Managing particulates in cell therapy: Guidance for best practice

DOMINIC CLARKE1,2,* JEAN STANTON3,* DONALD POWERS4, OHAD KARNIELI1,5, SAGI NAHUM6, EYTAN ABRAHAM1,7, JEAN-SEBASTIEN PARISSE1,8 & STEVE OH1,9

1International Society for Cellular Therapy Process and Product Development Subcommittee, USA, 2Charter Medical, Ltd., Winston-Salem, North Carolina, USA, 3Johnson & Johnson, Philadelphia, Pennsylvania, USA, 4Janssen Supply Chain, Spring House, Pennsylvania, USA, 5Karnieli Ltd, Tivon, Israel, 6Pluristem Therapeutics, Haifa, Israel, 7Lonza Walkersville, Inc., Walkersville, Maryland, USA, 8Aseptic Technologies, Les Isnes, Belgium, and 9Stem Cell Group, Bioprocessing Technology Institute, Centros, Singapore

Abstract
The intent of this article is to provide guidance and recommendations to cell therapy product sponsors (including developers and manufacturers) and their suppliers in the cell therapy industry regarding particulate source, testing, monitoring and methods for control. This information is intended to help all parties characterize the processes that generate particulates, understand product impact and provide recommendations to control particulates generated during manufacturing of cell therapy products.

Key Words: cell therapy, disposable, injectable, parenteral, particle, particulate

Background and introduction to particulate challenge

The cell therapy community is currently engaged in more than 2500 clinical trials around the world, with 15% of those being industry-sponsored clinical trials and the remainder sponsored by leading academic centers [1]. In addition to commercially available cell therapy products and those in development, cells have been used as a standard of care for decades in the medical practices of hematology and oncology. Stem cell transplantation, for example, continues to be routinely used in, and investigated for, an increasingly diverse and growing list of malignant and nonmalignant diseases. More than one million stem cell transplants have been performed globally to date [2]. When stem cell transplantation is used as a form of cancer treatment, the requirements for particulate control in the material used for the transplant have been limited to aseptic techniques used during production, visual inspections at release and immediately before administration and in-line filters utilized at the bedside.

Cell therapy products do not undergo final filtration steps (e.g., sterile filtration) as is routinely done for many biologic products; consequently, companies developing commercial cell therapy products and suppliers need to consider the potential risk of particulate matter contamination from the external environment, during the manufacturing process or related to cellular or protein degradations. The presence of particulates in cell therapy products currently represents a quality challenge and may pose safety concerns. The challenges of particulates include difficulties identifying the nature of the particulates found in the final or injected product, limited or no understanding of the impact of particulates on the cells and difficulty defining relative contributions of different types of particulates to any in vivo toxicity or immunogenicity effects. Together these issues mean that control of particulates is likely to require a combination of analytical methods [3].

The current pipeline of cell-based therapies represents a maturation of the science surrounding these products. As such, the next generation of products carries enhanced expectations of quality, safety, efficacy and commercial viability. Compared with the first generation of cell therapies, the products currently in clinical development must be better characterized to understand the contribution and potential impact of particulates.

A number of articles addressing particulate matter exist for the pharmaceutical and bioprocessing industries [4–6], but references to particulates and cell
therapies have been limited [7]. As highlighted by Clarke et al., there are many reasons the regulations, guidelines and practices used to manufacture traditional pharmaceutical drugs or biologic products do not apply equally well for cell therapy products. Traditional injectable products are sterile filtered before the final fill. These clarified solutions are much easier to assess with current visual inspection methods [7]. Efforts have also more recently been held directly or indirectly by academia with research and development–driven competences to aid in addressing the particulate contamination issue for cell therapies. One example, is the implementation of bioprocessing integrated strategies as mentioned by Cunha et al., in which process integration can reduce the potential source of personnel contamination [8].

To build on the general awareness brought forth in Clarke’s publication, this article describes the current challenges and understanding in the industry. Challenges discussed here include risks to patients, risks to product quality and compliance or regulatory risks. Current understanding includes particulate characteristics, measurement methods, composition and sources of particulates that can be discovered in the final formulation of a cell therapy product. This article also provides guidance and recommendations for elements to be included in particulate management programs for suppliers and companies developing cell therapies. The information is intended to assist companies with their characterization of the manufacturing processes and products to minimize particulate contamination in the final products and suggests a means for suppliers and companies developing cell therapy products to work toward improving manufacturing quality and minimizing risks to products and patients.

### Particulate risks to patients and products

It is critical to the success of cell therapy products in development that they be both safe and efficacious when used to treat a specific indication in relevant patient populations. Goals of product development are to understand the risks and benefits specific to the intended use of the product and, ultimately, to reduce the risks as the product evolves. Particulate risks related to a product’s final formulation are important for many reasons. The most obvious risk is the potential for adverse events due to particulates that occur after a product is administered to a patient. The second risk is the impact of particulates on the product quality itself, both during production and in a product’s final formulation. Particulates discovered in final products also increase a product’s risk of being recalled, leading to potential clinical trial delays or failure to maintain commercial inventory.

### Patient safety and medical risks

Although the expectation is always to produce safe and effective products, a variety of criteria should be evaluated when trying to determine possible patient risks. Despite the different sources and composition of particulates, there are several common types of pathogenic mechanisms for potential harm to patients. These mechanisms include inflammation due to infections caused by viable organisms, inflammatory responses caused directly or through associate leachates that trigger direct tissue injury, normal and abnormal immune responses to cellular debris, and tissue damage from thromboembolism [9]. Most of the adverse events in the literature related to particulates in parenteral drugs are based on animal studies, in vitro studies or human case reports. The nature of the injury and degree of risk depends on several factors, including the route of administration, frequency, particle size and number, particle composition and patient population [4].

The routes of administration mainly considered for this discussion are intravenous (IV), intra-articular and intrathecal because most cell therapy products are administered via these routes. The size of the veins changes as blood flows to the heart and then to the lungs. Most particulates administered intravenously will follow this route until they are trapped in the pulmonary capillaries within the lungs, the diameter of which is approximately 12–15 μm. Many of the cells used for cell therapy purposes are often greater than 20–30 μm in size. Their size often leads to significant entrapment. Unlike many of the intrinsic and extrinsic noncellular particulates discussed in this article, many of the cells will eventually pass through after multiple passes by the blood through the lungs. Cells are more flexible when it comes to moving through smaller diameters through a mechanism called “deformation.” Inhibitors of cellular adhesion molecules (CD49d) on the cell have also been created to reduce the number of passes it takes for cells to clear the lungs [10]. The most common consequences from these trapped intrinsic and extrinsic noncellular particulates are compromised oxygen transfer and impaired respiratory function. Another clinical complication potentially resulting from IV or intra-arterial product administration is granuloma formation, with symptoms of dyspnea and reduced pulmonary function. For example, a report by Garvan describes postmortem pulmonary vasculature granulomas due to cellulose fibers. In another report, a large pulmonary granuloma formed in a patient when a particle became lodged in an arteriolar wall and eventually eroded through to form a giant cell granuloma [9]. Granulomas in organs other than the lungs are found to be of negligible safety risk for patients [11]. Another
potential risk is related to intra-arterial administration of small particulates as opposed to larger particulates. Both particulate sizes can cause occlusion in the vessels, but smaller particulates are more detrimental to patients as they are capable of causing arteriole occlusions, which can lead to limb-threatening ischemia [12].

Case reports related to large particulates are not common, most likely because these particulates are identified during visual inspection and the units are rejected from the lot. However, large particulates may cause direct traumatic damage to the vein itself, as well as infection if the particulate is not sterile. Small particulates are generally phagocytized by macrophages and taken up by several organs of the body. Normally, if the amount of particulate is small, then there are no clinical consequences because most organs have reserves to manage the deposits. When the particulate load is high, however, it may affect patient health, often overloading the spleen and liver and causing infections [13]. Several studies have also suggested that the shape of the particulate matters. One such study showed that rabbits injected with cellulose died within 4 min of administration due to acute toxin response. In contrast, rabbits injected with cellulose spheres did not suffer the same result [7].

The number of particulates administered has been considered indirectly through clinical studies that looked at adult patients in the intensive care unit. These studies were conducted to evaluate the use of IV inline filters. The results indicate that use of these filters reduced the incidence and time of onset for particulate-induced phlebitis. An inference from these studies is that the level of risk increases as the particulate load increases [14].

Particulate composition is another factor with a potential to cause harm. Barber provides an overview of several animal studies that tested different particulate compositions, including glass, fiber and polystyrene [15]. The clinical effects ranged from minor to serious (local inflammation, granuloma and death). The particulates responsible for the more serious reactions were plastics, fiber paper and polystyrene. Patient-related sequelae attributed directly to glass particulates include pulmonary granulomas, phlebitis, adult respiratory distress syndrome and systemic inflammatory response [16].

Metal particulates are seen as one of the most dangerous contaminants identified in the literature, both for IV therapies and nutritionals. Stainless steel is most commonly associated with the pharmaceutical industry, whereas metal particulates will likely be observed much less frequently in cell therapies. Literature searches found no animal or in vitro studies associated with stainless steel. The records found were associated with product recalls [17]. Inherent particulates such as cellular debris or proteinase components have the risk of triggering an unintended immune response, as can be seen in the literature for both the blood industry and therapeutic proteins. Cell therapy products that are stored for any period of time outside of cryogenic temperatures run the risk of containing high levels of cytokines. Cytokines have the potential of activating pro-inflammatory pathways, as well as causing extravascular types of reactions [18]. Proteinaceous particulate matter also poses a risk of triggering host responses, resulting in antibodies to proteins expressed on cellular surfaces.

The patient populations most at risk for particulate-related sequelae include patients with existing tissue damage, critically ill patients and neonates. Because the majority of cell therapy products in development are for adult indications, neonatal populations will generally be excluded from this discussion. Cell therapy products are indicated for patients with specific tissue damage, such as spinal cord injuries and cardiac disease. In both cases, the products are used to rescue, replace and help repair the site of injury or infarct. Particulates passively administered to these patients with cell therapy products could potentially have major secondary sequelae at the damaged site.

Another critical patient population to consider when it comes to particulates is oncology patients. Many of these patients have impaired immune systems, either as a direct result of the disease or secondarily through treatments. If the particulates are nonsterile, then patients are at potential risk for infections. Particulates also have the potential to have a negative impact on a patient’s immune system through possible immune suppression or by activating the immune system abnormally.

Evaluating each cell therapy product requires a full understanding and identification of various factors; one size does not fit all. Risk assessment is commonly performed in accordance with recognized guidance documents and standards, with risk generally being defined as the combination of the probability of occurrence of harm and the severity of that harm. Table I is a visible depiction of some of the criteria described previously, which can be used to assess potential risks to the patient. Given the unique nature of many cell therapy products, especially autologous products, the risk of limited product availability should also be considered when assessing overall risk and particulate impact.

**Product risk**

Cell therapy products face a unique challenge as the cells themselves are the final product. Thus, there is an inherent limitation to the purification processes that
can be performed, and effective removal of particulates from the final formulations will be limited. It is the responsibility of the sponsor of the cell therapy product to characterize and understand the load, source, composition and potential impact of particulates in the final formulation of their product.

The impact of inert particulates on cells and cell cultures will vary greatly depending on the properties of the particulate and of the cell lines. Cellular adhesion can be affected by exposure to particulates, depending on whether the particulate is taken up by the cell and the basic topography of the particulate. When taken up by cells, specific particulates may affect a cell’s cytoskeleton, potentially affecting cellular adhesion. Several studies with silicon-based particles have demonstrated an induced membrane deformation when the silicon-based particulates come in contact with human-derived cells, leading to cellular lysis [19]. Inert particulates that remain external to the cellular membrane can have opposite effects on cellular adhesion. First, particulates can provide a surface for cellular adhesion and thus promote aggregate formation. Cellular aggregates can clog delivery systems and negatively affect the mechanism of action and efficacy of a product. A particulate’s surface features can also reduce cellular adhesion. Studies have shown that rougher surfaces are accompanied by changes in cell physiology such as integrin expression. This altered cell architecture has been shown to result in decreased synthesis of extracellular matrix (ECM) proteins, which are crucial to cellular adhesion [20].

Upon exposure to cells, particulates can be engulfed by cells through endocytosis. The act of endocytosis can have an impact on a cell and its environment in many ways. In addition to affecting cellular adhesion, endocytosis can also be an energy drain on the cell, increasing both respiration and metabolic activity [21]. In one study, metallic particulates were shown to be cytotoxic [22]. Another study demonstrated that presentation of processed particulate antigens by antigen presenting cells can lead to the inadvertent activation of cells via release of media-

tors into their environment [23]. Moreover, particulates taken up by cells can be released into the cytoplasm, which can result in cytotoxic effects due to energy depletion, membrane disruption and organelle destruction.

Particulates can also be detrimental to the viability and functionality of the cell culture. Leachables and extractables from these types of particles can alter the pH of the environment or produce compounds that are toxic to the cells—both immediately and over time—which could directly affect product stability.

Under the heading of product risks, it is important to include the risk of product recall. The presence of foreign visible or sub-visible particulate matter in parenteral formulations has been one of the most common reasons for product recalls. During the period 2008–2012, the U.S. Food and Drug Administration (FDA) reported that 22% of recalls for sterile injectable drugs were caused by the presence of visible particles [17]. As discussed earlier in this section, the presence of certain types or numbers of particulate matter can pose health hazards. Health authorities require that they be notified of product recalls and adverse events related to products, either clinically or commercially. Therefore, it is prudent for manufacturers and their suppliers to work diligently toward characterizing visible and sub-visible particulates for their products. The amount of unexpected particulate that is present in parenteral products is an indication of the pharmaceutical quality of the product. Some of the themes the FDA noted in the 483s issued to manufacturers in 2012 related to particulate matter and to the establishment of a maximum allowable reject rate, training and certification of personnel performing visual inspections; the need to conduct a thorough investigation regarding particulate matter; and identification of sources during production.

**Particulate characterization**

The type, size, quantity, composition and source of particulates are descriptions used in the pharmaceutical industry to characterize process and product
particulates. Particulates are classified as inherent, intrinsic or extrinsic as described in Table II [9]. Inherent particulates are defined as materials that are expected from the product formulation and are typically an accepted product characteristic. Intrinsic particulates are defined as materials that arise from sources related to the product formulation, packaging and processing. Extrinsic particulates are defined as materials that do not originate from product formulation, packaging or processing but are of foreign or unexpected sources [6].

As introduced earlier, particulate size is commonly categorized as being visible or sub-visible. The general consensus within various existing pharmacopeia monographs has established visible particulates as those greater than 100 μm. With respect to the accepted quantity of particulates, the U.S. Pharmacopeia USP <1> Injections “essentially free” along with the European “practically free” and Japanese “free from readily detectable” Pharmacopeias (EP 2.9.20 and JP 6.06, respectively) [24–26] have similar descriptions and requirements for visible particulates. USP <788> Particulate Matter in Injections is generally established as a means for setting limits on the size and number of particulates based on container volumes, and these limits are harmonized with EP and JP [27]. Currently, suppliers and cell therapy sponsors use these documents as guidance, but critical challenges exist.

Particulate evaluation methods

It should be noted that this section is not intended to address and describe in detail all particulate detection or characterization methods; rather, it provides a summary of some of these methods. A variety of methods and technologies are available to measure and identify particulates from the visible to the sub-visible level, and they have been previously described at length for many applications [5,7,9]. Furthermore, technological advances have been relatively limited, so this represents an area for future development.

Before any mitigation or particulate reduction steps can be assessed and implemented for a specific cell therapy product, process and product characterization is a priority. Particulate evaluation and measurement is commonly performed as part of the cell therapy manufacturing release process. Although a number of techniques exist, visual inspection is the method of inspection most commonly used by both suppliers and sponsors.

Different factors and criteria need to be considered to determine which particulate methods to use. The type of product and its intended application will influence the particulate requirements and methods. In general, methods can be separated into either quantification or characterization. Table III depicts some of the more common methods, but it does not represent all possible methods available. Some type of quantification is almost always performed as part of the qualification and release criteria of cell therapy production, both from the supplier and the cell therapy sponsor. Quantifying particulates is performed more often, given that some of the methods available are nondestructive. Visual inspection allows for the detection and quantification of larger particulates. For detection of smaller particulates, other methods are required such as USP <788> and ASTM F24-09 [28]. These methods are destructive in nature and cannot be applied to each final product; they are often performed along with the particulate characterization testing as part of the process and product validation. Aside from quantifying particulates, characterization of particulates is important to understanding the type, source and composition of the particulates.

Table II. Types of particulates.

<table>
<thead>
<tr>
<th>Type</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inherent</td>
<td>Expected from the drug formulation and thus represent a generally accepted characteristic of the product, within limits Examples: cell clumps, excipients, etc.</td>
</tr>
<tr>
<td>Intrinsic</td>
<td>Related to the formulation, packaging or assembly processes Examples: glass, plastic, rubber, etc.</td>
</tr>
<tr>
<td>Extrinsic</td>
<td>Foreign and unexpected, not part of the formulation, package or assembly process Examples: hair, fibers, paint, etc.</td>
</tr>
</tbody>
</table>

Table III. Common particulate evaluation methods.

<table>
<thead>
<tr>
<th>Quantification</th>
<th>Characterization (all destructive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visible inspection (nondestructive)</td>
<td>Scanning electron microscopy (SEM)</td>
</tr>
<tr>
<td>TAPPI (nondestructive)</td>
<td>Energy dispersive x-ray spectrometry (EDX)</td>
</tr>
<tr>
<td>USP &lt;788&gt; (destructive)</td>
<td>Magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>ASTM F24-09 (destructive)</td>
<td>Micro-Fourier transform infrared spectroscopy (FTIR)</td>
</tr>
</tbody>
</table>
Particulate composition and source

Recognizing the source of particulates in combination with establishing particulate composition can ultimately aid in identifying potential risks to both the cell therapy product and the intended recipient. Speaking in general terms, the sources of contaminant particles found in clean rooms used in the manufacturing of pharmaceutical products can be sorted into the same categories found in other industries [15]:

- Air handling and filtration system
- Particles shed by the room construction materials
- Personnel activity
- Production materials (including packaging)
- Equipment and instrumentation

On the basis of the literature, these same categories can be extended to production of cell therapy products. Each of the preceding sources contributes proportionally to the overall particulate load of the final product and to the level of risk. A number of authors have identified proportional contributions in clean areas. Figure 1 was created using the proportions provided by Barber [15]. Extrinsic particulates, specifically those from unknown sources, represent the greatest concern. Additionally, the challenge for product developers is that cell therapy products contain cells, and the majority of cell therapy products are not “clear” solutions, which makes commonly applied particulate inspections difficult to perform. This challenge points to the need to understand and characterize particulates for each cell therapy product and manufacturing process, which should aid in assessing risk and help to identify locations and strategies for particulate controls.

Particulate matter in cell therapy products can be generated from a variety of sources within the manufacturing and production processes, as is the case with both biological and drug manufacturing. The generic cell therapy manufacturing process flow diagram depicted in Figure 2 illustrates such a process, including multiple handling and processing steps. This figure reinforces earlier statements and highlights the large number of labor-intensive steps that commonly occur in this type of manufacturing. Personnel and their activities are generally the most significant sources of nonviable particles in controlled environments. On the basis of the rationale that people contribute high numbers of particulates and interact with materials, equipment and other sources, the highest particulate levels in the clean room will occur when the process is operating with people present [29].

As seen in the Austin Contamination Index (Table IV) from Dr. Philip Austin’s Encyclopedia of Cleanrooms, Bio-Clean Rooms and Aseptic Areas, humans emit huge amounts of potentially hazardous particles even while sitting and not moving [30]. Given the nature and complexity of cell therapy processes, particulates have potential ingress routes at every step. Health authorities expect that manufacturers are knowledgeable about the particulates and their sources, as well as their potential ingress routes. To further complicate matters, only a few products have actually reached commercialization, limiting the collective experience and data that can be disseminated and used by the industry to better understand their processes and improve the quality of their products.

Figure 1. Contributing sources of contaminant particulates found in clean rooms used in the manufacturing of pharmaceutical products.
Other common sources of particulates are the materials used in production. For the purposes of this article, materials include the starting materials or cells, raw materials, excipients, disposables and primary contact materials required for manufacturing. Figure 3 illustrates some of the more common sources of particulates both from the material suppliers and the cell therapy sponsor [5]. Given the nature of cell therapy products and the manufacturing process, particulates are usually additive and can accumulate throughout the manufacturing process, as is demonstrated in Figure 3. Particulates accumulate on the supplier side of the process with each step performed, resulting in the final container or assembly. If these particulates are not controlled and removed, the particulate load is then transferred to the cell

Table IV. Austin Contamination Index: particles ≥0.3 μm emitted/min in garment indicated.

<table>
<thead>
<tr>
<th>Personnel activity</th>
<th>Snap smock</th>
<th>Standard coverall</th>
<th>2-Piece coverall</th>
<th>Tyvek coverall</th>
<th>Membrane coverall</th>
</tr>
</thead>
<tbody>
<tr>
<td>No movement</td>
<td>100 000</td>
<td>10 000</td>
<td>4 000</td>
<td>1 000</td>
<td>10</td>
</tr>
<tr>
<td>Light movement</td>
<td>500 000</td>
<td>50 000</td>
<td>20 000</td>
<td>5 000</td>
<td>50</td>
</tr>
<tr>
<td>Heavy movement</td>
<td>1 000 000</td>
<td>100 000</td>
<td>40 000</td>
<td>10 000</td>
<td>100</td>
</tr>
<tr>
<td>Change position</td>
<td>2 500 000</td>
<td>250 000</td>
<td>100 000</td>
<td>25 000</td>
<td>250</td>
</tr>
<tr>
<td>Slow walk</td>
<td>5 000 000</td>
<td>500 000</td>
<td>200 000</td>
<td>50 000</td>
<td>500</td>
</tr>
</tbody>
</table>
therapy sponsor, where additional accumulation occurs as part of the manufacturing process (as indicated by the arrows in Figure 3).

One way to visualize the process inputs with respect to particulates is through the use of a quality tool, such as the fishbone diagram (Ishikawa diagram). Figure 4 illustrates how the fishbone diagram can be used to evaluate contributing factors or sources of particulates. The contributing factors can be evaluated from the supplier’s and sponsor’s perspectives. Contributions can be broken down into two categories: suppliers and the cell therapy manufacturing process. These categories can be further divided into additional sources of potential contribution, with each being affected by five main sources:

- Machine
- Methods
- Measurements
- Environment
- People

For example, considering the generic cell therapy manufacturing process depicted in Figure 2, a single step such as the wash/buffer exchange can be highlighted and analyzed for potential particulate contribution using the fishbone diagram. Breaking down the manufacturing step into the main contributing sources, probable sources can be mapped out as demonstrated in Table V. Particulate types can then be categorized as inherent, intrinsic or extrinsic. Further characterization can include the identification of potential ingress routes for particulates. One way to
understand possible failure modes or potential risks associated with the particulates is to conduct a failure modes and effects analysis (FMEA), which can provide a systematic inductive risks analysis of a system. This method can assist suppliers and sponsors in prioritizing strategic planning with regards to any studies or experiments necessary to further define sources and potential ingress routes of particulates and, from there, to determine potential control measures [31].

Table VI provides an example of such an analysis for the same step from Figure 2, used to demonstrate the fishbone diagram for the wash/buffer exchange step. As the table indicates, once a supplier or cell therapy sponsor has identified the failures and current controls, they can make decisions regarding any additional controls and/or mitigations needed (see Appendix, which describes a case study for particulate mitigation of a cell therapy product). It is apparent from the information described thus far that particulates can be found in most cell therapy products and that methods exist to help manage the potential risk.

### Particulate guidance and mitigation solutions

Cell therapy products will almost certainly have some level of particulate contamination due to the source of the starting material and the complexity of the process. The cell therapy sponsor has the responsibility of understanding the particulate profile of the final product (number, size, composition and source) and assuring its safety for the intended patient. Ultimately, it is the responsibility of both suppliers and sponsors to control and minimize the introduction of particulates. Although removal of all particulates is unlikely, each step can be assessed for particulate control by evaluating the sources described in the prior figures.

All parties are involved in the control and reduction or elimination of particulates, and alignment is imperative. Suppliers are responsible for knowing where particulates originate and must also be aware of the end user’s needs; this may be other suppliers or cell therapy sponsors. Depending on the life cycle of a specific component, the particulate requirements and controls may be vastly different. Medical-grade components will typically have significantly greater controls in place for particulate management when compared with research-grade materials. Although many components are manufactured in clean room environments—either an ISO 8/Grade D/Class 100,000 or an ISO 7/Grade C/Class 10,000—some components or stages of the component’s life cycle may actually be manufactured outside of a clean room [5].

As part of cell therapy manufacturing, the occurrence of some particulate matter is currently challenging to avoid. Health authorities expect the product’s sponsor to understand the amount, composition and source of particulate matter in the final product and that the knowledge on the topic increase over time during development. The end point of this evolution is that the commercial product will be well characterized for particulate matter. Although particulates are assessed on a regular basis, particulate acceptance standards specific to the cell therapy industry do not currently exist. Accordingly, more guidance in this burgeoning industry could prove useful in the following areas: particulate matter characterization, control of particulate matter contamination, and confirmation or verification of a control’s acceptable quality limit.

### Characterizing particulate load

As described in an earlier section of this article, particulate characterization includes the sources of and ingress routes for particulates (process) and the composition, morphology, size and amount (product). Several quality tools are available to identify process- and product-related particulate matter; these include process flow, fishbone diagram and FMEA. The process flow tool (Figure 2) is a picture of the separate steps of a process in sequential order. A process flow provides a visual description of the details included in a process, including potential sources of particulates generated during the production of a product, as well as the means to identify, detect and measure particles [31].
The exercise of creating process flow diagrams (PFDs), which are critical in the generation of standard operating procedures and batch records, may simply be a brainstorming session. The information generated from the exercise can be used to populate the next tool, which is a fishbone diagram. This tool is commonly used to prevent defects or to identify potential factors that cause a defect. Taking each of the individual steps and its specific elements from the process flow exercise, the fishbone diagram can be used to further break down the process to provide added insight into potential sources of particulates. The causes of a defect can then be grouped into categories, referred to as the five M’s, as depicted in Table VI. From the fishbone diagram, the information can then be used to initiate a FMEA. Upon completion of a FMEA, a sponsor can describe both process and product characterization of particulate matter (see Appendix, which describes a process for particulate mitigation of a cell therapy product).

### Manufacturing controls

Controlling particulates is as important as addressing methods to reduce particulates for cell therapies. Vigilance is necessary from the choice of raw materials to the delivery of the final product. The first step in control and reduction comes from the alignment of suppliers and sponsors, with understanding of requirements versus limitations. Numerous steps can be implemented to reduce particulate load throughout the life cycle, and the main factors involve the particulate sources described previously. Starting with the sources of particulates identified in Figure 1, controls can be described as mechanisms that act as barriers to particulates, being able to deny access to the products or control particles on components used in production. Examples of barriers are provided in Table VII.

Control measures needed to prevent particulate contamination in a product can be separated into two groups: current or existing controls versus those developed as part of efforts to remediate risks identified in a FMEA. Controls included as part of routine current Good Manufacturing Practice in an aseptic manufacturing environment include the following:

- Classification of the manufacturing area
- Positive pressure environments (airflow from clean to less clean areas)
- Airflow patterns and airflow velocity
- Filtered air (high-efficiency particulate arrestance [HEPA] or other)
- Suitable facilities, areas, equipment, and materials
- Trained personnel
• Adequate transport and storage
• Production flows (product, personnel, equipment, materials, waste, quality control)
• Cleaning

Verifying effectiveness of controls

Confirmation or verification of a control’s effectiveness enables manufacturers to assess the value of the process, procedures and equipment, as well as to direct further efforts in this regard. These verification activities include the following:

• Environmental monitoring
• Visual inspection process
• Filter testing (process, HEPA, etc.)
• Water/placebo runs
• Product quality complaints

Environmental monitoring programs verify multiple systems that can affect particulate matter control in a manufacturing facility, including cleaning/sanitization, personnel and facility/utilities. It is the last system regarding levels of nonviable contaminants or the particle burden in the air of a process room or production facility that verifies particle controls. Viable and nonviable particles are usually counted and sized with the use of light-scattering particle-counting instruments, also called optical particle counters. The objectives of such a program should include establishing suitable test limits and frequencies and conducting routine monitoring of air quality to determine the need for maintenance or filter testing and periodic evaluations to assess the overall effectiveness in maintaining suitable conditions.

Visual inspection has long been considered problematic in the biologics industry due to the heavy dependence on trained personnel to perform the analysis, leading to much subjectivity. For cell therapy products, the process becomes even more problematic, as was stated earlier in this article. For sponsors of cell therapy products, it will be prudent to develop a visual inspection procedure that can detect visible particulates within an opaque liquid. This process or procedure should include decision making for accepting or rejecting an individual product (autologous cells) or an entire batch (allogeneic cells), depending on the type of cell therapy product.

There are multiple types of filters used in a particle management program, one of which is a process filter used to remove nonviable and viable particles from liquids added to the process during production. Process filters should be tested after use to ensure they remained integral through their use. For cell therapy, these large porosity product filters (e.g., greater than 50 μm) can be used for removal of large particulates or aggregates. Additional filter types include air filters, which lower particle burden in a manufacturing area through the removal of particles from the air. Attaining cleanliness in a clean room requires air filtration at a high level of efficiency. There are several types of air filters, the most well known being the HEPA. These air filters must be tested periodically to check for leakage due to damage to the medium or air bypassing the filter seal.

After the mitigation plans have been executed and remediation efforts are in place, sponsors should conduct a placebo or water run. These runs are conducted to evaluate the whole process from end to end, and they use worst-case process conditions, including those that present the greatest potential for particle generation. It is recommended to run the process under the same manufacturing conditions that are planned for the product. Some common parameters to consider include the following [6]:

• Mixing speeds
• Number of connections made during production/exposure to the environment
• Clamping or valve use
• Pumping
• Rinsing, flushing and wash steps
• General handling practices

As part of a material controls program, sponsors of cell therapy products should include a process to qualify both materials and suppliers. Material qualification includes determining its fitness for use in production and how it was produced. As stated earlier, the grade of the material provides some indication regarding the controls in place during its manufacturing, with quality increasing from research grade to medical
grade. To verify a material’s quality, suppliers also need to be evaluated by a sponsor. Qualification of suppliers should include an onsite audit, if possible, when the sponsor has no history with the supplier. Supplier audits and qualification should be performed as early as possible to avoid any unknown challenges that might influence availability and commercialization.

**Supplier particulate verification, control and mitigation**

Verification, control, and mitigation of particulates for cell therapy products require the combined efforts of suppliers and manufacturers, and a number of possible solutions were previously described [6]. From a supplier standpoint, general methods can be employed—through FMEA, for example—to identify potential ingress routes and to establish controls to minimize particulates. Many of the same methods described in the earlier sections on particulate characterization and manufacturing controls apply. These analysis methods will again point to the contribution sources illustrated in the previous section (Figure 4A), and from there one can identify next steps.

For example, consider a general single-use storage container and tubing assembly and evaluate the methods that can be employed by a supplier to verify, control, and mitigate potential particulates, bearing in mind the cleanliness of the manufacturing. Using the methods described in the previous section, manufactured bags can be initially tested to determine particulate load (number and size distribution). More detailed studies can subsequently be performed to characterize the particulates, which can be used to determine their source. In this example, particulate control and reduction strategies can be investigated at two major levels: the final assembled product and the components. Each can then be broken down using a fishbone diagram to investigate ingress routes. This detailed analysis can reveal areas where controls and reduction can be applied:

**Final product assembly**

- 100% visual inspection of the final containers
- Particulate testing as part of product validation
- Some level of product lot testing for particulates (depending on volume)
- General particulate characterization for source identification
- Regular clean room monitoring and acceptance levels
- Cleaning practices for clean room, machines and incoming raw materials/components
- Evaluation and maintenance of gowning

**Component suppliers of final product**

- Audit suppliers on a regular basis
- Inquire about component particulate testing
- Manufacturing environment (class or grade)
- Type of packaging and packaging environment
- Control and mitigation procedures
- Personnel and gowning procedures/requirements
- If component is “dirty,” can same be sourced elsewhere (secondary source)?
- Cleaning procedure (not ideal, as validation is likely required)

Numerous steps can be considered and put into practice for each product—whether it is a single-use component, raw material or the final cell therapy product—but each requires a detailed analysis. Even if some of the steps described here are addressed and controlled appropriately, the overall particulate load for a given cell therapy product can still be reduced, as illustrated in Figure 3. Understanding the origin of particulates and their general impact on the product and the intended patient is necessary, and taking actions to control and reduce particulates will be key to continuous improvement. Because time and cost will both be significantly affected, cell therapy suppliers and sponsors need to consider the intended application of the products to determine the potential particulate risk.

**Concluding remarks**

Given the nature of the processes and products in cell therapy, particulates are common and likely unavoidable at some level. Currently there are no limits or standards specific to particulates and cell therapy products. On the basis of the information described in this review, a number of factors are important (size, number, source and infusion route) when determining possible risks, which include not only risks to the intended patient population but also risks to the cell therapy product itself. As demonstrated, particulates can be added during each manipulation and can continue to accumulate throughout the entire cell therapy manufacturing process. The first step is to know the particulate composition of the final product. With this information, risk can be addressed, and subsequent particulate controls and mitigation actions can be implemented as needed by both cell therapy sponsors and suppliers.

Although following current guidance and standards developed for other industries is common practice, we propose that the unique nature of cell therapy requires its own risk-based approach. The cell therapy industry needs to maintain its awareness and continue monitoring and controlling particulates, based on the general understanding of particulates and the...
literature documenting patient risk. This understanding and the supporting data will play a key role in determining suitable standards as the industry matures, ensuring the highest patient safety and maintaining the availability of cell therapies to treat illness and life-threatening disease.

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References


Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.jcyt.2016.05.011.

Appendix

Case study

Product P is a cell therapy product that is manufactured from a bone marrow donation. The product is manufactured in the following steps using the following components:

- Collection of 30 mL bone marrow aspirate into a sterile blood collection bag
Isolation of adherent cells (tubes, plastic tubes)
• Culturing cells for four passages (tissue culture flasks/multi-trays, tubes, and buffer/media bags)
• Cryo-banking of the cells (2-mL cryovials)
• Thawing the cells and passaging in a bioreactor for additional three weeks (medium bags, tubes, carriers)
• Downstream processing (centrifuge kit, tubes, buffer bags)
• Final formulation (spinner flask, tubes, bags, containers)
• Cryopreservation (vial or cryobag)

The suggested approach taken in this case study, based on the aforementioned strategy, was divided into four main steps:

1. Identification of the source and nature of the particulates
2. Risk analysis and risk ranking
3. Mitigation, including defining measures to reduce and control the amount of particulates
4. Long-term trend analysis and control

**Step 1: Identification of the particulate source and nature**
To understand the baseline amount and nature of particulates in the raw materials and disposables, three full kits including all raw materials, tubes and disposables were prepared. Filtered water (0.22 μm) in a clean and closed container was used to fill the disposables. The water from each disposable was carefully tested to see whether particulates could be observed, quantified and identified. This initial step was used to map the source and baseline amount of particulates that do not originate from the culture.

**Step 2: Risk assessment**
The disposables were ranked by two main categories: their step in the process and the quantity/nature of particulates found (Supplemental Table SI). The closer the process step in which the disposable was used was to the final formulation, the higher the risk rank it received. Additionally, as volumes change, a score for the impact was determined. This impact score took into account the total number of particulates that the disposable introduced to the process; for example, a media bag of 10 L with 10 particulates received a lower score than a 1-L buffer bag that introduced 15 particulates, but the 10-L bag received the same score as a 20-L bag with the same amount of particulates because of the introduction of the same total number of particulates to the product. Additionally, the nature of the particulate and its safety was assessed and scored.

**Step 3: Mitigation**
Following the risk assessment, options for mitigation were then established. Examples are shown in Supplemental Table SII.

**Step 4: Long-term trend analysis and control**
To ensure the quality of each batch, a trend analysis of particulates identified during the visual inspection of the process steps must be logged and analyzed. Every new batch of material that is introduced to the manufacturing process must be initially tested for the base level of particulates in the material, similar to the study described here; this can also determine a maximum particulate level.

It is important to work with suppliers to set criteria that are achievable and acceptable to both parties. Establishing criteria that are too low or to which a supplier cannot commit must be defined, and further in-house mitigation steps (such as filtration) should be introduced.