CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

# Can primary packaging selection help mitigate particulate risks in cell and gene therapy manufacturing?

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Primary containers are critical components of cell and gene therapy manufacturing that are a known source of particulates. This article will discuss primary container criteria that may help mitigate the risk of visible particulates within cell processing and evaluate closed-system containers as alternatives to standard cryobags.

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## INTRODUCTION

Particulates in injectable or infusible drug products are an important concern for cell and gene therapy developers, posing a risk of embolization, contamination, or host immune reaction. While there is some variability in wording between regulators, the expectation for injectable products is that they should be free of visible particulates.

Recalls are an important means of measuring the impact of particulates to therapy manufacturers that particulates pose. In the last five years, there have been 189 drug recalls in the United States alone, of which 20 were due to the presence of particulates. In at least 35% of drugs withdrawn for the presence of particulates, the particulates were thought to originate from the container (e.g., glass or silicone particles). Clearly, particulate contamination is still a major issue within the wider pharmaceutical industry.

Control of extrinsic particles is definitive—any contamination from the environment is generally unacceptable. However, there is recognition by regulators, developers, and suppliers that cell and gene therapy products have inherent particulates, and that

management of intrinsic particles is challenging. Moreover, critical single use systems are commonly made of plastics and tend to be electrostatic and attract particles. In the context of sterile, ready-to-use components, it is difficult to eliminate components of manufacturing without introducing additional risks. A particulate management plan should be developed that incorporates a life cycle control plan and includes containers, components, raw materials, and critically, risk analysis.

## POTENTIAL PARTICULATE SOURCES AND PREVENTION MEASURES

Many currently used containers carry significant challenges on both performance (i.e., residual risk of particulates) and detectability of particulates. When considering final product containers, different types of containers have significant differences in particulate rate, ranging from ~0.5% for vials, ~1% for bottles and up to ~15% for bags (based on internal testing performed by the authors). Bags are the greatest challenge, since films tend to attract particles, and the welding at the bag edges can easily trap particles. In bags, the particulate range for the released product is highly variable and can change over time.

Other sources include all components used in the manufacturing process. A filter can be used to remove particles but also carries a risk of introducing particles flowing off the filter itself. Tube sets are another potential source, especially since the tube material is often less controlled than the plastics used in the containers. Finally, the filling process must be considered, including the cleanroom environment and closed processes where appropriate.

Prevention of particulates starts with having the right conversations with suppliers. Developers and suppliers must align on acceptance criteria for incoming material, to ensure consistency. Without alignment, an end user risks setting unreliable specifications for their processes. Another means to control particulate risks is to source new containers, tube sets, etc., with lower particulate rates. Once the limits of control are reached, filtration can be applied to reduce particulates further.

BioLife Solutions supplies both final containers and reagents. As part of the process of continual improvement, the company regularly evaluates different containers for its reagents. Table 1 shows the results from a particulate assessment of a variety of bags. A wide percentage range of particulate detection was observed, demonstrating that the choice of container is a critical factor to consider.

## THE IMPORTANCE OF INSPECTABILITY

As part of a particulate control strategy, inspectability is a key factor in choosing a final product container. Choosing a container

**TABLE 1**

**Particulate evaluation of bag types.**

| Bag type | Number of bags filled | % of units with detectable particulates |
|---|---|---|
| EVA Bag 1 | 38 | 30% |
| LDPE Bag 1 | 40 | 50% |
| EVA Bag 2 | 10 | 30% |
| LDPE Bag 2 | 10 | 100% |
| FEP Bag | 10 | 60% |
| ULDPE | 15 | 53% |
| Fluoropolymer | >50 | <10% |

EVA: ethylene-vinyl acetate; FEP: fluorinated ethylene propylene; LDPE: low-density polyethylene; ULDPE: ultra low density polyethylene.

that is easy to inspect can reduce time and increase the accuracy of inspections. Figure 1 demonstrates the ease of inspection of two different containers and shows that the newly developed CellSeal® CryoCase™ from BioLife Solutions offers significantly better inspectability versus a standard bioprocessing container. It is crucial to evaluate and define particulate risks early in the development and manufacturing process, ensure robust inspection process development, and consider alternatives when selecting containers and equipment.

## PARTICULATE CONTROL DURING FILL/FINISH

The fill/finish process contains several risks for particulate generation. Equipment manufacturer Xiogenix carried out a design failure mode and effect analysis (DFMEA) to assess potential risks associated with particulate generation with the automation equipment and manifold of their fill/finish system, ARES™ X20.

The analysis revealed that key potential risk points included:

- Environmental particulate generation from the mechanical action of the pump;
- Intrinsic particulate generation within the manifold from the interaction between pump and tubing;
- Extrinsic contamination of the single-use manifold.

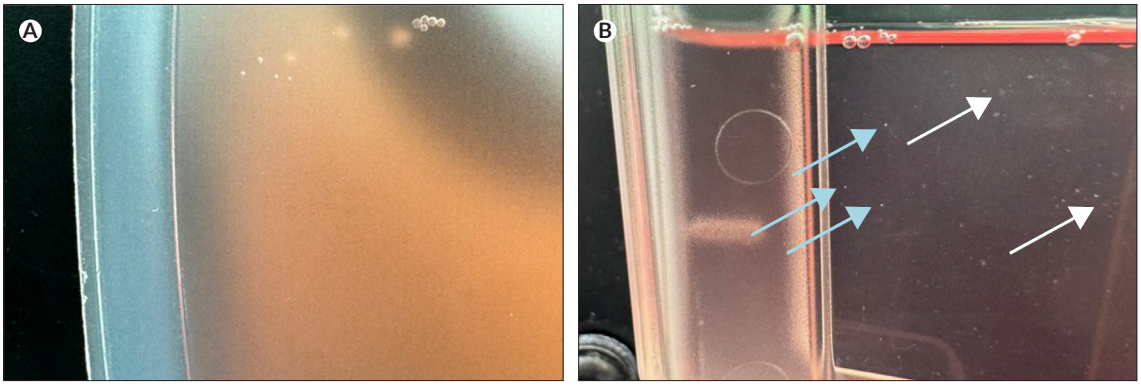
Mitigation strategies were employed, including choosing:

- A pump designed to isolate the moving mechanical components from the external environment and minimize particulate generation;
- A closed-system manifold with fully or functionally closed configurations available;
- Tubing that introduces minimal intrinsic particulate generation during operation.

Further studies are ongoing to identify intrinsic and extrinsic particle sources and to further refine the system to ensure the mitigation of any particulate contamination risks. The ARES™ X20 system is a versatile fill/finish solution for multiple different processes. One key feature is that the system is compatible with the CellSeal CryoCase, providing a convenient solution with minimal particulates and good inspectability.

## FIGURE 1

**Images of a commercially available bioprocessing container (A) versus the CellSeal CryoCase (B).**




Images were taken at identical distances under identical 2× magnification. Both containers were filled with non-filtered cell culture media. Floating aggregates or fibers (white arrows), and microbubbles (blue arrows) were detectable in the CryoCase but were undetectable in the bioprocessing container.

## CASE STUDY: IMPLEMENTING CELLSEAL CRYOCASE IN CGT MANUFACTURING

Users at CGT developer adthera bio found that the filling of the CellSeal CryoCase is straightforward—transferring a volume to the CellSeal CryoCase, clearing the line, and transferring to a controlled-rate freezer were easily managed by a single operator, in contrast to the two operators often required for bag filling. CellSeal CryoCase also offered greater consistency in fill volume, nucleation point, and freezing rate, with a slower but more uniform latent temperature reduction.

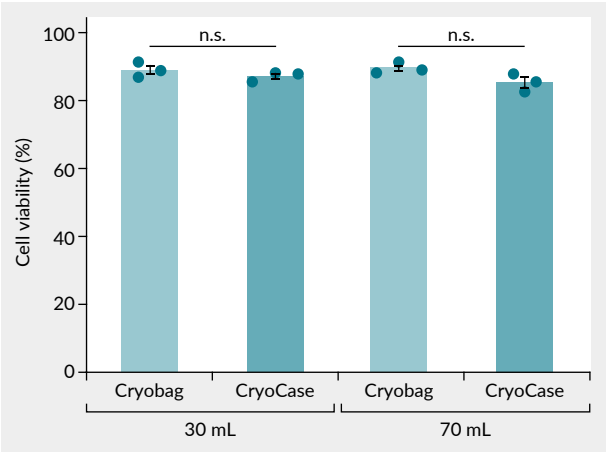
The post-thaw viability of the cells was the same in CellSeal CryoCase versus cryobags (Figure 2).

## BENEFITS OF EARLY AUTOMATION AND DIGITIZATION

Currently, the majority of steps in CGT manufacturing require manual operations but automation and digitization of processes can bring many advantages, including lower cost, the ability to capture data throughout the process, more consistency in finished product quality, and easier scale-out. The CellSeal CryoCase is designed for closed-system automation, whereas the handling of flexible bags is likely to prove challenging to automate.

## FIGURE 2

**Viability of T cells 24 h post thawing.**




## CONCLUSION

Particulates are a growing challenge in cell and gene therapy manufacturing, creating demand for innovative container options and new controls in manufacturing processes to lower particulate occurrence. The CellSeal CryoCase is an alternative to the standard cryobag with several advantages. It is designed to generate fewer particulates than cryobags, offer superb inspectability, and be compatible with closed-system automation systems. Adthera bio recently tested the CellSeal CryoCase alongside their automation equipment and found it easier to use than cryobags, with the same cell viability after freeze/thaw.

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## AUTHORSHIP & CONFLICT OF INTEREST

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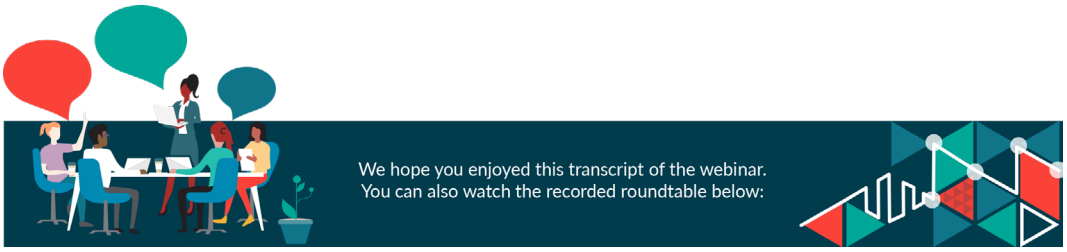
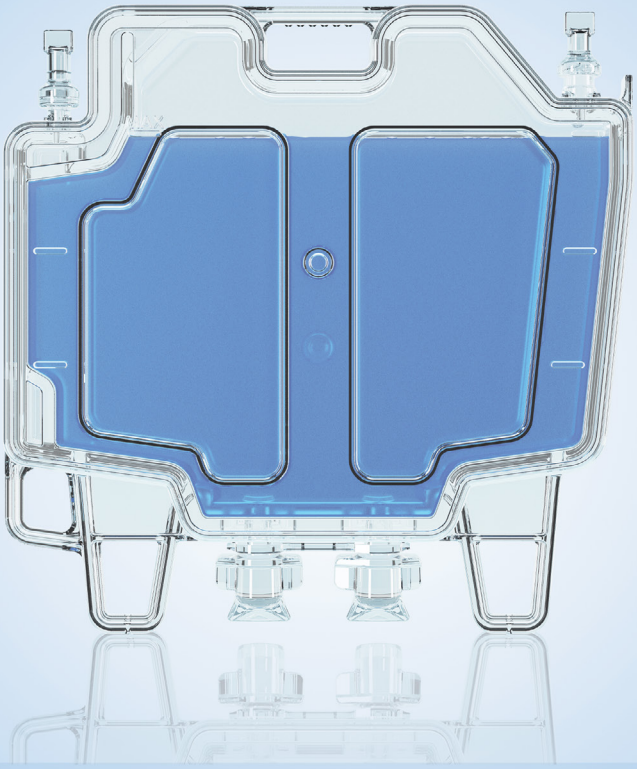


CellSeal®
CryoCase™

# CryoCase™ and conquer

An innovative solution for smarter CGT



Rethink the standard; **replace the bag!**

## Safer

Fracture resistant and capable of withstanding multiple freeze-thaw cycles.

## Stackable

Easier to handle, ship, and store.

## Easier to inspect

Transparent design boosts efficiency.

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