Cytotherapy 24 (2022) 1195–1200

Contents lists available at ScienceDirect

CYTOTHERAPY

journal homepage: www.isct-cytotherapy.org

Short Report

Particulates are everywhere, but are they harmful in cell and gene therapies?

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ARTICLE INFO

Article History:
Received 8 February 2022
Accepted 31 July 2022

Introduction

Cell and gene therapies (CGTs) are a broad class of revolutionary new treatment modalities with enormous potential to impact multiple intractable diseases. In recent years, the sector has reached a breakthrough point, with unprecedented approvals of cell- and gene-based treatments such as adipose-derived mesenchymal stromal cells (Alofisel, manufactured by Takeda Pharma A/S), chimeric antigen receptor T-cell therapies (e.g., Yescarta, Kite Pharma) and gene therapies (e.g., Luxturna, Spark Therapeutics) and lipid nanoparticle (LNP) vaccines against severe acute respiratory syndrome coronavirus 2 virus (Spikevax by Moderna and Comirnaty by Pfizer-BioNTech). However, when considering particle separation and product purification strategies, these diverse and complex product types pose new manufacturing challenges compared with their biologic (monoclonal antibodies and recombinant proteins) and small-molecule predecessors. These include characterization of particulates generated in the manufacturing process, understanding how these particles impact the safety and efficacy of the therapy and what standards regulators expect various CGT manufacturers to meet.

Particulates in therapeutic preparations can vary widely in terms of size and are composed of undissolved organic or inorganic materials that are intrinsic to the drug itself, inherent to the manufacturing process or extrinsically derived from a contaminating source (Table 1) [1]. Although large particulates can be removed via filtration or centrifugation methods under current regulatory guidance, smaller subvisible particles measuring 25 μm or less are a concern because of their potential to cause patient harm [2] and the difficulty associated with separating them from the drug product. Additional challenges are presented when the drug product itself can be considered a particulate, as is the case for many advanced therapeutics, such as living cells [3], viral particles [4], drug components encapsulated in lipid vesicles [5] and extracellular vesicles (EVs) released from mesenchymal stromal cells [6] or in combination (device–drug) products [7].

A typical approach when developing products intended for human use (e.g., medical devices) is to implement clean manufacturing processes to minimize particulate generation and contamination. This approach allows for careful consideration to add cleaning or filtration processes as mitigations where required. Because of the lack of applicable standards, best practices currently involve implementation of process controls to limit and characterize particulates within the product system followed by assessment of final (or clinically representative) product biocompatibility in relevant *in vitro* tests and/or *in vivo* models to verify product safety.

Of top concern are the addressable risks particles pose to patients [8]. Particle size is important because drug products delivered directly into the circulation may contain smaller particles measuring 6–8 μm that then become lodged in capillaries [9]. Similarly, one could envisage that inorganic particulate matter may accumulate in phagocytic cells, which eventually perish, releasing sequestered particulate matter back into the tissue environment and/or potentially causing inflammatory reactions, as demonstrated with nanoparticles [10]. In addition, repeated intravenous injections or local

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https://doi.org/10.1016/j.jcyt.2022.07.014

Table 1
Typical particles found in parenteral drug products.

Type		Size	Source
Inherent	Protein aggregates	Subvisible	Drug formulation
	Air bubbles	Visible	Drug formulation
	Cell aggregates	Visible	Drug product
	Cell debris	Subvisible	Drug substance
Intrinsic	Beads	Subvisible	Manufacturing processes
	Micro-carriers	Visible	Manufacturing processes
	Polymeric	Visible	Single-use plastics and closures
	Elastomeric	Visible	Containers and transfer devices
Extrinsic	PPE-derived	Visible	Gowns, gloves and other PPE
	Dust	Visible	Room air
	Silicone oil	Subvisible	Syringe components
	Polymer fibers	Visible	Filtration
	Raw materials	Subvisible and visible	Containers and transfer tubing
	Metal	Visible	Manufacturing equipment
	Suspended particles	Subvisible and visible	Raw materials

PPE, personal protective equipment.

administrations at the same anatomical region may lead to accumulated particles within local tissues, contributing to chronic inflammation or other deleterious responses [11,12].

For all the potential harms presented by particulates, an obvious mitigation is to limit particle generation by focusing on reducing particle size and quantity to the lowest reasonable levels depending on the nature of the drug product, manufacturing process and packaging. Practical guidance on navigating particulate risks in advanced therapy medicinal products would necessitate the measurement of particles generated along the single-use value chain in a typical drug product specific use case. The collection of case studies by drug product and method of particulate monitoring used in the typical manufacturing process would benefit therapy developers, suppliers and service providers. In addition, more comprehensive analysis of particulate-related side effects could be performed by the health care community. Table 2 illustrates some potential adverse effects to consider when assessing particulate risks along with additional mitigation strategies.

Current regulatory guidance provides recommendations on how to reduce particulate load through chemistry, manufacturing and control process improvements, but these standards have largely been written for and applied to traditional pharmaceuticals and biologics. Clarke *et al.* [13] outlined the risk mitigation strategies that could be considered to reduce particulate load in CGT products. However, in the last 5 years, the field has expanded dramatically, with unprecedented approvals. There is now a greater need to move the discussion toward particulate management in cell- and gene-specific regulatory guidance.

The Challenge for Updating Regulatory Guidance

As CGTs move through the clinical pipeline toward approval and commercial-scale manufacturing, the risk of product contamination with particulate matter needs to be carefully considered. The following key factors, which set advanced therapies apart with respect to particulate identification and removal, need to be considered.

Biological particles as therapies: cells, viral particles, EVs and others

Visible and subvisible particles are defined as particles measuring $\geq$100 μm and <100 μm, respectively (Table 1) [1]. Cell types typically used in cell-based therapies, including both hematopoietic and stromal variants, can range from 5 μm to 15 μm, with some larger cells, such as mesenchymal stromal cells, reaching up to 25–30 μm in size [14]. Some cellular medicines are delivered as aggregates that may contain hundreds or thousands of cells, typically varying in size from approximately 50 μm to 250 μm. In addition, cells naturally shed particulate matter through cellular processes such as exocytosis, via release of EVs when proliferating and when reacting to their micro-environment or dying (apoptotic bodies) [15–18]. Gene therapies based on small active particles such as LNPs (LNPs, EVs and viruses broadly range between 20 nm and 800 nm in size) (United States Pharmacopeia [USP] <1046>) and some viruses are oblong or irregular in shape. This poses a challenge when enumerating visible and subvisible particles per milliliter for release testing (European Pharmacopoeia 2.9.19, USP <788>). Cells and cellular material are considered particulate matter under current guidance, raising issues as to how terminology related to visual inspection methods and acceptance specifications should be defined in the release and quality control of a CGT.

Other subvisible particles that may impact CGT therapeutics

The authors opine that in certain advanced therapy drug types like LNPs and micro-volume gene therapies, potency and safety may be affected by intrinsic particles found in the manufacturing process. Particle generation from systems and consumables employed in the manufacturing process should be controlled during product development by suppliers in the manufacturing industry, such as those supplying single-use cell culture and liquid handling systems, sterile connectors, containment solutions in the cold chain and in-process

Table 2
Potential harms and mitigations related to particulates in human drug products.

Potential harm	Mitigation
Tissue implantation	
Local inflammation	Characterize particulates (inert or biodegradable, biocompatible materials may be acceptable)
Local tissue toxicity (necrosis)	
Pyrogenicity	
Allergic response (e.g., anaphylaxis due to latex particulates from PPE)	
Tissue-specific, including immune-privileged sites (brain, spinal cord and eyes)	
Eye (particulates injected into vitreous humor cause visible "floaters")	Limit visible and opaque or colored particles
Brain and spinal cord	Limit particulate size and quantity
Occlusion of arachnoid villi restricts absorption of cerebrospinal fluid	
Chronic inflammation due to reduced immune response (e.g., limited particle phagocytosis)	
Access to or communication with vascular system	A cell product itself may present a higher embolic risk; *in vivo* safety testing should address this
Embolism (particulates clog blood vessels)	Hemocompatibility testing
Thrombus formation (particulates cause clot formation)	
Thromboembolism (particulates cause clot that embolizes)	

PPE, personal protective equipment.

analytics. Some sources of subvisible particles may be inherent to the cell therapy manufacturing process. One such example is the magnetic beads that are routinely used to isolate, sort and expand cell products, which potentially can become engulfed, attached or otherwise embedded in the finished product. Release assays are routinely performed to ensure that the final cell product has a defined level of purity, free from these exogenous materials (Table 1) [19].

In an age of nanomaterials, coatings, laminations and other contaminant mitigation solutions, process designers should consider inorganic material shedding and accumulation utilizing applicable standards supplemented with analyses regarding the impact from the cold chain and freeze–thaw processes widely practiced with current CGT drug products [20–24]. In addition, the effects of cell or gene delivery particle aggregation on CGT products have been investigated and may vary significantly depending on the product [25,26]. Although efforts are made to prevent or reduce aggregation that impacts therapeutic efficacy or manufacturing, it is unclear how to best approach cell or gene particle aggregates in the context of particulate testing for product release. Likewise, the impact of detection and safety limits on drug product stability and efficacy should be considered. Guidance and subsequent standardization of such limits will be important in the translation and regulation of CGTs specifically.

CGT-based combination products as therapeutics

Combination CGT–medical device products are leading to real-world particulate issues. For example, implanted medical devices utilized to encapsulate cells may contain inorganic particulates from the device manufacturing process. Other particulate challenges arise when creating organic particulates as therapeutic EVs, aggregated protein or inorganic–organic precipitate. The presence of such particulates may be relevant to clinical efficacy, such as in the case of tissue-engineered products, further complicating acceptance criteria and specifications for these types of therapeutics.

Case Study Example: International Organization for Standardization 10993 Extraction—Particulate Observation

Several compendial biocompatibility tests defined in International Organization for Standardization 10993 involve assessment of extractions using polar and non-polar solvents. During a routine extraction process, the technician observed that a couple of particles were present in the extract and noted this in the report. The test was performed per protocol and satisfied acceptance criteria, resulting in a pass. A regulatory reviewer who had requested the full report questioned the origin and type of particulate. Despite a standard memo, generated years earlier, indicating that sample extraction was not performed in a cleanroom environment and therefore particulate contamination was not uncommon, the regulator insisted that the company perform a root cause analysis. This required a repeat of the test under cleanroom conditions, with the source of the particulate being identified as personal protective equipment worn by test technicians

CGT manufacturing approaches: batch size, single-use systems and purification

Biologics such as antibodies, cytokines and receptor decoy therapies emerged and expanded rapidly by leveraging small-molecule process development and applied learning of protein-based therapeutics utilizing similar master cell banks and bioreactor production methods. Today's CGT growth encompasses multiple product types with numerous production methods that rely on production processes firmly rooted in research methods, lacking the benefit of having been previously translated to manufacturing using Good Manufacturing Practice standards for consistent execution and analysis. Figure 1 illustrates one of these products, highlighting possible particulate generation and elimination points in a generalized cell therapy manufacturing process.

How do we, as an industry heavily reliant on single-use systems, define the significance of particulate matter in our therapies? Upscaling technologies such as bioreactors pose new questions with respect to potential system-specific intrinsic particulates (e.g., dissolvable micro-carriers or particles shed by filter cartridges). The requirement to maintain sterility throughout manufacturing, from collection to administration, creates unique challenges since typical methods used to sterilize traditional final drug products cause harm to the living and fragile drug products that define CGTs. Similarly, purification strategies such as in-line filtration methods pose challenges of particle shedding, as sterile filtration requires a polymer membrane that can also shed into the "clean" fluid path. Sterile connectors that puncture, create welds or require tear-off films may also contribute to particulate accumulation, but additional data are required to confirm this. Although particulates introduced from single-use systems can be filtered out of molecule-sized products, this cannot be as easily accomplished for many CGTs, and whereas advanced filtration

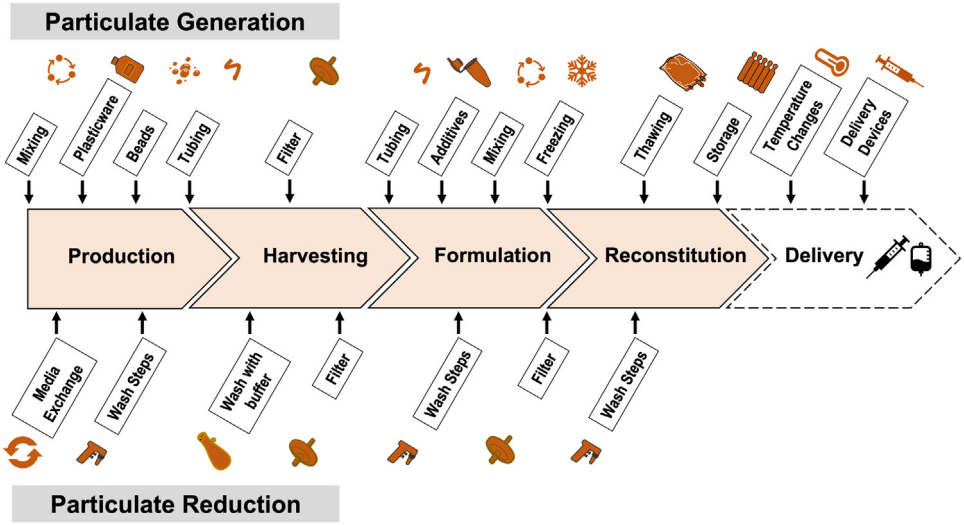

Fig. 1. Schematic depicting points of particle generation and reduction during the development of a generalized cell therapy manufacturing process. Figure created using elements of Servier Medical Art by Servier, licensed under CC BY 3.0 (https://smart.servier.com/). (Color version of figure is available online).

methods may work for some, the challenge lies in defining and isolating a clean cell product that is free of intrinsic particles. How can such release criteria and limits be standardized with such vastly different production processes employed?

Recently revised recommendations from the Bio-Process Systems Alliance regarding particulates in single-use systems provide helpful guidance [27], and task groups within ASTM Committee E55 are currently working on new standards. Running a process cycle with purified water or buffer alone can aid in the determination of sources of particulates released during processing, and single-use systems may benefit from flushing prior to application, especially components like filter cartridges. Importantly, risk scenarios vary widely, as small batch sizes and single lots can pose a challenge. For example, the production of 20 000 doses (1 mL) of a vaccine using a 20-L single-use bag with 20 visible particles yields a potential increase of only 0.1% in the rejection rate during a visual inspection. However, application of a small single-use bag with "only" a few visible particles for a CGT drug product could result in 100% rejection of the drug product during a visual inspection.

Route of administration for CGTs is likely to impact regulatory guidance

The source, material, size and quantity of foreign particulate matter will impact human health based on the therapeutic route of administration. Micro-volume injections in the eye or central nervous system are likely to pose increased risks from particulates due to the nature of the therapy [28], whereas particles contained in infusates administered into the vasculature pose an embolic risk [11]. Potency of cell-based implants may not be affected by inherent particle generation, but the quality of implanted cells may be adversely affected by particles potentially embedded during the additive manufacturing process, as in the case of tissue-engineered medicinal products. Antifouling technologies such as nanomaterials, coatings and laminations could also negatively affect the immune privilege of implanted materials because of erosion or delamination over time, possibly shedding particles throughout the life span of the product. To the authors' knowledge, both the impact of such long-term product decay and the methods used to assess the impact are not widely known in combination advanced therapy medicinal product drug–devices.

Promising Separation, Process and Product Technologies

Particle detection methods

Real-time detection of the type and quantity of particulates could enable small-scale fill and finish operations to quickly release products in a manner amenable to both product quality (e.g., size distribution of LNPs and weight distribution of viral particles) and patient safety (absence of large or aggregated particulates). Similar technologies could also be used to detect unacceptable particulates in single-cell and aggregate suspensions as well as implanted combination devices, where the therapy itself is considered a particulate. Publication of USP chapters <790> and <1790> and European Pharmacopoeia chapter 5.17.2 for visual inspection of final drug products has provided more clarity on current regulatory requirements (Table 3). However, many questions remain unanswered, including detection capabilities, training and how these requirements should be interpreted for individual CGTs. The International Conference on Harmonization (ICH) tripartite guidelines, specifically ICH Q5C, state that for stability of biotechnological/biological products, visual appearance of the product should be routinely assessed, which in the case of solutions and suspensions should be an assessment of color and opacity. When considering the use of light obscuration techniques described in USP <788>, the guidelines state that "applicants should consult the appropriate regulatory authorities on a case-by-case basis to determine guidance for testing" (ICH Q5C). This further highlights the lack of standardization with respect to how best to evaluate these parameters and how to overcome the complexity associated with classifying CGTs as drug therapeutics.

Table 3
Current regulatory guidance on particulates in injectables.

Reference Standard	Document Title
Ph. Eur 2.9.20	Particulate contamination: visible particles
Ph. Eur 2.9.19	Particulate contamination: subvisible particles
Ph. Eur 5.17.2	Recommendations on testing of particulate contamination: visible particles.
USP <1>	Injections and implanted drug products
USP <788>	Particulate matter in injections
USP <789>	Particulate matter in ophthalmics
USP <790>	Visual particulates in injections
USP <1788>	Particulate matter in injections and ophthalmic solutions
USP <1790>	Visual inspection of injections
USP <1044>	Cryopreservation of cells
ISO 23565	ISO/TS 23565:2021—biotechnology—bioprocessing—general requirements and considerations for equipment systems used in the manufacturing of cells for therapeutic use; section referencing particulates (7.3.4)

USP outlines documentary standards relied on by the FDA to ensure safety and quality of food and drugs. Ph. Eur is a single reference work for the quality control of medicines. The official standards it contains provide a scientific basis for quality control during the entire life cycle of a product.
FDA, Food and Drug Administration; ISO, International Organization for Standardization; ISO/TS, International Organization for Standardization Technical Specification; Ph. Eur, European Pharmacopoeia.

Challenges posed to therapy developers during manufacture are currently therapy-specific. Flow cytometry, other light obscuration technologies and optical systems have been used successfully to provide in-line, near real-time particle data in research [29–31]. Some of these existing technologies should be leveraged into equipment and processes that enable on-demand, batch-oriented cell products to be manufactured cost-effectively. In addition, there has been significant movement toward the development of automatic inspection systems; however, significant challenges remain regarding how best to employ these systems with respect to parameters for quantification and validation.

Contemporary automated cell and particle counting instrumentation relies heavily on software for determination of singlet, doublet and aggregated particles. Artificial intelligence is poised to dominate the discriminatory methods for determining particle size, distribution and type as detection and characterization methods improve. Technologies such as photometric mass determination combined with high-speed image processing may offer a significant improvement over pure human visual observation or simplistic light obscuration. A concerted effort to improve these technologies to permit discrimination between intended drug product components and contaminating particulates is required.

Recommendations for Consistent Standards

Because of a lack of consensus particulate standards for CGTs or the medical devices used to deliver them, the industry has leveraged existing standards intended for parenteral drug products such as injectables for intravenous or intrathecal delivery (e.g., USP <787>, USP <788>) (Table 3). However, these standards were not designed to establish acceptance limits for CGTs or delivery into other anatomical sites. To develop new or updated standards, the industry should identify the challenges faced, including:

(i) What type of particulates need to be measured?

(ii) What size and quantity of particulates are acceptable based on dose ranges, route of administration and therapeutic mechanism of action?
(iii) How is this affected by the material or source of the particles?
(iv) How does the anatomical delivery site affect acceptance limits?
(v) What are the acceptable final release assays for particulate testing of the final drug product?
(vi) What are the potential implications to patient safety?
(vii) How should we measure different particulates, particularly within cell suspensions?

CGT manufacturers and their suppliers need to identify and implement mitigations to limit particulates throughout the manufacturing process so that finished products can meet these future standards. Limitations exist around current drug purification processes, as they create challenging physical and environmental requirements for CGTs that need to maintain potency and, ultimately, clinical efficacy. Many CGTs inherently involve delivery of particulates into the patient, including cells and cellular aggregates in suspension. Can pre-clinical testing, including biocompatibility assessments and *in vivo* safety studies, be utilized to establish the safety of these products in a manner similar to that of verification and validation of medical devices?

Consistent with the fundamental philosophy of medical product development, a quality risk management approach (ICH Q9) should take a central role in determining risks associated with particulates while balancing the benefit to patients. Risks associated with these emerging therapeutics and manufacturing paradigms are not always clear, and we need to work together in an open and transparent way to identify best practices in particulate prevention and assessment to ensure CGTs get to patients in a safe and timely manner.

Although efforts are underway to establish advanced therapy-specific standards and guidance (Alliance for Regenerative Medicine Standards Coordinating Body), there is a lack of consensus in current guidelines, largely based on existing non-specific standards applied to legacy processes borne of research. Product-specific requirements and route of administration should guide product development and manufacturing process optimization based on specific particulate-related quality attributes. Related acceptance limits should consider the clinical administration procedure, number of injections/implants, total dose and likelihood of inadvertent delivery into vascular or intrathecal structures as well as particulate materials, size and quantity.

To uncover the potential challenges that particles pose to CGTs, we, as an industry, need to openly and willingly share our challenges so that the entire community can learn and improve. Without direct feedback from stakeholders and the regulatory community, increasing approvals of advanced therapies are at risk.

Funding

No funding was received.

Declaration of Competing Interest

The authors have no commercial, proprietary or financial interest in the products or companies described in this article.

Author Contributions

Conception and design of the study: All authors. Acquisition of data: All Authors. Analysis and interpretation of data: All authors. Drafting or revising the manuscript: SM, MS, and SD. All authors have approved the final article.

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