Live Cell Transport – The New Way of Transportation

A new generation of active temperature-controlled and CO_2 conditioned transport devices are challenging the existing cell shipment methods. In routine cell culture transport protocols, it is commonplace to thaw, plate and recover cryopreserved cells. When using this method, some cells survive the transport quite well, while others either do not survive the transport at all, or they come back from a near-death experience- not quite the same afterwards. Cells that are exposed to suboptimal life-sustaining conditions encounter severe stress and have a reduced survival rate.

The ever-evolving landscape of regenerative medicine and drug discovery is driving the development of innovative and structurally complex biological research tools and living therapies. Simultaneously, the number of business relationships, networks and consortia in which scientists, industry professionals and physicians from hospitals, research institutes and biobanks are cooperating with one another, is growing globally. The demand from these professionals is ever increasing for the proper handling and transportation of sensitive material under optimal physiological conditions.

Temperature-controlled Logistics take the Stage

In recent years the logistics industry has made a shift from what was known as cold-chain transport to temperature-controlled transport. This can be attributed to the increasing number of regenerative medicines, clinical trials, targeted therapies and cell-based products that need to be shipped over greater distances, under tightly regulated conditions. The complexity and composition of these materials demand a wider spectrum of transport temperatures and as a result specialty logistics companies now offer services that can be divided into cryogenic temperatures (-80°C and -150°C), extremely low temperatures (-20°C), cold-chain (+2 to +8°C), controlled ambient temperature (+15 to +25°C) and body temperature (+37°C).[1] The transport temperatures of these kinds of shipments are not only stringently regulated, but also monitored and the data is logged in order to provide evidence that the conditions were maintained throughout the trip. This is of utmost importance when considering that in many of these instances, the transported products will be administered to patients. Where the integrity of the shipment has been compromised, it may negatively impact a patient's health on the receiving end, or in the worst case it could even lead to a loss of life.

Shipping Cells and Tissue: The status quo

Traditionally, cells and other biological material have been stored and transported at low to cryogenic temperatures. Under these conditions, cellular degradation is limited, because the biological and chemical activity in the cells are either slowed dramatically or brought to a halt by cryopreservation.[2] With this in mind, the temperature needs to be selected based on the type of material being shipped, the composition of the solution wherein the material is stored during shipping, and what type of packaging is used during the shipment.[3] As low temperatures only slow the onset of cellular degradation, cryopreservation is the golden standard for the majority of cells and tissues that need to be sent on long journeys.

Cryopreservation: A Love-hate Relationship?

Cryopreservation in itself is a process that has revolutionised both the biological sciences and modern medicine, by providing a long-term storage and distribution solution for biological material. Nevertheless, it is not without its own shortcomings and constraints, since most living organisms don't survive freezing.[2] During freezing, cells are damaged by what is known as cryoinjury. The damage, at least in part, can be ascribed to two reasons. The first being the intracellular formation of ice crystals, which results in the degradation of intracellular structures and the second is the solution effect, which is caused by the osmotic stress experienced by the cells.[2] To counter cryoinjury, researchers add cryoprotectants during the cryopreservation. Cryoprotectants are biologically acceptable fluids with a low toxicity that can penetrate the cells in an attempt to prevent cryoinjury from occurring.[4] Following the addition of the appropriate cryoprotectant, cells are either rapidly frozen by vitrification or they are slowly frozen in a controlled manner using a freezing device. To revive the cells from the low temperature, a thawing procedure is implemented. Thawing generally involves rapid heating of the cells to 37°C, which prevents prolonged exposure to the toxicity of the cryoprotectant.[5] After the rapid heating of the cells, the cells are taken up in fresh culture media and allowed to recover in an incubator. Although remarkable progress has been made in developing strategies that are compatible with diverse cell and tissue types, the toxicity of the cryoprotectants, the costly freezing devices used, the time intensive processing and the ultimate loss of cell viability during the freeze-thaw cycles are considerable drawbacks.

Cell Losses: Expected, but a Real Headache

In general, the temperature-controlled logistics solutions being used today are adequate for the transport of any relatively robust cell lines and cell cultures, however, after the shipment the loss in cell viability could be as high as two-thirds of the total population. To examine the cell damage and resulting viability, investigators studied the effect of cryopreservation on cell cultures of NIH3T3 (Mouse Embryo Fibroblasts), HEK293 (Human Embryonic Kidney Cells) and K562 (Human Leukaemia Cells). Cells which have been cryopreserved at -80°C in the presence of a cryoprotectant, DMSO, showed that the damaged cells represented 20% (NIH3T3), 66% (HEK293) and 55% (K562) of the respective totals.[6] In the same study, slightly better results were observed when using DMSO in combination with liquid nitrogen (-196°C), with damaged cells amounting to 17% (NIH3T3), 61% (HEK293) and 49% (K562) of all cells.[6] In the control experiment, the cells were maintained under

standard incubation conditions in a CO_2 incubator, as opposed to being cryopreserved and recovered. In standard cell culture applications, incubation conditions are defined as a temperature of 37°C and a gaseous environment of 5% CO_2 for example. The optimal temperature of 37°C is based on the physiological body temperature of the organism involved, while the CO_2 is supplied to maintain pH of the bicarbonate buffered cell culture media. A bicarbonate buffered system is preferred for cell culture, as it is the most important natural buffer that maintains the pH of mammalian blood. Under these conditions, fresh cell cultures of NIH3T3, HEK293 and K562 only showed a proportion of 15%, 14% and 2% of the damaged cells respectively.[6] The aforementioned losses, due to cryopreservation, are considered within an acceptable range, since the remainder of the surviving cells are well preserved and can be recovered and re-cultured for downstream applications.[5]

Considerations for Drug Discovery and Healthcare

While a loss in cell viability can be seen as an acceptable compromise during shipments, there are applications where cryopreservation is incompatible or where cell recovery rates are unsatisfactory. Not only does cell viability need to be considered, but inadequate cryopreservation may introduce variations between different batches or could even cause genetic and epigenetic modifications.[5] These concerns are becoming more prevalent where research projects and drug discovery workflows are dependent on the delivery of highly viable fragile cells, co-culture, engineered cell/tissue constructs and 3D cultures, such as spheroids and organoids. A very interesting example is the effect of cryopreservation and storage on peripheral blood mononuclear cells (PBMCs). PBMCs are collected from a patient's blood during the apheresis process and they are important components of basic research and clinical trials.[7] They are also the source from which pure T-cells can be isolated for CAR-T research. The recommended handling procedure is to cryopreserve and then store these cells until they are needed. This however raised some concerns as to the effect of cryopreservation and long-term storage of these PBMCs. To address these concerns, researchers found that the viability of the cells was affected and the gene expression

patterns were significantly altered when comparing the fresh and cryopreserved cells. More than a thousand genes were differentially expressed and a substantial amount of them mapped to pathways such as stress response, immune activation and cell death.[7] These effects may be less significant when the cells undergo shorter periods of storage, but it would be reasonable to assume that cryopreservation and shipping may have an influence on the outcome of *in vitro* assays which are dependent on PBMCs as its starting material. When cells are destined to be used as treatments in healthcare and therapeutics, the viability, cell quality, and reproducibility are even more crucial factors. Medical products are subject to strict regulations and any potential threat to the efficacy of the final product should be addressed and verified before it is administered to a patient.[5] It may also be sensible to explore the testing requirements that viable and healthy cells should conform too. Basic tests applied to cells directly after thawing may underestimate the proportion of damaged cells, since a sizable number of cells will experience a delayed-onset cell death and start to die 24 hours post-thaw. Assays that stain for membrane integrity or other simple dye-based assessments could also be misleading, as they provide very limited information regarding the molecular physiology, health and quality of the cells. Cells may appear viable, nevertheless they may have lost their ability to renew themselves or differentiate from precursor cells.[5] Furthermore, selective pressure applied under low temperature conditions may favour subpopulations, which are epigenetically and genomically distinct from the original culture.[5]

Made to Survive

Shipping cells under non-physiological conditions will influence the cell's quality and viability. If the benchmark for cell survival in most tests is fresh/live material maintained under optimal laboratory conditions in a CO_2 incubator, would it not be sensible to also ship them under these conditions? Even though this is not a completely new idea, as Styrofoam boxes with 37°C gel packs or 37°C PCM (phase change material) containers have been used before to maintain the near physiological temperatures[1,3], these methods do not provide the needed stable CO_2 environment for the bicarbonate buffer system. In addition, these methods cannot and are not regulated. To circumvent the need for CO_2, bicarbonate buffers may be exchanged for an alternative, but this could eventually be toxic to sensitive cells and it introduces another parameter which may affect the cell's quality. To adhere to the regulated conditions used in laboratory incubators, cells should be shipped in a portable CO_2 incubator which maintains not only the ideal temperature for the cells, but also the appropriate CO_2 concentration. Technological constraints have been the limiting factor for the development of such regulated shipping incubators in the past. Now, however, the first generation of portable and truly autologous CO_2 incubators have been brought to the market, due to clever engineering. Innovative manufacturers already offer both flight and ground portable incubators.[8] With these two methods of transport, shipments are made possible to any destination worldwide (depending on local regulations). The cells can therefore be kept at regulated and user-defined incubation conditions for more than 24 hours in transit. When the shipment is handled by a specialty logistics provider, this transit period can even be extended up to 48 hours, thereby ensuring the optimal solution for sending cells to distant locations.

The Impact and Future

Portable CO_2 incubators can have a tremendous impact on the conditions of the cells which are delivered for research collaborations, drug discovery projects, clinical trials and therapies in the future. In the case where losses in cell viability and cell quality are completely unacceptable, these devices guarantee shipment under optimal laboratory conditions. Moreover, every shipment can be verified, due to comprehensive and accessible data logs that provide detailed evidence of the transport conditions. Taking all of the benefits into account, it becomes relatively simple to integrate these shipping incubators into distribution and supply chains where end-to-end monitoring is of utmost importance. The future might be here already! Portable CO_2 incubators are currently changing the way in which fragile cells are being transported in the fields of neurodegenerative diseases, cardiovascular diseases, oncology and fertility. Expansion into other medical use fields are underway. For example, initiatives to provide expanded cell therapy related capabilities to transport cell-based products for clinical application, (i.e. device and software upgrades, packaging solutions designed to guarantee sterility and lack of cross-contamination, Device Master File submission with the FDA and implementation of a QMS are already in development.[8] Although live cell shipments have many known benefits, they are not intended to replace cryopreservation, rather offer new possibilities.

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