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# Particulates in Preclinical Testing: Understanding the Impact on Overall Device Biological Safety

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# Importance of Particulates in Biological Safety Testing

In today's medical device industry, manufacturers are creating new devices at an exceptionally rapid pace—this is due to the ongoing drive to treat patients in the most effective and least burdensome manner. While some of the advancements in medical devices revolve around the development of new materials, others simply involve creating innovative ways to use materials that already have a long, safe history of clinical use. Regardless, confirming the biological safety of a new medical device has been, and will continue to be, one of the most important aspects of medical device development.

Oftentimes, to demonstrate the biological safety of a new device, some form of testing is needed; this may include chemical characterization and/or testing that involves in vitro or in vivo test methodologies. If testing is warranted, the ISO 10993 series of standards and the U.S. Food & Drug Administration (FDA) both recommend that testing be conducted on a medical device in its final finished form. Not only does this confirm (or not) that the primary materials of construction are biocompatible, but it also helps to ensure that the manufacturing, sterilization and packaging processes do not alter the overall biocompatibility of the device. Testing the device in the final finished form is intended to demonstrate that the product, transitioning from raw materials to a patient-ready device, is safe to use when subjected to clinical and biological conditions.

One of the most common approaches to biocompatibility testing involves the use of device extractions. These extractions, through the choice of vehicles and extraction conditions, are intended to exceed (or sometimes mimic) the conditions that a device will be subjected to during clinical use. Commonly, devices are extracted in polar solvents (e.g., purified water, saline) and non-polar solvents (e.g., vegetable oils, such as sesame oil); extraction temperatures and durations can depend on the study being performed and the device's materials of construction.

According to ISO 10993-12 guidelines, test samples (medical devices being tested) are prepared using a ratio of device surface area to volume of extraction vehicle, while also considering device thickness (e.g., 6 cm2 per 1 mL of extraction vehicle, for a device that is < 0.5 mm thick). These ratio and extraction conditions were developed so an exaggerated clinical use condition would be used to facilitate an optimal extraction process. Extracts are then applied to the test systems to evaluate if any leachable substances are present at levels that induce a biological response.

One challenge when preparing device extracts for testing, however, surrounds the instance when particulates are observed in test extracts. Not only could the presence of particulates impact the study being performed, but it could also be an indication of a potential problem for device performance.

In regards to a potential impact on a study, the presence of particulates in an extract that is intended to be administered intravenously (e.g., intravenous administration of polar extracts for Systemic Toxicity and Material Mediated Pyrogen testing) could cause harm to the test system used for in vivo testing that, in many cases, would be clinically irrelevant to the device in question. The ISO standard guidelines indicate that the prepared extracts be administered without any additional processing such as filtration, centrifugation or other methods to remove suspended particulates, unless justified. While one would certainly have justification to allow to settle or remove the particulates from an extract sample that is intended to be administered intravenously (to avoid intravascular dosing of particulates so as to prevent harm to the test system), regulatory agencies may require additional rationale for why removal of the particulates did not invalidate the study.

Further, one is still left with several questions: "Where did the particulates come from?" and "Does the presence of particulates indicate a potential problem with the device, manufacturing or cleaning processes?" Particulate formation under the exaggerated extraction process is not uncommon, and there are numerous contributing factors that need to be accounted for when particulates are observed. Some common examples of particulate formation are:

- Subdivision of the device prior to extraction
  - Many times, devices are cut into small pieces in order to meet the required device surface area to volume of extraction vehicle ratio, and to ensure the device is fully submerged
- Oxidation or corrosion of components
  - For example, metal components being extracted in 0.9% sodium chloride solution
- Precipitation of extractable chemicals/compounds upon cooling of the extracts to room temperature or refrigerated conditions
- Incomplete manufacturing and/or cleaning processes

The observance of particulates could be a sign of unanticipated degradation, leaching of colorants, unanticipated or unacceptable levels of corrosion or residues from manufacturing or packaging processes.

A summary of these commonly observed particulate sources is provided in Table 1. Typically, particulates due to subdivision can be identified visually as pieces of the device (e.g., white particulates observed from a white device that was subdivided prior to extraction). In this case, the presence of particulates in the extract is not of high concern, as these can be considered clinically irrelevant. However, in other cases, the observance of particulates could be a sign of unanticipated degradation, leaching of colorants, unanticipated or unacceptable levels of corrosion or residues from manufacturing or packaging processes, all of which could cause concern for device performance/failure or have direct adverse patient affects (e.g., devices that have direct contact with circulating blood or respiratory devices).

When particulates are observed that are clearly not resultant from device manipulation, a more in-depth analysis should be performed to determine their identity and source, and ensure their presence did not impact or invalidate the study being performed.

It is logical that particulates derived from a device (not from external sources; e.g., environmental background) would have a similar extractable and leachable profile to the overall device; as such, it can be concluded that these particulates would not invalidate a study. However, this argument requires characterization of the particulates using appropriate analytical techniques to determine their composition and, thus, the source of formation/origin.

**Table 1. Summary of Commonly Observed Particulates**

| Type of Particulate Observed | Probable Sources | Techniques for Identification |
|---|---|---|
| Fragmentation | Subdivision of Test Article | Visual Inspection |
| | | Surface Analysis Techniques |
| Precipitation | Degredation | Surface Analysis Techniques |
| | Oxidation/Corrosion | |
| | Colorant Leaching | |
| | Incomplete Manufacturing Practice | |
| | Incomplete Cleaning Practice | |

## Steps for Particulate Characterization

The most commonly used methods for particulate identification are surface analysis techniques, and the choice of a specific technique is dependent on whether the particulate is organic or inorganic in nature. In certain situations, an educated guess can be made about whether particulates are organic or inorganic based on the composition of the device and the polarity for the extraction vehicle. For example, it would be logical to speculate that particulates observed in a saline extraction of a stainless steel implant are inorganic iron oxide formed due to corrosion. Similarly, it would be reasonable to think that white particulates observed in a hexane extraction of a hydroxyapatite-coated metal implant are from hydroxyapatite (due to dissolution under exaggerated conditions and subsequent precipitation). In these situations, NAMSA would recommend submitting the particulate directly for confirmatory tests such as Scanning Electron Microscopy-Energy Dispersive Spectroscopy (SEM-EDS) surface analysis for inorganic particulates.

Conversely, the majority of modern medical devices are multicomponent and complex in nature. Consider the materials of a pacemaker which might include metal, plastic and adhesives—particulates observed in the pacemaker extraction would be considerably more difficult to speculate as being organic or inorganic without additional testing. Therefore, in a typical scenario, Fourier Transformed Infrared Spectrophotometer (FTIR) analysis is recommended first as it is a quick, economical and non-destructive technique. Additionally, SEM-EDS analysis is recommended only after the determination is made that the particulates are not organic in nature.

As indicated above, in NAMSA's experience surrounding organic particulates, FTIR is the most preferred analytical technique for characterization, and use of this technique for particulate characterization is widely accepted by regulatory agencies across the globe. The advantage of FTIR is the high degree of specificity and the identification of unique functional groups through a library match, as well as the fact that it is economical and non-destructive.

Attenuated Total Reflectance Fourier Transformation Infrared (ATR-FTIR) Spectroscopy is the technique of choice for organic particulate surface analysis due to the simplified sample preparation requirements and speed of analysis. A typical procedure involves isolation and drying of particulates from the extracts followed by analysis and library match. Since minimal to almost no sample manipulation is required to obtain high quality spectrum using this technique, it has gained special use in characterization of particulates formed during extraction (as isolated particulates can be easily and directly analyzed, and results can be compared

with the device and/or library reference spectra). NAMSA's global laboratories have utilized this technique for qualitative identification on a wide variety of particulates with successful demonstration of effectiveness and utility of this technique during medical device regulatory approval submissions.

Alternatively, for particulates that are inorganic in nature, SEM-EDS is a commonly used method for surface analysis. The EDS technique is based on the principle that, when bombarded with high energy electrons, an atom emits x-rays of specific energy. The detection and measurement of emitted x-ray energy forms the basis of the SEM-EDS technique.

Simplistically, in a typical procedure, a high-energy electron beam (i.e., SEM) is used to irradiate an isolated particulate surface. The incident energy excites and ejects an electron from an inner discrete electron shell (e.g., K-, L-, N-shell, etc.) of an atom. The ejected electron creates an electron hole in the shell which is filled by an electron from an outer higher energy shell filling the hole. This transfer of electron from a higher energy shell to a lower energy shell leads to the release of excess energy (dispersion of energy) in the form of an x-ray. The energy differences between electron shells are atomic mass related and atom specific. Therefore, each atom/element in the periodic table emits a characteristic x-ray of specific energy, and the detection and measurement of the number and energy of the x-rays by an energy dispersive spectrometer allows construction of elemental composition of the surface of a scanned particulate. The disadvantage of SEM-EDS, as compared to FTIR, is that it requires expensive instrumentation and maintenance, highly specialized technical expertise for data interpretation and the method is destructive. Regardless, this method may be required for particulate characterization in some instances.

## Particulates' Impact on Overall Device Biological Safety

Once particulates are identified, potential biological effects have to be evaluated. Whether the particulates are related to the 1) device itself, 2) device manufacturing/packaging or 3) device sample preparation, an evaluation is necessary to determine if the observed particulates will have an impact on the biological safety of a device.

For example, one commonly observed and identified particulate is cellulose. Because cellulosic materials are omnipresent in our daily life (e.g., clothing, packaging, etc.), revelation of cellulose particulates from an FTIR analysis is commonly linked to device packaging materials and/or environmental background. From a biological safety point of view, cellulose is naturally occurring and we are exposed to it every day without any known physiological harm. Thus, in many cases, it can be argued that the presence of cellulose particulates, regardless of the source, do not indicate any clinical risk for the majority of device types. However, if the analysis results indicate the particulates are device-related, then the clinical indication and exposure route of a device are important factors to consider when assessing the impact of particulates on the biological safety of that device.

Particulates observed for an oral device would likely be of little biological concern as risk potential will be negligible. In contrast, particulates from a cardiovascular device, regardless of particulate composition, could have serious biological consequences that need to be considered for the overall biological safety of the device. Additionally, presence of colorant(s) as leachable(s), which are likely to precipitate out in polar extraction vehicles due to relatively low water solubility of most of the color additives, may warrant colorant additive assessment as part of overall biological risk assessment.

## Conclusion

Emphasis on characterization of particulates observed following the extraction process is a critical factor in the biological evaluation of a medical device. NAMSA has observed an increase in regulatory body requests for identification of observed particulates. For this reason, it is critical to understand infrared and EDS surface analysis techniques and their role in effective, device-specific risk assessments and successful pre-market approval outcomes.

Whether the particulates are related to the 1) device itself, 2) device manufacturing/ packaging or 3) device sample preparation, an evaluation is necessary to determine if the observed particulates will have an impact on the biological safety of a device.

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Amarjit "Al" Luniwal holds a PhD in Medicinal Chemistry, a Master of Science (Pharmaceutical Chemistry emphasis) and a BS in Pharmaceutical Sciences. Dr. Luniwal has several years' experience in laboratory services with primary expertise in the areas of synthetic organic chemistry, analytical chemistry, mass spectrometry data interpretation and toxicology/biocompatibility testing. He conducted postdoctoral research in molecular biology and drug design and development at the Center for Drug Design and Development, University of Toledo prior to joining NAMSA's Analytical Services Department as a Senior Chemist. He has published his research in several peer-reviewed scientific journals and has co-authored a book chapter in 'Analogue Base Drug Design III' on Selective Estrogen Receptor Modulators (SERMs) and is a co-inventor for a granted patent from the U.S. Patent and Trademark Office. Dr. Luniwal has been with NAMSA for over six years, where he currently serves as a Board Certified Toxicologist (DABT) and focuses on materials characterization and toxicological evaluation of extractables and leachables from biomaterials used to make medical devices, as well as risk assessments for biocompatibility and biological safety.

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Dr. Fusby is a Biosafety Scientist in the Biological Safety Department at NAMSA. Her experience includes medical device biological safety and preparation of biological and toxicological risk assessments for submission in countries complying with EU and U.S. FDA regulations. She holds a PhD in Molecular Biology from the University of Colorado Denver Anschutz Medical Campus and a BS in Chemistry from the University of Nebraska Kearney (magna cum laude; minor in Molecular Biology and an emphasis in Health Science).

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Dr. Kent holds a BS in Biochemistry from San Francisco State University and an MS and PhD in Chemistry from the University of California, San Diego; he is also a Diplomate of the American Board of Toxicology. He has been working in the medical device toxicology field for over 15 years, developing expertise in the interpretation of biocompatibility and chemical characterization data in the context of the risk assessment of medical devices and materials. Dr. Kent has performed risk assessments and developed biological safety evaluation plans for a wide variety of medical devices and materials in support of successful regulatory submissions to the U.S. FDA, notified bodies and a variety of other regulatory agencies.

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Mike Hendershot currently serves as NAMSA's Manager of U.S. Biological Safety & Validation Services. He has been with the organization for over 15 years, working in the area of biological safety evaluation for the last year. Mike is a member of AAMI, SOT and ACT, and his areas of expertise are in test selection and sample preparation. He has also held positions at NAMSA ranging from National Accounts Manager, Senior Biocompatibility Advisor and Senior Medical Research Manager, prior to being named to his current role. Mr. Hendershot has presented globally on the topics of test selection, sample preparation and the biological safety evaluation of medical devices.

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Dr. Smiraldo is a Toxicologist in the Biological Safety Department at NAMSA. His experience in the medical device field encompass toxicology, biological safety, preclinical study design and extensive preparation of biological and toxicological risk assessments for submission in countries complying with EU and U.S. FDA regulations. Prior to being appointed to his current role, he was a Study Director (NAMSA) overseeing special/custom preclinical functional studies, preclinical safety studies and simulated-use chemistry studies.

Before joining NAMSA, Dr. Smiraldo was a Staff Toxicologist at WIL Research, where he was a Study Director of preclinical safety studies for pharmaceutical- and chemical-based products. He holds a PhD in Molecular and Cellular Biology from the University of Toledo Medical Center (formerly Medical College of Ohio) and a BS in Biology from Bowling Green State University (summa cum laude; with minors in Chemistry and Italian Language). He was a Postdoctoral Fellow at the University of Texas Southwestern Medical Center (awarded two grants to support his research), authored several articles that were published in peer-reviewed journals (regarding his research of DNA repair in mammalian cells) and authored a book chapter appearing in Telomerases: Chemistry, Biology, and Clinical Applications.

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 | doc-id: [ WP.028.00 ]. Publish Date: February 2019