How the AT-Closed Vial® Technology reconciliates CCI and cryo-storage of cells

Introduction

With 1078 on-going clinical trials within the field of cell and gene therapy globally, reported by the Alliance of Regenerative Medicine[1], the cell and gene therapy market is witnessing unprecedented level of financing, despite the impact of CoViD-19 on the global economy. With the current clinical success rates as well as analyzing the pipeline, the United States Food and Drug Administration (US FDA) anticipates that 10-20 cell and gene therapy products will be approved yearly by 2025[2], allowing an even larger patient population suffering from various severe diseases and conditions to access these transformative treatments.

In order to deliver the cell and gene products while preserving their critical quality attributes, low temperature storage and cryopreservation techniques are used at multiple points of the manufacturing process.

In 2014, more than 80% of the Mesenchymal Stem Cell (MSC)-based product submissions to US FDA described cryopreservation as storage and delivery method[3]. Cryopreservation is the process of cooling and storing cells, tissues, or organs at very low temperatures to maintain their functional and structural integrity. According to the United States Pharmacopoeia (USP) Chapter ⟨1044⟩, the clinical material is recommended for storage in the vapor phase of liquid nitrogen, not warmer than -150°C.[4]

In the field of gene therapy, adeno-associated virus (AAV) is the most commonly used drug delivery technology: in 2019, 89% of in vivo gene therapies developed in the United Kingdom (UK) used AAV vectors in clinical trials[5]. Meanwhile important effort is made in the field of formulation development of viral vectors for gene therapy, the current typical practice in the field remains storage at -80°C.[6] For example, recent publications on ValRox (Valoctocogene Roxaparvovec, BMN 270) by Biomarin, the near-term AAV-based gene therapy approval, refers to storage of the product at -80°C until use.[7] Some of non-AAV soon-to-be approved gene therapy products describe storage at even lower temperatures, such as Zynteglo by BlueBird, a genetically modified autologous drug containing hematopoietic stem cells (HSC) transduced with lentiviral vector (LVV), with a shelf life of 1 year at ≤-140°C.[8]

The following list highlights the advantages of low temperature storage of the drug product:

- Insurance of the product shelf life;
- Possibility of quarantine in case of necessity of extended release procedures;
- Easiest product transportation between facilities;
- Flexibility in scheduling of the treatment;[9]
- In general, more robust supply chain in case of force-majeure.

Among numerous technical challenges related to freezing and cryopreservation the choice of the container system remains a priority when considering its impact on the manufacturing, storage, logistics, and clinical strategy of the product.

[1] source: ARM Global Regenerative Medicine & Advanced Therapy Sector Report, H12020 , Source : http://alliancerm.org/wp-content/uploads/2020/08/ARM_1H-Report_-FINAL.pdf
[2] source: “Statement from FDA Commissioner Scott Gottlieb, M.D. and Peter Marks, M.D., Ph.D., Director of the Center for Biologics Evaluation and Research on new policies to advance development of safe and effective cell and gene therapies”, https://www.fda.gov/news-events/press-announcements/statement-fda-commissioner-scott-gottlieb-md-and-peter-marks-md-phd-director-center-biologics)
[3] source: Mendicino M, Bailey AM, Wonnacott K, Puri RK, Bauer SR. MSC-based product characterization for clinical trials: an FDA perspective. Cell Stem Cell. 2014 Feb;14(2):141–5.
[4] USP 1044 https://www.usp.org/sites/default/files/usp/document/our-work/biologics/resources/gc-1044-cryopreservation-of-cells.pdf
5 The Cell and Gene Therapy Catapult UK clinical trials database https://ct.catapult.org.uk/clinical-trials-database
6 Source: Development of formulations that enhance physical stability of viral vectors for gene therapy, MA Croyle, X Cheng & JM Wilson, Nature, https://www.nature.com/articles/3301527
7 The Impact of Pre-existing Immunity on the Non-clinical Pharmacodynamics of AAV5-Based Gene Therapy, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6513774/
8 Zynteglo: summary of product characteristics, https://www.ema.europa.eu/en/documents/product-information/zynteglo-epar-product-information_en.pdf
9 Technical Considerations in the Freezing, Low-Temperature Storage and Thawing of Stem Cells for Cellular Therapies, Hunt C.J https://www.karger.com/Article/Fulltext/497289

The selected container system is expected to be fit for purpose, implying that its materials should be suitable for cryogenic temperatures and allowing minimization of the safety risk related to leachables. Moreover, the container system shall ensure the container closure integrity (CCI) during long term storage at different cryogenic temperatures.

The CCI is referred to as the ability of the closure system to provide a sterile barrier against various contaminants and maintain the product quality throughout the shelf life.[10] While designing a cell or gene therapy manufacturing process, a holistic approach to CCI should be considered.

Importance of Container Closure Integrity and the Regulatory Environment

The container system is subject of qualification with a view to demonstrate how its different components react and interact during its complete life-cycle. In case of cell and gene therapies, it understands the storage at -80°C and at cryogenic temperatures.

Therefore, the selected test shall be capable of validating, in a deterministic way (i.e. methods that follow a predictable chain of events and can be controlled), how the container system behaves during the storage, while generating science-based CCI data.

Over the years, the Regulatory bodies around the world have emphasized that CCI as a critical quality parameter for sterility and stability of the product.

Specific definitions and guidance are provided in the EudraLex - Volume 4 - Good Manufacturing Practice (GMP) guidelines Annex 1 or more specifically in Part IV - GMP requirements for Advanced Therapy Medicinal Products, in USP Chapter <1207> and in Packaging Integrity Evaluation of Sterile Products of the Japanese Pharmaceuticals and Medical Devices Agency, amongst others.

“The suitability of primary packaging materials should be ensured having regard to the characteristics of the product and the storage conditions.

For production of authorized Advanced Therapies Medicinal Product (ATMP), selection, qualification, approval and maintenance of suppliers of primary packaging materials should be documented. ATMPs should be suitably packaged to maintain the quality of the product during storage, handling, and shipping. Particular attention should be paid to the closure of containers so as to ensure the integrity and quality of the product.”[11]

In the Japanese Pharmacopoeia, the container integrity is defined as: “Package integrity is the ability of a package to prevent the loss of preparations, to prevent microorganism ingress, and to limit entry of detrimental gases or other substances, thus ensuring that the product meets all necessary safety and quality standards. "Container closure system integrity" and "container integrity" mean "package integrity".[12]

In regards to testing during production, an emphasis is put on the risk control rather than testing. “Moreover, advances in the application of Process Analytical Technology (PAT), Quality by Design (QbD) and Quality Risk Management (QRM) principles to pharmaceutical development and manufacturing have shown that an appropriate combination of process controls together with timely monitoring and verification of pre-established material attributes provides greater assurance of product quality than finished product testing (conventionally regarded as the end-product testing) alone.”[13]

10 10.5731/pdajpst.2015.01071Access the most recent version at doi: 461-46569, 2015 PDA J Pharm Sci and Tech Scott Ewan, Min Jiang, Chris Stevenson, et al. Integrity Testing during Routine Manufacturing

11 https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2017_11_22_guidelines_gmp_for_atmps.pdf

12 Packaging Integrity Evaluation of Sterile Products https://www.pmda.go.jp/files/000229747.pdf

13 EudraLex The Rules Governing Medicinal Products in the European Union Volume 4 EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use Annex 17: Real Time Release Testing and Parametric Release

Facing the fill & finish challenge for novel therapies: the AT-Closed Vial® Technology

The fill and finish process of cell and gene therapy products shall be designed with an awareness of their particular features, in comparison to more common biological products. Special considerations shall be taken regarding contamination management (especially for extra small batch filling in which many manual operations are made), scalability and rapidity of the process as well as the CCI during cryopreservation.

Aseptic Technologies S.A. was created in 2002 with the aim to address the issues faced by the biopharmaceutical industry during aseptic processing. One of the developed solutions is the
AT-Closed Vial® Technology, widely used for the fill and finish of the cell and gene products, since 2009.

The AT-Closed Vial® Technology consists of two core elements: 1) the specially designed ready-to-fill polymer vial, molded and closed in ISO 5 conditions, and 2) the validated filling process, minimizing the contamination risks.

The AT-Closed Vials are delivered sterilized and ready-to-use at the end-user site after being released by Aseptic Technologies based on several tests, including CCI. The aseptic filling process is performed manually or automatically in grade A environment in three steps: first, the stopper is pierced by a sterile needle to inject the drug product into the vial. Once the needle is withdrawn, the stopper mechanically recloses as a result of the elasticity of the stopper material. Subsequently, the closure integrity of the vial is restored by applying a one second laser shot on the top surface of the stopper, re-sealing the needle trace. Finally, a sterile cap is snapped-on to protect the septum of the vial until its use at the clinical site.

These three steps allow:

- to maintain a functionally closed filling process, avoiding the risks of airborne contamination from the environment which can occur with a traditional open process (e.g., open glass/plastic vials);
- natural scalability from manual to fully automated process;
- fast filling, minimizing the product exposure to the cryoprotectant;
- robust Container Closure Integrity during the cryogenic storage.

Once filled, visually inspected and labelled, these vials can be frozen (usually down to -80°C) using different techniques and then, if required, stored in liquid nitrogen dewars before being shipped to the clinical site for the therapy administration to the patient.

How the AT-Closed Vial® resists to (ultra) cold temperature storage

First and foremost, the major factor contributing to the assurance of the AT-Closed Vial® closure integrity is the selection of the materials; the body of the vial is made of COC (Cyclic Olefin Copolymer) and the stopper is made of a proprietary TPE (Thermoplastic Elastomer). These polymer materials are molded, which allows extremely tight tolerances on critical dimension of the constitutive elements of the AT-Closed Vial®.

Secondly, a specific method is used to close these vials: right after simultaneous molding of the body and the stopper under ISO 5 conditions, high precision robots (±0.02 mm repeatability) assemble those parts together. Lastly, the top ring, is added to compress the stopper against the vial body and to secure these two parts together by snap-fit.

Such precise positioning and tolerances of the molded parts, ensure the right assembling of the different parts and the required compression between them (Figure 1).

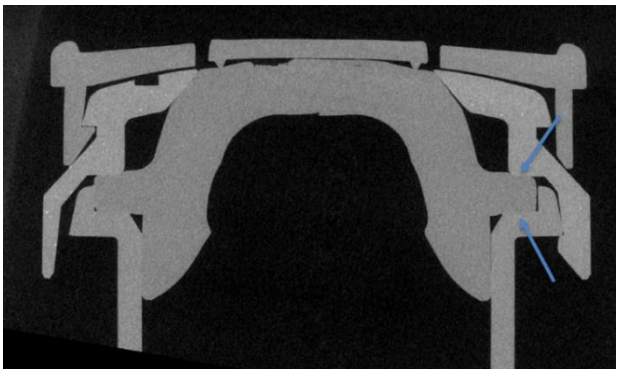

Figure 1: computed tomography scan of a processed AT-Closed Vial® at room temperature.

After packaging, the vials undergo sterilization by gamma irradiation. The gamma irradiation, in combination with the compression of the stopper on the vial body, creates a chemical bonding between the COC and TPE, which plays an important role in maintaining the CCI while the vials are frozen down. This was highlighted by an external specialized laboratory using technologies such as Scanning Electron Microscope equipped with elemental composition detector, Raman spectroscopy and mass spectrometry, who detected significant trace of material transfer on the complete contact circumference where the stopper is compressed to the body (Figure 2).

After the fill and finish process at the user's site, the AT-Closed Vials are frozen by various means and stored at different temperatures. Independently from the storage temperature (weather it is -80°C or -196°C), exclusive usage of polymers for the different container parts allows them to have similar behavior.

The conjunction of similar deformation at low temperature with chemical bonding between the stopper and the vial (Figure 3), ensure the uncompromised closure integrity of the container, even when stored at cryogenic temperature, unlike traditional container, as demonstrated by Laser Headspace Analysis (LHA) when a drop of oxygen content in the headspace proves a leak during storage in $vpLN_2$ (Figure 4).

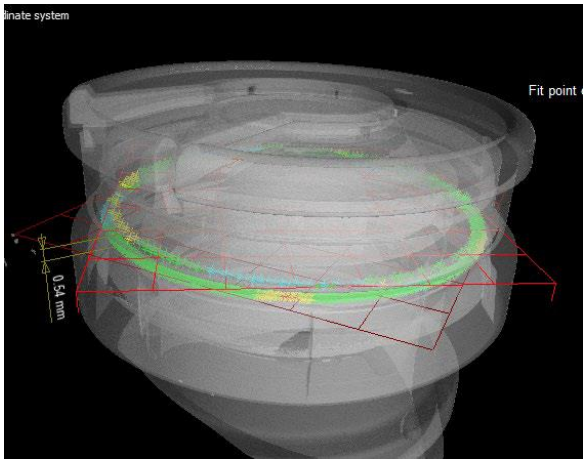

Figure 2: zoom on the zones analyzed by elemental composition detector: the chemical bonding between AT-Closed Vial® body and stopper

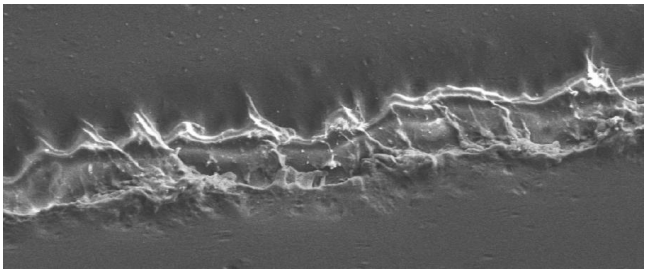

Figure 3: computed tomography scan of a processed AT-Closed Vial® at<-130°C, showing bonding all around between stopper and body.

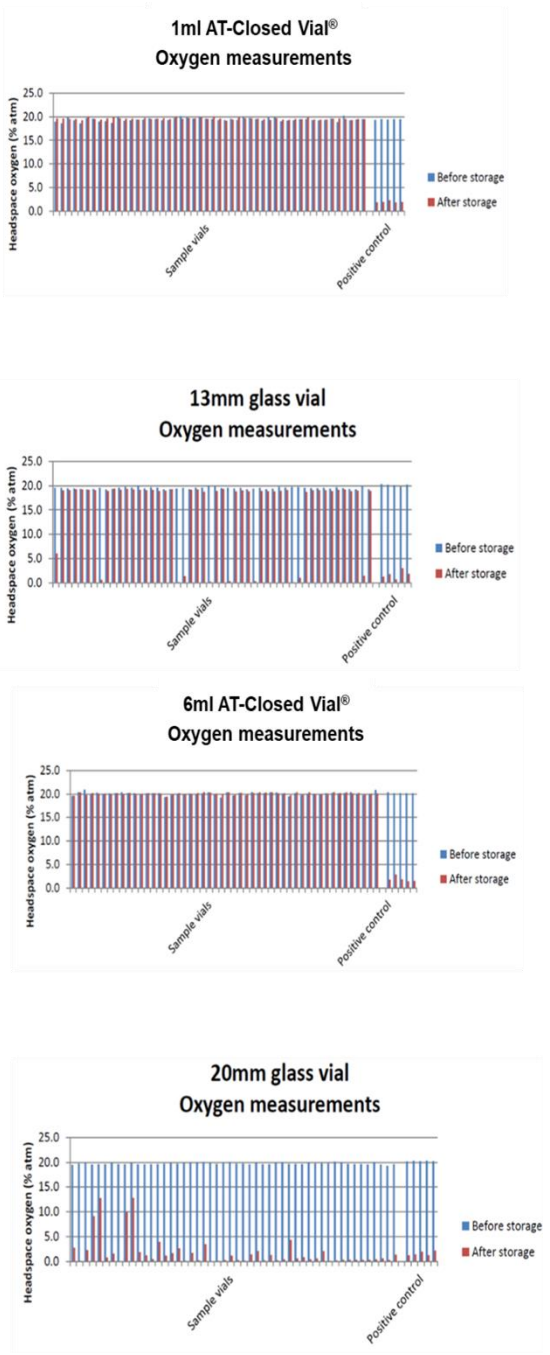

Figure 4: LHA performed by LightHouse Instruments, LLC (report 20141029), showing 20 to 100% failure of glass vials and no failure of AT-Closed Vial®

Validations of AT-Closed Vial® resistance to (ultra) cold temperature storage

Over the years, Aseptic Technologies has developed a Validation Master Plan (VMP) package to share with users an overall validation strategy, which can be provided to regulators to serve as clear justification for the validation effort.

The VMP is an integral part of the company's Quality Management System. It gathers number of test results and validation data that Aseptic Technologies produced to demonstrate the suitability of the AT-Closed Vial® Technology. The scope of the VMP addresses all activities related to equipment, processes, systems, and procedures that may impact the stability of the drug product.

The CCI is specifically addressed in the VMP as being one of the most important features of the AT-Closed Vial®.

The following data are part of the "Low Temperature" CCI package in the VMP:

1. Dye ingress test made after storage of vials at ultra-low temperature:

A large number of vials was stored at different cryogenic temperatures (e.g., at -80°C and in liquid nitrogen) for up to 15 months and have been tested by dye ingress to demonstrate that the CCI of the AT-Closed Vial® is maintained following long-term storage at low temperatures.

2. Closure integrity of AT-Closed Vials stored at different temperature and humidity conditions:

In order to cover as many scenarios as possible, Aseptic Technologies stressed empty AT-Closed Vial® at different temperatures (-20°C, 4°C, 25°C, 30°C and 40°C) and humidity conditions (40%RH, 35%RH and 25%RH) for four years.

A dye ingress test (based on international guidelines) was performed on the vials stored at each temperature & humidity condition, demonstrating that the CCI is maintained even after challenging storage conditions.

The vial appearance and container closure integrity are not modified by 4 years of storage at all these different storage conditions/configurations.

3. Closure integrity using a laser-head space analyzer on AT-Closed Vials stored in $vpLN_2$.

Several drug products, such as live viral vaccines or products containing active cells, generally require storage at cryogenic temperatures. Storage at such low temperatures can potentially lead to loss of container integrity during the storage period. One problem that can arise for pharmaceutical stoppered vials is that commonly used butyl stoppers lose their elastic properties at these low temperatures as a result of their glass transition temperature (Tg) which is typically in the range of -55°C / -70°C.

Traditional container closure integrity testing would not be appropriate in determining leaks at low temperatures as the tests are usually performed at room temperature, i.e. when the container temperature is back above Tg of the stopper, which can therefore regain its elastic properties and reseal the sample.

Effective detection of a leak during storage is possible by non-destructive test of headspace analysis. A leak during storage in $vpLN_2$ implies nitrogen ingress in the headspace which can then be detected by measuring a change in headspace oxygen level and/or an overpressure in the vial.

The study using a method known as Frequency Modulation Spectroscopy developed by Lighthouse Instruments revealed an uncompromised integrity of the AT-Closed Vial® after storage in the gas phase of liquid nitrogen (-165°C).

None of the samples showed modified oxygen levels in their headspace, or increased pressure, meaning they remained completely tight during cold storage.

In comparison, 20% to 100% of glass vials closed using butyl stopper and crimped (manually or at a monitored set force) showed signs of leakage during storage in the same conditions.

4. Analyze of the Chemical bonding of the stopper and vial body:

Chemical bonding is one of the most basic fundamentals of chemistry: chemical bonds hold molecules together and create temporary connections between materials. Aseptic Technologies analyzed the chemical bonding of the stopper (TPE) and of the vial body (COC) after gamma-irradiation to demonstrate how it can bring a benefit the CCI of the AT-Closed Vials.

Based on these and given the importance of the CCI for its users, Aseptic Technology built a specific set of information to holistically cover the subject and allow AT-Closed Vial® users to implement, qualify and eventually file new drug application to the relevant Authorities.

Conclusions

A growing number of cell and gene therapies demonstrate huge potential to be the source of revolutionary treatments ultimately curing a variety of diseases.

These advanced therapies bring with them new challenges such as cryopreservation, which impact several aspects of the life cycle of the drug product from development to its commercialization and from manufacturing to its infusion to the patient.

CCI is a key aspect of it, implying the selection of the right primary container for the drug, considering its storage at ultra-low temperatures.

CCI is defined as the ability of the container system to provide a sterile barrier against contamination and to maintain the product quality throughout the shelf life.

With that in mind, the AT-Closed Vial®, developed by Aseptic Technologies, brings a comprehensive solution for the fill and finish step of the cell and gene therapy products manufacturing.

AT-Closed Vial® provides the absolute resistance to cryopreservation, that has been validated and demonstrated, by the following methods:

- Analysis by Scanning Electron Microscope equipped with elemental composition detector;
- Analysis by Raman spectroscopy and mass spectrometry;
- Analysis by Computed Tomography Scan;
- Dye ingress test made after storage at ultra-low temperature;
- Closure integrity after storage at different temperature and humidity conditions;
- Closure integrity using a laser-head space analyzer after storage in VpLN2 (Analysis by Frequency Modulation Spectroscopy).

Combined to the process scalability and the reduction of the risk of contamination, the assurance of CCI at cryogenic temperatures makes AT-Closed Vial® Technology particularly suitable and widely used solution for the cell and gene therapy products.

About Aseptic Technologies S.A.

Aseptic Technologies, part of SKAN Group, is a globally acting developer and manufacturer of fill and finish solutions for novel biotechnology products.

Within last 10 years, AT has accompanied over three hundred sponsors in the cell and gene therapy field, contributing to their scaling-out or scaling-up manufacturing strategy either in-house or transferred to a third party, gaining a tremendous experience in de-risking of the process and providing absolute resistance of the filled vials during cryopreservation.

http://www.aseptictech.com/