



## Particulates in Cell and Gene Therapies

Over the past few months, I've had numerous conversations at work and conferences about particulates in cell and gene therapies. These discussions have highlighted to me two things. Firstly, the importance of identifying both inorganic and organic particulates in cell-based therapies. Secondly, many in the field may not be fully aware of the existing options for this identification, which has significant implications for the quality and safety of these advanced therapies.

### Challenges in Cell-based Therapeutic Products

Building on these insights, it's clear that manufacturing finished products for cell-based therapies presents unique challenges. These therapies often involve living cells, introducing complexities not found in traditional pharmaceuticals. The additional complexity arises from the presence of living cells and their surrounding secretome. For some therapeutics, this secretome is essential for their function and naturally contains organic particulates. Despite these complexities, ensuring the purity and integrity of these products is crucial, as any contaminants, including particulates, can significantly impact their efficacy and safety.

Another challenge caused by the presence of living cells and their surrounding secretome is that it is often not possible to test finished product for sub-visible particles. Therefore, it is essential that a baseline for sub-visible and visible particulate levels is established. To create a baseline, a comprehensive suite of testing of all materials and processes involved in the manufacture of finished product is typically performed. To ensure that the whole manufacturing process is tested, a mock run where the entire manufacturing process without growing cells allows the flushed fluid to be tested for particulates. At this stage, sub-visible, visible and even endotoxin testing may be performed. The data from this mock run helps in understanding the typical levels of particulates present, assessing the risk each component poses and implementing strategies to control this risk. It will also give the reassurance and confidence that baseline particulate levels are acceptable before the actual product is processed.

While the complexity of a cell-based therapeutic poses challenges when assessing particulates in the final product, in addition to establishing a baseline level generated by the manufacturing process, incoming raw materials should still be assessed for particulates. The materials used to produce the therapeutic will vary but often include common reagents such as DMSO, DPBS, and EDTA. These reagents should be assessed for particulates upon arrival and by screening the reagents before they are used in the manufacturing process means that additional particulates are not being introduced into the process.

When the likely particulate level produced during a manufacturing run has been determined, incoming raw materials analysed, and the risk is at a low and acceptable level. The finished product would still need to be assessed for the presence of any visible foreign bodies. During this analysis, any extrinsic object in the samples should be investigated. Often these bodies are a fibrous material and could contain protein, silicone and non-silicone based plastics or a range of all three. Identifying these foreign bodies are crucial as they may be indicative of a fault or contamination occurring during the manufacturing process.

With foreign body analysis, the presence of living cells and their secretome, again adds complexity. Typically, analysis would involve running the sample through the particle counter and isolation of the foreign body would require passing the sample through various filters. In the case of cell-based samples, both the particle counter and filters with small pore sizes would clog. In addition, if the cell-based sample is run through a filter with a bigger pore size to avoid clogging, there would be an inherent risk that some particles are lost.

Once the foreign body has been isolated, work on identifying it can proceed. A plethora of analytical techniques can be implemented to determine the chemical or elemental composition of the extrinsic material. For elemental analysis, SEM and X-ray microanalysis are typically performed. For chemical composition, two complementary methods Fourier-transform infrared (FTIR) or RAMAN spectroscopy can be used. When single use plastic manufacturing consumables are used, identification





can be further enhanced by collecting and keeping a library of all components used in the manufacturing process. Once a foreign body has been isolated, both the foreign body and library database can then be spectroscopically analysed. If an exact match is generated, then this can help determine where the source of the particulate has come from.

### Techniques and Approaches

After addressing the challenges, it is crucial to understand the advanced techniques and approaches used to identify particulates:

- **Fourier-transform Infrared (FTIR) Spectroscopy:** FTIR spectroscopy is a powerful analytical technique used to obtain the infrared spectrum of absorption or emission of a solid, liquid, or gas. It measures the intensity of infrared light absorbed by a sample at different wavelengths. This method is particularly effective for identifying inorganic particulates, as it can detect specific vibrational modes of chemical bonds. Each inorganic particulate has a unique spectral fingerprint, allowing for precise identification. By creating a library of all inorganic compounds found in the manufacturing process, the source of particulate contamination can be identified quickly and efficiently.
- **Raman Spectroscopy:** Complementary to FTIR, Raman spectroscopy is especially useful for materials rich in water, which can be challenging to analyse using FTIR. Raman spectroscopy provides detailed information about molecular vibrations, making it effective for identifying both organic and inorganic particulates.
- **Scanning Electron Microscopy (SEM):** SEM examines the surface morphology and ultrastructure. It provides high-resolution images and, when combined with energy-dispersive X-ray spectroscopy (EDS), determines the elemental composition of the particulates.
- **Light Obscuration and Flow Imaging:** These techniques count and size subvisible particulates in a sample. They are particularly useful for monitoring the presence of particulates in therapeutic proteins and other biopharmaceuticals.
- **Confocal Microscopy:** Confocal microscopy allows for high-resolution imaging of particulates and their distribution within a sample. It is often used in conjunction with fluorescent stains to differentiate between different types of particulates.
- **HIAC Particle Counting:** For therapeutic proteins, smaller volumes can be tested using HIAC particle counters to measure protein aggregation and particulate formation.

### Practical Considerations

Implementing these techniques requires careful planning and execution. Here are some practical considerations to ensure effective particulate identification:

- **Sample Collection and Preparation:** Samples must be collected and prepared in a way that prevents additional contamination. This includes using clean containers and avoiding procedures that might introduce particulates.
- **Method Validation and Standardisation:** Ensuring that the methods used for particulate analysis are validated and standardised is crucial. This ensures consistency and reliability in the results.

- **Comparing to Controls:** Comparing particulate samples to controls is important to determine they are intrinsic to the product or extrinsic contaminants.
- **Rapid Identification and Response:** Once particulates are identified, it's important to respond quickly to address the source of contamination. This might involve adjusting manufacturing processes or sourcing materials from different suppliers.

### Future Directions

Looking ahead, the field of particulate identification in cell and gene therapies is continuously evolving. Advances in technology and methodologies are improving the accuracy and efficiency of particulate analysis. Some future directions include:

- **Automation and High-throughput Analysis:** Developing automation and high-throughput analysis techniques can speed up the process of particulate identification and characterisation, allowing for faster and more efficient quality control.
- **Advanced Imaging Techniques:** Emerging imaging techniques, such as super-resolution microscopy, provide even greater detail and accuracy in particulate analysis.
- **Integration with Other Analytical Methods:** Integrating particulate identification with other analytical methods, such as proteomics and genomics, offers a more comprehensive understanding of the sources and impacts of particulates in cell and gene therapies.

### Conclusion

In summary, identifying particulates in cell and gene therapies is a critical aspect of ensuring the quality, safety, and efficacy of these advanced treatments. By combining advanced analytical techniques and practical approaches, manufacturers can effectively identify and address particulate contamination. This ultimately leads to delivering safer and more effective therapies to patients. Establishing a baseline through thorough testing of all materials and processes involved in production is essential. This proactive approach helps maintain the integrity of the final product and adhere to stringent regulatory standards.

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