

CRYOPRESERVATION CRITICAL PROCESS PARAMETERS: IMPACT ON POST-THAW RECOVERY OF CELLULAR PRODUCT

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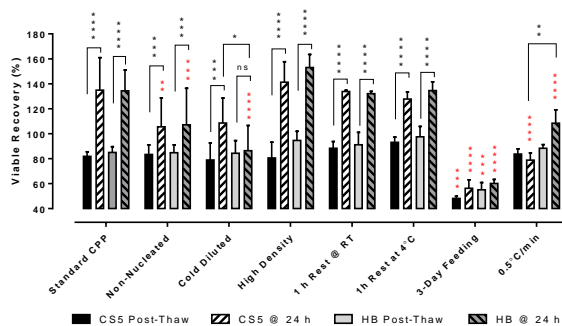
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Technological advances have transformed cells from mere drug targets into potent 'living drugs' with the potential to cure formerly incurable diseases such as cancer. Such Regenerative Medicine Advanced Therapies (RMATs) require stringent and complex vein-to-vein support to deliver their intended function. Cold Chain pertains to strategies designed to ensure the product potency and efficacy during ex-vivo transition, and includes various components starting from the source material collection, to culture and expansion process, formulation, fill-finish and packaging, storage, transportation, chain of custody, and delivery. Biopreservation is the overarching theme of the cold chain and refers to strategies to slow down or fully suspend the biological clock to allow logistical considerations. The two main modes of biopreservation are hypothermic storage and cryopreservation. This presentation aims to map the connection between a specific biopreservation strategy, namely cryopreservation, and formulation and fill-finish, and how implementation of Biopreservation Best Practices can improve the outcome of Cold Chain.

There is more to Biopreservation than storage on ice or freezing at a rate of $-1^{\circ}\text{C}/\text{min}$ in 10% DMSO. To comprehend the rationale behind Biopreservation Best Practices, a basic understanding of cellular response to cold and freezing is essential. In this study, we highlight critical process parameters (CPPs) of cryopreservation, such as freezing and thawing rates, storage and post-thaw stability, and container type, among others. Using a Jurkat T-cell model, we will discuss the impact of these CPPs on critical quality attributes (CQAs) such as viability, yield, proliferation rate, and return to function. We will also discuss the connection between variability in CPPs and characterization assay results. In general, implementation of best practices in formulation can directly address multiple process bottlenecks, including GMP compliance, minimizing freezing damage, support stability during storage and against transient warming events, support post-thaw stability, and excipient use. The CQAs may also be significantly improved by adjusting a few parameters in the freezing profile. For example, a missed or improper nucleation step during freezing may result in decreased recovery and increased variability in post-thaw proliferation rates. We have also found that the feeding timeline prior to freezing can have a profound impact on post-thaw viability and recovery in Jurkat T cells. While discussing these results, we will also review the underlying biophysics of such phenomena. The basic knowledge of designing a freezing profile may introduce degrees of freedom to process engineers to minimize the DMSO concentration in the formulation, and improve the CQAs of "hard-to-freeze" cells such as Natural Killer (NK) cells. We will also discuss the interplay between the cryopreservation CPPs and the choice of container format and how it may impact the CQAs.



Cryopreservation CPPs and their impact on post-thaw recovery of Jurkat T cells

Incorporation of Biopreservation Best Practices conveys important advantages upstream and downstream of cell manufacturing, including: (1) Assisting in the selection of the right delivery model, i.e. fresh vs. frozen, and the specific infrastructure and personnel requirements of each, based on which a commercial model is structured, (2) Reducing the Quality/Regulatory footprint of Cold Chain, and eliminating the burden of process changes when advancing to clinical trials, and (3) Improving the product CQAs, all of which could potentially improve safety and efficacy of RMAT-based clinical trials. As such, early incorporation of Biopreservation Best Practices in cell manufacturing is highly recommended.