OVERCOMING LIMITATIONS OF ORGAN-ON-CHIP (OOC) TECHNOLOGIES TO ADVANCE THE CHARACTERIZATION AND MEDICAL MANAGEMENT OF CHEMICAL AND BIOLOGICAL (CB) THREATS

Cryopreservation Improvements For Cap Organs-on-chip Increase Viability And Reproducibility To Improve Data Sets On Cb Threats And Counter Measure Development

Warren Hoeffler HOF Therapeutics

OoC systems are complex 3-D cell culture products that require expertise to produce and use. Simplifying their use will reduce the training required, while improving data reproducibility. A notable additional benefit is that the technology would be adaptable to tissue banking. Keeping living cells alive is simplified if they can be frozen solid (cryopreservation) for long-term (multi-year) storage (liquid nitrogen -196oC –210oC) or short-term (1-2 month) storage (dry ice -78.5oC), but more complex biological constructs like tissue biopsies, organs, and 3D lab organoids typically show poor survival. Despite progress in organ freezing using vitrification, directional freezing, or other technologies, tissue and organ freezing is still in the early stages of development. The need of T-cell immunotherapies for cryopreservation in their workflows, has led to improvements in freeze/thaw techniques for cells in suspension, with a better appreciation for the benefits of both controlled-rate freezing and thawing. Nucleation of ice crystals outside of the cell, and fuller ice crystal formation improves the uniformity of solutes within cells leading to improved viability upon thaw. Solid tissues, as present in all of the organs of the body, require additional expertise due to the difficulty in getting cryopreservatives to surround and penetrate attached cells. We developed a new freezing method, Cryophase®, which includes a new step to adapt tissues and organs to freeze/thaw, and uses our observations on beneficial rates of cooling/heating using programmable controlled rate equipment. We also designed a special appliance for holding plates during freezing. Importantly, our method is being optimized and customized for our 3-D cell organoids termed CAP (cell assembly programmed) organs, with the goal of simplifying correct handling and improving data reproducibility. 96-well plates containing epithelial sheets were propagated to confluence and used to optimize Cryophase® freezing of cells directly onto plates without trypsinization. Cell viability from freeze/thaw cycles was assessed using MTT assays, which measure metabolic activity, indicating a 2-to-5-fold increase in viability using Cryophase®, compared to other standard freezing techniques on attached cells. We propose to conduct a similar set of freeze/thaw assays on 96-well plates of the CACO design, which is the standard for robotic high-throughput complex biological assays used in the pharmaceutical industry. The plates will contain the CAP (cell assembly programmed) organs we have developed. Further indications of the sophistication of our method include our success with freezing/thawing small organisms. C. elegans is a microscopic worm used in genetic research. The full-sized adults can be frozen/thawed using Cryophase®, whereas only eggs and occasional hatchlings survive using currently available methods. Additionally, human skin biopsies cryopreserved using Cryophase® show an improvement in viability post thaw, especially for keratinocytes, where the improvement is several-fold. Biopsies from other organs need to be tried, and if improvements in cell viability are seen, larger organ samples should also be investigated. The efficiency of freeze/thaw for Cryophase® will be evaluated and optimized for routine use on CAP organs to make them a practical way to screen for countermeasures, which will improve the data for analysis using AI/ML platforms.

The ideas presented were developed at HOF Therapeutics, where future work will be conducted.