices identify the process parameters, and investigate the characteristics that can impact the critical quality attributes of the product. Within the considerations of biopreservation, broader process best practices may overlap to more focused Biopreservation Best Practices that can serve as a guiding approach applicable to CGT manufacturing (Figure 7).

Often, the early-stage development of a product understandably focuses on the high-level product efficacy (recovery, viability, and perhaps some measure of functionality). Admittedly, if the feasibility of that aspect is not established, the other parameters may be moot considerations. The ability to manufacture the product tends to be an early translational focus, and as the product progresses along potential clinical or commercial development there is increasing scrutiny to Quality and/or Regulatory Risk considerations. Areas of overlap with focus on Biopreservation Best Practices may include:

1. Ability to integrate a biopreservation tool (media, equipment, method, etc.) into the CGT manufacturing process, including risk from process change.
2. Cost-effectiveness of those tools and technologies, such as pre-formulated biopreservation media or controlled rate freezer.
3. Efficacy of the tools, methods, and cell product.
4. Impact to Quality and Regulatory footprint, such as safety of biopreservation media and consideration to qualification for excipient application. Also, consideration to alignment with Good Manufacturing Practices (GMP).

FIGURE 7

Biopreservation Best Practices



Adapted from [9].

5. Qualification and validation of the tools, technologies, or methods.

6. Supplier reliability, risk, expertise, and qualification alignment. Also, supply chain security of the tools and technologies.

ADDITIONAL BIOPRESERVATION PROCESS PARAMETERS

As an extension of the number of critical steps within the cryopreservation process (Figure 6), there are Biopreservation Critical Process Parameters (BCPP) throughout the CGT manufacturing process, and including where biopreservation and stability might impact the quality attributes of the process/product (Figure 8).

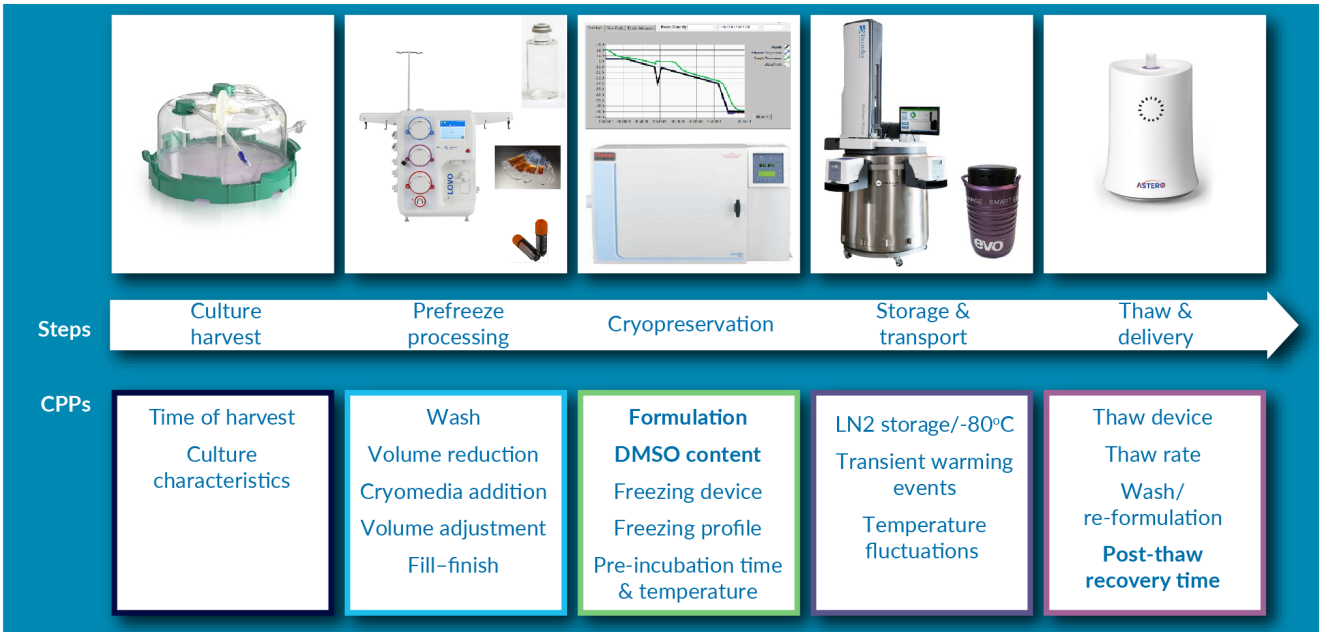
Cold chain management

Advances have been made in cold chain management systems, and monitoring of this critical part of the CGT workflow. Innovations in insulating materials have overcome shortcomings in insulated packaging performance. 'SMART' shippers with improved cloud-based data tracking and software technology have enhanced management of time-critical and temperature-sensitive products. Technology innovations have improved packaging, monitoring, logistics practices, data collection and data management; and incorporated them into unique, innovative, and self-contained systems [31–33].

SMART cold chain technologies such as Liquid Nitrogen (LN2) "dry vapor" SMART shippers and longer-range dry ice shippers are increasingly being utilized by late-stage clinical trial and commercialized therapy providers. The temperature monitoring and control, location tracking, chain of custody monitoring, and long temperature life of these shippers addresses a critical part of the supply chain biologistics [33]. With LN2 shippers, traditional LN2 dry vapor shippers experience reduced performance when not maintained upright, they may require palletization, and therefore may be restricted to wide-body aircraft and limited to large airport channels. New shipper technologies look to maintain temperature under some tilting, accommodate loading onto smaller

FIGURE 8

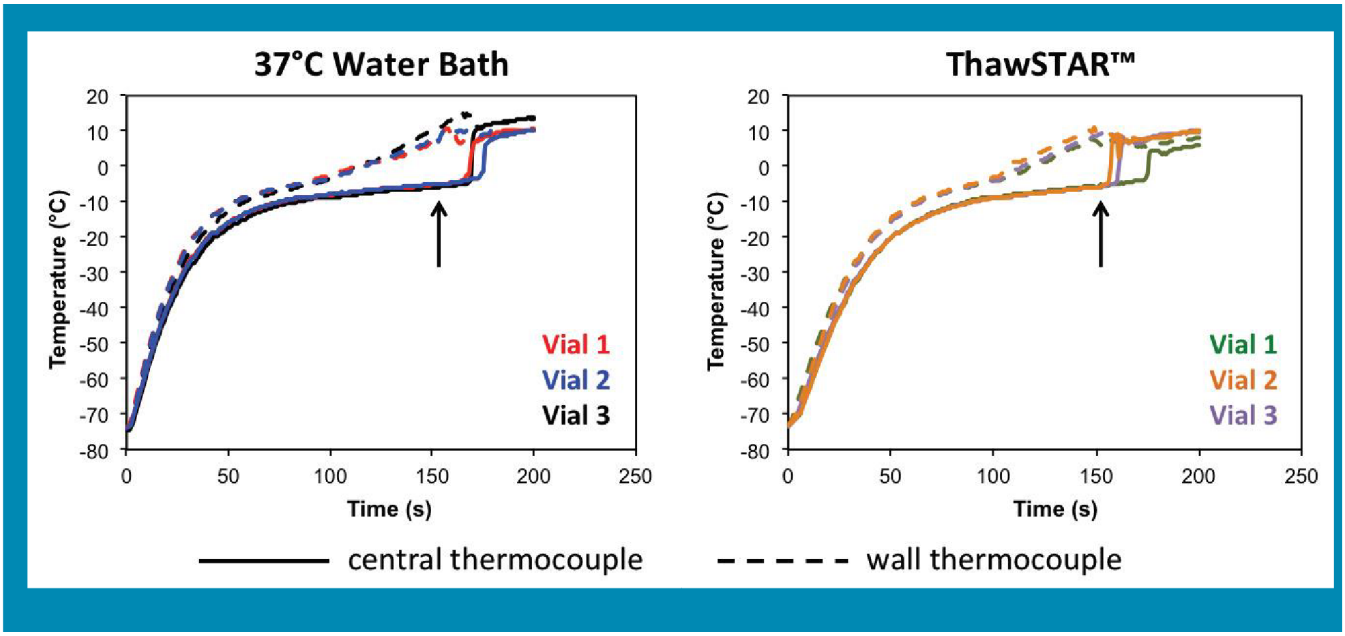
Biopreservation critical process parameters.



DOI: 10.18609/cgti.2020.150

FIGURE 9

Thermal profile of vials thawed in a water bath or ThawSTAR System.



Frozen vials were thawed in a 37°C waterbath (left panel) or in the ThawSTAR System (right panel). The temperature profiles recorded by both thermocouples were very similar for both the waterbath thaw and the ThawSTAR thaw. For the waterbath thaw, the vials were removed from the bath when a pea-sized ice chunk remained (arrow) and then gently tapped to melt the chunk. Similarly, ThawSTAR ejected the vial at the point where a pea-sized ice chunk remained (arrow). The final vial temperature is ~5–10°C.

regional aircraft that cannot support palletized cargo, and enable greater flexibility during transport [33].

Thawing

In order to transition from cryopreserved samples/product to application of the cells, the intermediate step is returning cell samples/products to the non-frozen state. Optimal thawing of these cells may be critical to successful downstream applications. Thawing rate and temperature may be parameters for potential optimization for cell size and volume, cell type, and cryopreservation media.

The most common and well-accepted method for rapidly thawing cryopreserved cell samples is partial submersion of the sample in a 37°C waterbath. There are several reasons for using this approach: waterbaths are relatively cheap and easily available, and they allow efficient heat transfer from the water to the sample due to the high heat capacity and thermal conductivity of liquid water. However, there are potential risks to using a waterbath for thawing, particularly in a clinical environment. These potential risks include:

1. Lack of scalability post-manufacturing.
2. User-to-user variability in subjectively determining thaw recognition times, final vial temperature, and ending point of ice.
3. Overthawing, or excessive warming, of samples.
4. No data management or chain-of-custody connectivity.
5. Contamination of sample contents.
6. Challenge in using a waterbath as part of a sterile process inside a biosafety cabinet or clean environment.

7. Restrictions in using waterbaths in GMP or clinical environments.

To overcome some of the limitations of using waterbaths for thawing, researchers and process engineers have explored other options such as dry bead baths or heat blocks [34,35]. Unfortunately, these solutions have inefficient thermal contact, resulting in reduced heat transfer, and may require 2–3 times longer (~7 minutes in a dry bead bath vs. ~2.5 minutes in a 37°C waterbath for a standard cryovial) to thaw samples. This slower rate of thaw may be negatively impactful to the cell product.

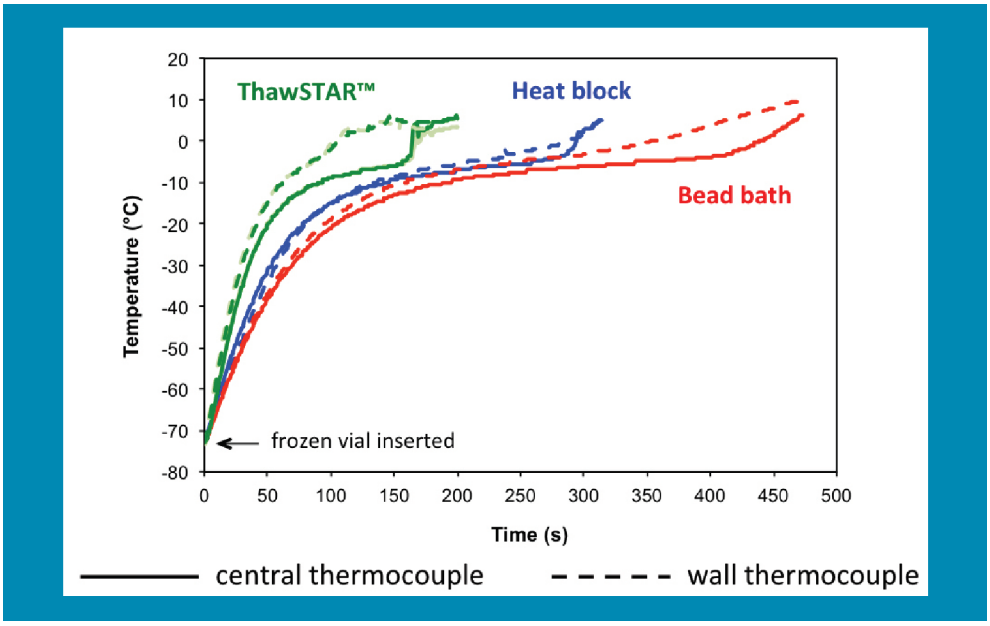
Innovations in water-free automated thawing technology have enabled sample thawing with similar thawing rates as waterbaths (Figure 9), more efficient thawing in comparison to other dry heat methods (Figure 10), cessation of active heating upon product transition from solid to liquid state, and physical separation of sample from heating interface upon thaw [36]. Equivalent post-thaw cell recovery and cell viability have also been demonstrated between newer water-free thawing technology and traditional waterbaths (Figure 11).

CONCLUSION

Cell and gene therapies are demonstrating clinical efficacy, and exhibiting early potential for commercial viability. The manufacturing and supply chain for cell and gene therapies would still benefit from substantial development and innovation, in order to model the robustness and efficiencies as experienced in the more mature fields of small molecule pharmaceuticals and large molecule biopharmaceuticals. Successful optimization of product development would benefit from a broad analysis of the product lifecycle and workflow. A methodical and diligent review of cell-based materials stability risk points (in essence, a Biopreservation Quality by Design, or BQbD), consideration to Biopreservation Critical Process Parameters (BCPP), and identification of Biopreservation Critical Quality Attributes (BCQA); would serve

FIGURE 10

Rapid vial thawing with ThawSTAR compared to dry bead bath or heat block.

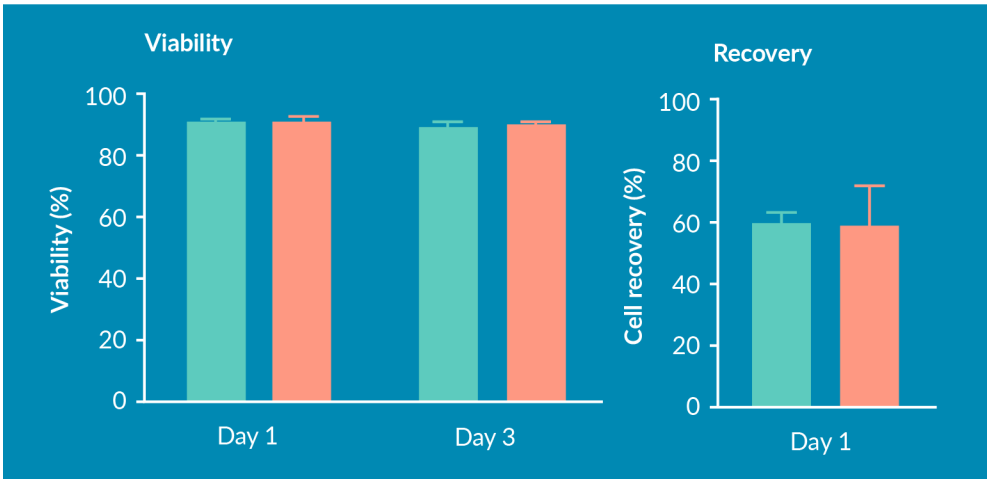


Frozen vials were thawed in either a ThawSTAR System (green traces), a 37°C bead bath (red traces), or an aluminum heat block equilibrated to 37°C (blue traces). The ThawSTAR System thaw time is 2-3X faster than these other dry thawing methods.

DOI: 10.18609/cgti.2020.150

FIGURE 11

Post-thaw cell recovery and cell viability with newer water-free thawing technology versus traditional waterbaths.



to identify stability gaps, increase system robustness, and optimize the overall CGT manufacturing and supply chain workflow. Optimizing the end-to-end Process utilizing Biopreservation Best Practices, and integrating the latest tools and technologies related to biopreservation media, controlled rate freezing and cryogenic storage, cold chain shipping management, and automated water-free thawing; would facilitate optimization of the CGT Product, and increase the probabilities for clinical and commercial success.

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AFFILIATIONS

Todd CJ Berard
BioLife Solutions Inc.

Aby J Mathew
BioLife Solutions Inc.

DOI: 10.18609/cgti.2020.150

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *Todd CJ Berard and Aby J Mathew are employees of BioLife Solutions, Inc. Todd CJ Berard is Chief Marketing Officer. Aby J Mathew is Executive Vice President & Chief Scientific Officer.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

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Article source: *Invited.*

Draft submitted: *Aug 19 2020;* ***Revised manuscript received:*** *Oct 20 2020;* ***Publication date:*** *Nov 4 2020.*

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