Using cryotechnology to advance personalised medicine

SUB-ZERO ARCHIVES

The future of medical research rests in the icy repositories of biobanks. Tissue, tumour and stem cells hold information that could lead to more exact diagnoses of diseases and the development of individual therapies. To ensure this valuable biomaterial can be referenced in future, it must be kept frozen. Linde offers cryotechnologies for the entire cold chain, enabling samples to remain cryogenically preserved without damage for decades.

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Imagine an ice-cold library – that's exactly where many scientists work. Medical biobanks are home to millions of human cells. Blood samples, stem cells and tumour tissue are all kept in a cryogenic deep sleep at a temperature of minus 196 degrees Celsius, waiting for the day when they can be used. At such low temperatures, organic material can be stored for decades and kept as a vital bioresource for future generations. This is because cryogenically frozen cells are still alive. And once they have been thawed, the important information that they store can be used to study diseases or solve crimes. Scientists can, for example, use tissue samples to track the different stages of a disease, clarify the results of examinations and develop therapies.

All this is only possible because of cryostorage – a term derived from "cryos", the Greek word for cold. Researchers have been focusing on how organic materials react at extremely low temperatures since the middle of the last century. When cells are cryogenically preserved, all of their internal metabolic processes come to a halt. While frozen, they do not age, grow or separate. Cell activity stops completely. Yet this can only be achieved if the cryogenic temperature is maintained during the entire time the cells are preserved. "Cryopreservation is only possible with reliable and precise cooling," explains Peter Mawle, Global Business Manager for Cryostorage at Linde. Liquid nitrogen provides the low temperatures, as low as minus 196 degrees Celsius, that scientists need. "Linde has a wealth of expertise in this area," states Mawle. This know-how could play a key role in personalised therapies. The icy bio-databases are an important part of the equation here. Based on a patient's genetic profile, cryogenic tissue samples and analyses of those samples and the biodata stored for that patient, medics can create a clear clinical picture of the individual's medical condition and develop a personalised therapy. There are huge differences between different types of cancer, for example. Every tumour develops in its own particular way.

FREEZING CANCER CELLS TO DEVELOP INDIVIDUAL THERAPIES.

Thanks to cryo research, doctors across the globe can now use patient tissue samples to diagnose illnesses and develop therapies. Frozen bone marrow stem cells, for example, are used to fight leukaemia, while heart valves can be used to save lives years after they were donated. Frozen sperm banks have already been around for a long time. Fertility doctors are now also able to freeze ova and ovarian tissue. One of the greatest cryotechnology success stories in the field of reproductive medicine took place back in 2004 in Louvain, Belgium, with the birth of a baby conceived using frozen ovarian tissue. This process gives hope to women whose ova have been damaged by chemotherapy, for example, and who would otherwise be unable to have children. Universities and research institutes are at the forefront of these and other medical advances.

Deep freeze treasure trove: *Tissue samples are archived in biobanks. Decades later, the frozen cells can play a key role in medical research.*

However, it is not all plain sailing. "Handling biological samples is not easy. The material is extremely sensitive. Cryotechnology enables it to be transported without damage or quality impairment," states Shivan Ahamparam, Market Segment Manager Chemicals and Energy at Linde. Linde supplies cryobanks around the world with liquid nitrogen and also offers a full range of vessels, from large sample storage volumes to small transport containers. On request, Linde's "cryo" experts also deliver turnkey facilities with specially designed freezers connected to automatic liquid nitrogen re-filling units. Building on its biobank in the Dutch town of Hedel, Linde offers the full range of cryoservices to university hospitals, blood banks and biomedical and pharmaceutical companies in Belgium and the Netherlands. "Our service portfolio ranges from secure transport through material-specific storage solutions to 24-hour monitoring," explains Will Kremers Commercial Manager Hospital-/Cryocare at Linde Healthcare Benelux.

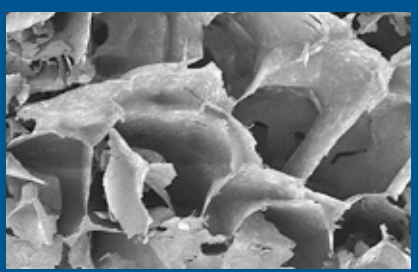
PRECISION FREEZING FOR SENSITIVE TISSUE SAMPLES.

"We transport tissue samples and biomaterial, for example, in special low-temperature containers," continues Mawle. The frozen material is then safely stored in the continuously monitored repositories of cryobanks. Cutting-edge technology and trained personnel ensure that these valuable biological resources are kept at the requisite temperature with a continuous, automatic supply of nitrogen, and that the entire process from freezing to sample removal can be accurately traced at all times. Biobanks have become increasingly important, and are now internationally networked. Which makes it more crucial than ever that they comply with the same high quality standards the world over. This is the only way of ensuring that research, science, industry and hospitals can collaborate seamlessly. "It therefore makes sense for biobank operators to have 'cryo' specialists on site to manage storage," explains Linde expert Ahamparam. Professional management is essential to ensure that the valuable samples are not prematurely awakened from their cryogenic slumber.

Linde also provides biological storage vessels that use liquid nitrogen in the gas phase. The samples are evenly cooled in the cold vapour atmosphere and are also easier to handle while minimising any risks of cross contamination. "DryStore®" is another storage vessel used, for example, by Linde Group member BOC in its cryobank in the UK. These containers feature a double wall that contains the ice-cold liquid nitrogen. This liquid nitrogen "jacket" keeps the samples in the container cool, and reduces the risk of coolant contamination. "Linde may not be a biotech company," explains Mawle. "But our innovative technologies and in-depth know-how on cryostorage make us the ideal port of call for biotech players,

FREEZING MIST ENHANCES PHARMACEUTICAL APPLICATIONS

Drug products, which are often protein based, must remain effective not only through the production process, but more importantly when administered into a patient's body. These substances are expensive, fragile, and can lose their efficacy during storage. Lyophilisation (freeze-drying) is a dehydration process for stabilising these valuable medical substances and prolonging their shelf life. It is a relatively expensive, complex, yet gentle procedure that involves freezing many vials at the same time and then removing this frozen water via sublimation. The temperature at which a vial freezes (ice nucleation temperature) is a critical parameter that impacts not only operating times but also the quality of the final product. However, there is as yet no commercially feasible way of achieving uniform ice nucleation across all vials within a batch, leading to long operating cycles, reduced yield and non-uniformity within a batch. "This is where Linde's cryogenic expertise and process knowledge provides the solution, resulting in more robust lyophilisation cycles and improved product quality," explains Beatrice Chinh, Head of the Pharmaceutical Industry Segment at Linde. The company has now developed a solution in collaboration with freeze drying equipment manufacturer IMA Life, formerly BOC Edwards. The new approach uses a sterile freezing mist (ice fog) that rapidly spreads throughout the lyophilising chamber and causes all vials to freeze at the same time, and at the desired temperature. The vial-to-vial uniformity in ice nucleation promotes product homogeneity and prevents wastage. The control of the ice nucleation temperature produces the preferred ice structure within the product, leading to shorter drying times during sublimation. The approach is feasible for both small-scale development and large-scale aseptic manufacturing. Prerona Chakravarty, Project Manager Pharmaceuticals, Fine and Specialty Chemicals, maintains that through this improvement in lyophilisation, a crucial downstream operation, Linde's proprietary induced nucleation technology will help scientists and pharmaceutical manufacturers set higher standards for quality control in drug manufacturing.



Frozen structures under the microscope: *Linde's technology enables shorter process cycles by enabling larger pores to form in sugar alcohol.*

Cryogenic freezing with nitrogen: *Biomaterial can be stored without damage in cryogenic storage banks and tanks until it is needed (above). All cell activity stops when samples are submerged in liquid nitrogen at minus 196 degrees Celsius (right). Scientists can thaw the biomaterial decades later and use it for medical research (left).*

universities and research institutes looking for advice and support in their search for cryogenic solutions."

Cryo-Save, one of Europe's leading stem cell banks, is a major customer for Linde's "cryo" specialists. Stem cells, however, need to be frozen in a special way. Linde can also supply computer controlled freezing equipment to ensure this is achieved in a precise manner. For many medical professionals, these cells represent a great opportunity in the development of specific medical answers to diseases.

Controlled cooling for stem cells

The pharmaceutical industry also uses frozen biomaterial, for example, in the search for new medicines and in high-throughput screening (HTS). HTS involves running thousands of experiments in parallel to determine, for example, whether a medical compound reacts with specific cells. "Demand for high-quality tissue samples is rising," explains Stephen Thibodeau, Professor of Laboratory Medicine at the prestigious Mayo Clinic in Rochester, Minnesota. Most sub-zero archives are often just small freezers in a basement. However, scientists are increasingly cooperating with external cryobanks that specialise in widespread diseases such as cancer or Alzheimer's. The world's largest brain bank, the Harvard Brain Tissue Resource Center is located in the US at the McLean Hospital near Boston. Working with biobanks can help doctors adopt a highly systematic approach to determining the causes and biological roadmap of diseases in the future. This will then give them the insights to develop more tailored, individual therapies.

Despite massive advances in cryotechnology, there are still major challenges to preserving life at extremely low temperatures. "Entire bodies or even organs cannot be frozen for extended periods of time. Transplant hearts are only cooled during transportation," explains Mawle. This is because organs and larger cell structures take much longer to freeze and also freeze at a more uneven rate than red blood cells or stem cells. In addition, cryopreserved material cannot be used for regular blood transfusions as not enough studies have been carried out in this area. But that could change. One thing is sure: Future medical progress hinges on controlled freezing and cryogenic storage as much as it does on advances in biotechnology. And we are only just beginning to realise the potential of cryobiology.

LINK:
www.cryo-save.com