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Biological evaluation of medical devices

Part 22: Guidance on nanomaterials

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National foreword

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Part 22: Guidance on nanomaterials

Évaluation biologique des dispositifs médicaux —

Partie 22: Lignes directrices sur les nanomatériaux

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 194, *Biological and clinical evaluation of medical devices*.

A list of all parts in the ISO 10993 series can be found on the ISO website.

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Introduction

This document is intended as guidance for the biological evaluation of medical devices that contain, generate or are composed of nanomaterials. Multiple definitions have been developed for the term nanomaterial. For the purposes of this document, the ISO definition will be used: A material is considered a nanomaterial when it has a size at the nanoscale including external and internal dimensions, i.e. when it has a size or is composed of structures with a length of approximately between 1 nm and 100 nm (ISO/TS 80004-1:2015). For regulatory purposes, it is advisable to check if specific national or regional regulatory definitions are applicable. It should be realized that other characteristics (e.g. nanospecific properties) might also be included in such definitions.

Morphological structures created on the surface of a medical device can also have sizes in the nanoscale. Therefore, possible effects of such structures on the biological response to the device also need to be considered.

Nano-objects having a length range from 1 nm to 100 nm can be generated during the life cycle of a medical device, so the evaluation of possible adverse effects due to the generation of nano-objects either from preparation, use, wear or degradation of medical devices needs to be addressed. This applies to medical devices manufactured using nanomaterials and medical devices that are manufactured not using nanomaterials but having the potential to generate nanoscale wear and/or degradation particles. For the biological evaluation of medical devices, knowledge on the potential generation and/or release of nano-objects from such materials is essential.

The procedures as described in the ISO 10993 series for the biological evaluation of medical devices can be used for the biological evaluation of those medical devices that contain nano-objects that are not released from such a device as they are an integrated part of the device. However, when release of the nano-objects is possible, a safety evaluation should also be performed on the released nano-objects. In addition to evaluating a medical device, nanomaterial components or constituents can also be separately evaluated.

This document provides trained professionals, in the context of medical device evaluation, a general approach to biological evaluation of nanomaterials and addresses how the other parts of the ISO 10993 series can be used when dealing with the evaluation of nanomaterials. It is likely that the various assays as described in the ISO 10993 series are not always appropriate as such in the testing of nanomaterials. Nanomaterials by themselves can be present as powders or colloid dispersions, but also can be present in medical devices while incorporated in a matrix, as nanostructured material or as surface structures on materials and/or medical devices. In general, nanomaterials themselves need to be evaluated instead of extracts as usually used when testing biomaterials or medical devices. Nanomaterials pose specific challenges when applying test systems commonly used for medical device evaluation and when interpreting test results.

The field of nanotechnology, development of nanomaterials and the evaluation of potential toxicity of such materials are emerging fields and this document represents only the knowledge at the time of writing. Although appropriate tools and methods for evaluation of nanomaterials are still under development, data on the characteristics and biological effects of nanomaterials should be provided in order to address safety issues in their application in the medical device field, taking into consideration a risk/benefit analysis.

This document provides guidance on how to perform a biological evaluation for those medical devices that contain, generate, or are composed of nanomaterials within a risk management process as described in ISO 10993.

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Biological evaluation of medical devices —

Part 22: Guidance on nanomaterials

1 Scope

This document describes considerations for the biological evaluation of medical devices that are composed of or contain nanomaterials. In addition, this guidance can also be used for the evaluation of nano-objects generated as products of degradation, wear, or from mechanical treatment processes (e.g. *in situ* grinding, polishing of medical devices) from (components of) medical devices that are manufactured not using nanomaterials.

This document includes considerations on the:

— characterization of nanomaterials;

— sample preparation for testing of nanomaterials;

— release of nano-objects from medical devices;

— toxicokinetics of nano-objects;

— biological evaluation of nanomaterials;

— presentation of results;

— risk assessment of nanomaterials in the context of medical device evaluation;

— biological evaluation report;

— nanostructures on the surface of a medical device, intentionally generated during the engineering, manufacturing or processing of a medical device.

The following are excluded from this document:

— natural and biological nanomaterials, as long as they have not been engineered, manufactured or processed for use in a medical device;

— intrinsic nanostructures in a bulk material;

— nanostructures on the surface of a medical device, generated as an unintentional by-product during the engineering, manufacturing or processing of a medical device.

NOTE Examples of unintentional nanostructures on the surface of a medical device are extrusion draw lines and machining/tool marks.

This document is intended to provide a general framework and highlights important aspects which need to be considered when assessing the safety of medical devices composed of, containing and/or generating nano-objects. Additionally, the document identifies several common pitfalls and obstacles which have been identified when testing nanomaterials compared to bulk materials or small molecule chemical species. As a technical report (TR), this document represents the current technical knowledge related to nanomaterials. No detailed testing protocols are outlined or provided. This document can serve as a basis for future documents containing detailed protocols with a focus on nanomaterial testing.

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2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 10993 (all parts), *Biological evaluation of medical devices*

ISO/TR 13014, *Nanotechnologies — Guidance on physico-chemical characterization of engineered nanoscale materials for toxicologic assessment*

ISO 14971, *Medical devices — Application of risk management to medical devices*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 10993 (all parts), ISO/TR 13014 and ISO 14971 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at http://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
aggregate
particle comprising strongly bonded or fused particles where the resulting external surface area is significantly smaller than the sum of calculated surface areas of the individual components

Note 1 to entry: The forces holding an aggregate together are strong forces, for example, covalent bonds, or those resulting from sintering or complex physical entanglement.

Note 2 to entry: Aggregates are also termed secondary particles and the original source particles are termed primary particles.

[SOURCE: ISO/TS 80004-2:2015, 3.5, modified — definition and Note 1 to entry changed]

3.2
agglomerate
collection of weakly bound particles or *aggregates* (3.1) or mixtures of the two where the resulting external surface area is similar to the sum of the surface areas of the individual components

Note 1 to entry: The forces holding an agglomerate together are weak forces, for example van der Waals forces, or simple physical entanglement.

Note 2 to entry: Agglomerates are also termed secondary particles and the original source particles are termed primary particles.

[SOURCE: ISO/TS 80004-2:2015, 3.4]

3.3
engineered nanomaterial
nanomaterial (3.7) designed for a specific purpose or function

[SOURCE: ISO/TS 80004-1:2015, 2.8]

3.4
incidental nanomaterial
nanomaterial (3.7) generated as an unintentional by-product of a process

Note 1 to entry: The process includes manufacturing, bio-technological or other processes.

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Note 2 to entry: See "ultrafine particle" in ISO/TR 27628:2007, 2.21.

[SOURCE: ISO/TS 80004-1:2015, 2.10]

3.5
manufactured nanomaterial
nanomaterial (3.7) intentionally produced to have selected properties or composition

[SOURCE: ISO/TS 80004-1:2015, 2.9]

3.6
nanofibre
nano-object (3.8) with two similar external dimensions in the *nanoscale* (3.12) and the third dimension significantly larger

Note 1 to entry: A nanofibre can be flexible or rigid.

Note 2 to entry: The two similar external dimensions are considered to differ in size by less than three times and the significantly larger external dimension is considered to differ from the other two by more than three times.

Note 3 to entry: The largest external dimension is not necessarily in the nanoscale.

[SOURCE: ISO/TS 80004-6:2013, 2.6]

3.7
nanomaterial
material with any external dimension in the *nanoscale* (3.12) or having internal structure or surface structure in the nanoscale

Note 1 to entry: This generic term is inclusive of *nano-object* (3.8) and *nanostructured material* (3.17).

Note 2 to entry: See also 3.3 to 3.5.

Note 3 to entry: For regulatory purposes, it is advisable to check if specific national or regional regulatory definitions are applicable. It should be realized that different size ranges or other properties might be included in such definitions.

[SOURCE: ISO/TS 80004-1:2015, 2.4, modified — Note 2 to entry modified and Note 3 to entry added]

3.8
nano-object
discrete piece of material with one, two or three external dimensions in the *nanoscale* (3.12)

Note 1 to entry: The second and third external dimensions are orthogonal to the first dimension and to each other.

[SOURCE: ISO/TS 80004-1:2015, 2.5]

3.9
nanoparticle
nano-object (3.8) with all external dimensions at the *nanoscale* (3.12) where the length of the longest and the shortest axes of the nano-object do not differ significantly

Note 1 to entry: If the dimensions differ significantly (typically by more than 3 times), terms such as *nanofibre* or *nanoplate* may be preferred to the term nanoparticle.

[SOURCE: ISO/TS 80004-2:2015, 4.4]

3.10
nanoplate
nano-object (3.8) with one external dimension in the *nanoscale* (3.12) and the two other external dimensions significantly larger

Note 1 to entry: The smallest external dimension is the thickness of the nanoplate.

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Note 2 to entry: The two significantly larger dimensions are considered to differ from the nanoscale dimension by more than three times.

Note 3 to entry: The larger external dimensions are not necessarily in the nanoscale.

[SOURCE: ISO/TS 80004-6:2013, 2.4]

3.11
nanorod
solid *nanofibre* (3.6)

[SOURCE: ISO/TS 80004-2:2015, 4.7]

3.12
nanoscale
length range approximately from 1 nm to 100 nm

Note 1 to entry: Properties that are not extrapolations from larger sizes are predominantly exhibited in this length range.

Note 2 to entry: Properties impacting biocompatibility can also occur at larger sizes, e.g. between 100 nm and 1 um.

[SOURCE: ISO/TS 80004-1:2015, 2.1, modified — Note 2 to entry was added]

3.13
nanoscale phenomenon
effect attributable to the presence of *nano-objects* (3.8) or *nanoscale* (3.12) regions

[SOURCE: ISO/TS 80004-1:2015, 2.13]

3.14
nanoscale property
characteristic of a *nano-object* (3.8) or *nanoscale* (3.12) region

[SOURCE: ISO/TS 80004-1:2015, 2.14]

3.15
nanoscience
study, discovery and understanding of matter, where size- and structure-dependent properties and phenomena manifest, predominantly in the *nanoscale* (3.12), distinct from those associated with individual atoms or molecules, or extrapolation from larger sizes of the same material

[SOURCE: ISO/TS 80004-1:2015, 2.2]

3.16
nanostructure
composition of inter-related constituent parts in which one or more of those parts is a *nanoscale* (3.12) region

Note 1 to entry: A region is defined by a boundary representing a discontinuity in properties.

[SOURCE: ISO/TS 80004-1:2015, 2.6]

3.17
nanostructured material
material having internal *nanostructure* (3.16) or surface nanostructure

Note 1 to entry: This definition does not exclude the possibility for a *nano-object* (3.8) to have internal structure or surface structure. If external dimension(s) are in the *nanoscale* (3.12), the term nano-object is recommended.

[SOURCE: ISO/TS 80004-1:2015, 2.7]

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3.18
nanotechnology
application of scientific knowledge to manipulate and control matter predominantly in the *nanoscale* (3.12) to make use of size- and structure-dependent properties and phenomena distinct from those associated with individual atoms or molecules, or extrapolation from larger sizes of the same material

Note 1 to entry: Manipulation and control includes material synthesis.

[SOURCE: ISO/TS 80004-1:2015, 2.3]

3.19
nanotube
hollow *nanofibre* (3.6)

[SOURCE: ISO/TS 80004-2:2015, 4.8]

3.20
representative test material
RTM
material, which is sufficiently homogenous and stable with respect to one or more specified properties, and is implicitly assumed to be fit for its intended use in the development of measurement and test methods that target properties other than those for which homogeneity and stability have been demonstrated

[SOURCE: ISO/TS 16195:2013; 3.1, modified — Notes 1 and 2 to entry deleted]

4 General principles

4.1 General considerations

Nanomaterials are manufactured and used because of the specific properties that can be associated with the decrease in size accompanied by an increase of surface area. Also, materials with dimensions in the size range >100 nm or <1 micron can elicit properties different from those in the macroscale (>1 micron). For these types of particulate materials, it might be considered to perform an assessment similar to nanomaterials in the size range between 1 nm and 100 nm.

The biological evaluation of any material or medical device intended for use in humans should form part of a structured biological evaluation program within a risk management process in accordance with ISO 14971 and ISO 10993-1. The risk management process is applicable to devices that contain or are composed of nanomaterials. The risk management process is also applicable to devices that generate nano-objects as products of degradation, wear, or from mechanical treatment processes (e.g. *in situ* grinding, polishing of medical devices). Similarly, if there is release of nano-objects, there are specific challenges in the safety evaluation of such products. The safety evaluation and risk assessment of nanomaterials requires a special focus as various nanomaterials consisting of the same chemical substance can have a different toxicological risk profile depending on a number of variables, including size, surface chemistry, physicochemical properties and intended application. For medical devices that are composed of or that contain nanomaterials, the safety evaluation program should specifically address issues related to the safety evaluation of nanomaterials. The ISO 10993 series, ISO/TR 13014, ISO 14971, and References [5],[14],[15],[16],[21],[23],[24],[28],[46],[47] and [49] deal with biological evaluation of medical devices and various aspects of nanomaterials.

Nanomaterials have sizes similar to structures at subcellular levels including DNA, and thus (theoretically) can reach and interact with such structures. Also, medical devices utilizing materials with nanoscale internal structures or with surface nanoscale features associated with coatings, functionalization, or with other topographical features on the nanoscale, that are intended as part of the functionality of the device, can have specific and unique properties that might need to be addressed in the biological evaluation. For example, it has been shown that nanoscale surface topography can influence cell alignment, cell morphology, cell signalling, gene expression and extracellular matrix [52] [53] [54].

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The release of nano-objects and the use of free nanomaterials are considered to pose the highest potential for risk in view of the potential internal exposure that can occur.

4.2 Biological evaluation of nanomaterials

ISO 10993-1:2009, Annex A, provides a framework for the development of an assessment program of the biological risks that should be considered depending upon a device's type and duration of body contact. This framework is also generally applicable to devices that contain, generate or are composed of nanomaterials. Such testing should be based on each device's merits. Special considerations apply to the ISO 10993 series of tests due to the presence of nanomaterials, as outlined in this document.

ISO 10993-1 provides guidance on the risk management process, which includes hazard identification, exposure assessment and risk estimation. This process is generally sufficiently robust and flexible to provide a basis for evaluation of nanomaterials, even though they can have properties that can be different from conventional ones. This process, including biological evaluation strategy, program content and acceptance criteria of the risk related to the nanomaterials as required by ISO 10993-1, should be planned, carried out and documented by knowledgeable and experienced professionals. The initial step in the biological evaluation of nanomaterials is to gather existing information on that particular nanomaterial according to the general approach as described in ISO 10993-1. Literature review of clinical and non-clinical data should be carried out according to ISO 10993-1:2009, Annex C to provide a rigorous and objective summary of available information about the nanomaterial and its intended application. Reference [55] has summarized several places where information about nanomaterials can be found. Following the logic of ISO 14971 and ISO 10993-1, if the biological safety assessment concludes from existing data that the identified risks are acceptable, no further testing is needed. Otherwise, additional information should be obtained. In order to use existing data for the biological evaluation, demonstration of nanomaterial equivalence is necessary (see 4.4).

4.3 Categorization of nanomaterials

The exposure assessment and hazard identification should be based on the characteristics of the finished medical device and the intended use. Hazard identification should consider the physicochemical and toxicological properties of the nanomaterial, including additives and processing aids. Exposure assessment should consider the concentration of nanomaterial used in the medical device, intended use and exposure route, and the rate and pattern of release and estimated patient exposure. The manner in which the nanomaterial is incorporated into the finished medical device can significantly alter the exposure characteristics[56]. General considerations for different categories of nanomaterials and medical devices are presented in Table 1. Certain devices might fall into more than one category, in which case evaluation appropriate to each category should be considered. The evaluation of any device that does not fall into one of the categories described should follow the general principles contained in ISO 10993-1, along with any special considerations outlined within this document.

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Table 1 — Considerations for biological evaluation of medical devices that contain, generate, or are composed of nanomaterials

Category[a]	Type of nanomaterial in the medical device	Considerations in addition to the biological evaluation according to ISO 10993-1
1	Surface nanostructures	— Consider potential cellular or tissue effects due to direct interaction with surface nanostructures (beneficial or adverse). — Consider potential of structures to be released (break off) from the surface. — Consider potential of nano-objects to be generated by degradation, wear or mechanical treatment processes. — Consider characterization of nanostructures (see Clause 5).
2	Nano-objects bound to or incorporated within a medical device; without intention to be released	— Consider potential cellular or tissue effects due to direct interaction with surface-bound nano-objects/nano-materials (beneficial or adverse). — Consider potential of nano-objects to be released from the device. — Consider potential of nano-objects to be generated by degradation, wear or mechanical treatment processes. — Consider characterization of physicochemical properties of the nano-objects (see Clause 5).
3	Nano-objects/nanostructures on the surface of or within a medical device; with intentional/expected release from the device	— Consider release kinetics (rate and quantity) of the nano-objects and contact duration of the medical device. — Consider potential cellular or tissue effects due to direct interaction with nano-objects/nanomaterials (beneficial or adverse). — Consider characterization of physicochemical properties of the released nano-objects (see Clause 5). — Consider toxicokinetics and tissue distribution of the nano-objects (see Clause 8). — Consider biological evaluation of the nano-objects (see Clause 9). — Consider potential of nano-objects to be generated by degradation, wear or mechanical treatment processes.
4	Nano-object medical device	— Consider characterization of physicochemical properties of the nano-objects (see Clause 5). — Consider toxicokinetics and tissue distribution of the nano-objects (see Clause 8). — Consider biological evaluation of the nano-objects (see Clause 9).

[a] A device can contain nanomaterials in more than one category.

[b] Nano-objects can be generated from a medical device that does not contain nano-objects.

[c] Degradation, wear or treatment of a medical device containing nano-objects can generate new or unintended nano-objects.

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Table 1 *(continued)*

Category[a]	Type of nanomaterial in the medical device	Considerations in addition to the biological evaluation according to ISO 10993-1
5	Nano-objects[b] released from a medical device as product of degradation, wear, or from mechanical treatment[c] processes (e.g. *in situ* grinding or polishing)	— Consider characterization of physicochemical properties of the nano-objects (see Clause 5). — Clause 7 describes additional considerations for nano-objects released by wear or generated by in situ processing. — Consider toxicokinetics and tissue distribution of the nano-objects (see Clause 8). — Consider biological evaluation of the generated nano-objects (see Clause 9). — Consider contact duration and release kinetics (rate and quantity).

[a] A device can contain nanomaterials in more than one category.

[b] Nano-objects can be generated from a medical device that does not contain nano-objects.

[c] Degradation, wear or treatment of a medical device containing nano-objects can generate new or unintended nano-objects.

4.4 Nanomaterial equivalence

Proper identification and characterization of the nanomaterial is essential. For nanomaterials, equivalence is dependent on multiple factors. Chemical composition alone is not sufficient to demonstrate equivalence as nanomaterial-specific properties can also be influenced by a number of other factors such as size, shape and surface properties of the nanomaterial and/or the source (manufacturer) of these nanomaterials, manufacturing process and storage conditions. Equivalence can only be claimed if properly demonstrated and justified by accompanying data.

In general, extrapolation of results by using existing data from other products using/containing similar nanomaterials, or from the corresponding parent compound of the same substance is not applicable, although such products can give an indication of possible safety concerns. If testing is considered necessary, it should be performed on the actual product and/or any nanomaterials which can come into contact with patients.

5 Characterization of nanomaterials

5.1 General considerations

Knowledge of the physicochemical properties of nanomaterials is essential to understanding their behaviour in biological systems. The physicochemical characterization is necessary for the identification of a specific nanomaterial. Characterization of the physicochemical properties of nanomaterials/nano-objects incorporated in a device and/or created by degradation, wear or mechanical treatment processes of the device is thus an important step in completing its biological evaluation. Physicochemical characterization can also be useful in the screening of potential new nanomaterials for suitability in a medical device for a proposed application. In addition, a proper characterization is necessary to establish or confirm the specifications of a nanomaterial in a defined medium and conditions.

Physicochemical characterization addresses three fundamental questions about a nanomaterial used in or released from a medical device.

— **Chemical composition:** What is it made of?

— **Physical description:** What does it look like?

— **Extrinsic properties:** How does it interact with the surrounding environment?

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The physicochemical properties associated with these questions encompass a wide range of nano-object characteristics. In keeping with guidance for evaluation of conventional materials used in medical devices, the physicochemical characterization of nano-scaled materials is targeted to properties that are relevant to the biological evaluation and the intended use of the device (clinical application and duration of use). General principles for chemical, physicochemical, morphological and topographical characterization of materials used in medical devices are covered in ISO 10993-18 and ISO/TS 10993-19. The identification and quantification of degradation products in medical devices are addressed in ISO 10993-9, ISO 10993-13, ISO 10993-14 and ISO 10993-15. Detailed guidance specifically for the physicochemical characterization of nanomaterials is emerging. Recently published guidance includes ISO/TR 13014 and ISO/TR 14187 and guidances published by the European Commission for food and feed, cosmetic ingredients and medical devices[57] [58] [59].

ISO/TR 13014 lists the following as properties of engineered nanomaterials to be characterized in the context of toxicological testing:

— chemical composition;

— purity;

— object size and size distribution;

— aggregation and agglomeration state;

— shape;

— surface area;

— surface chemistry;

— surface charge;

— solubility;

— dispersibility.

These properties should be viewed as a starting point for the evaluation of nanomaterials used in a device; characterization of additional properties might be indicated depending on the design, intended use and wear characteristics of the device. Examples of other physicochemical properties that might be considered on a case-by-case basis include:

— crystallinity;

— porosity;

— redox potential;

— (photo)catalysis;

— radical formation potential;

— octanol/water partition coefficient (might not be applicable for solid materials).

In order to obtain the required data as indicated above, multidisciplinary collaborations among toxicologists, physical chemists, engineers and other subject-area experts are necessary in developing a relevant and reliable characterization program for a specific nanomaterial containing device.

Specific guidance for characterization is available for certain nanomaterials, e.g. single and multiwall carbon nanotubes (SWCNT, MWCNT), nanoscale calcium carbonate powder, nanoscale titanium dioxide powder, gold and silver nanoparticles, and nanoclays[1)].

1) For details, see http://www.iso.org/iso/home/store/catalogue_tc/catalogue_tc_browse.htm?commid=381983

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Devices incorporating nanostructured surfaces can require characterization of morphological features in addition to characterization of the physicochemical properties listed above. Modification of medical device surface structure at the nanoscale continues to be explored with the objective of modifying cell and microbial interactions with devices. The measurement parameters required for the adequate characterization of surface architecture depend on the specific application[60] [61]. For example, Webb et al.[42] propose the use of a minimum of three statistical parameters as a standard for describing the vertical and horizontal nanoarchitecture of surfaces in the context of bacterial adhesion.

— Average surface roughness (*Ra*): the average deviation of surface height values from the mean plane.

— Surface area difference (*R*sa): the increase in surface area caused by roughness, i.e. the percentage difference between the actual surface area and the projected surface area. This parameter can be used if it is possible to precisely measure the surface area.

— Peak count (*R*pc): the number of peaks in the measured profile.

In addition, peak height and peak-to-peak distance can be useful spatial parameters.

Nanoporous materials are being developed for use in device applications including drug delivery and tissue scaffolding. Useful information for characterization of porous materials includes:

— size and structure of pores or voids;

— density of pores or voids;

— distribution of the pores or voids.

Nanomaterials should be characterized in the form they will be delivered to the end-user, i.e. as the final device. Representative samples from the final device or materials processed in the same manner as the final device can also be characterized, in order to directly evaluate a nanomaterial component. In addition, characterization of the nanomaterial as manufactured might be necessary when conducting toxicological studies and biocompatibility testing. For biological safety evaluation, the above referred physicochemical characteristics of the nanomaterials should be determined also in the biological medium used in the test system. The interaction between these media and the nanomaterials can have a profound influence on the behaviour of the nanomaterials in the test system. This factor should be taken into account during the test procedures and during the evaluation of the test results.

The contact with body fluids can alter the surface characteristics of nanomaterials and thus their biological behaviour which can have an impact on the hazard caused by a nanomaterial (see 8.2.2).

The isolation and characterization of nano-objects generated by wear of medical devices remains a challenge. *In vitro* methods for simulating the *in situ* generation of nanomaterials/nano-objects should be representative of the clinical use.

5.2 Characterization parameters and methods

Table 2 summarizes fundamental parameters that are useful as a starting point for characterization of nanomaterials used in medical devices. Detailed information on the relevance of these parameters to biological evaluation is provided in ISO/TR 13014.

Table 2 also provides examples of methods that can be used to provide quantitative and/or qualitative data for each of these parameters, based on information provided in ISO/TR 13014. This list should be considered a dynamic compilation, as development of best practices for nanomaterial characterization is an area of ongoing research and discussion. The listed methods include some developed for conventional particle analysis, as well as others developed specifically for nanoscaled materials.

Multiple methods are often available for characterization of a specific physicochemical parameter. A single characterization method might not provide an accurate assessment of the parameter (e.g. size distribution, surface composition). In such cases, a complementary method, if available, might be necessary to provide an adequate assessment of the property to be characterized; two independent methods for characterization might be needed. It is important to note that the results obtained

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for a particular property using different methods might not be directly comparable and that there are currently few harmonized methods for physicochemical assessment of nanomaterials to aid development of a robust test plan. The method(s) selected for characterization should be justified on the basis of the nanomaterial type and form, and proposed use of the device. For example, for nano-object size determination, at least one microscopy technique [e.g. transmission electron microscopy (TEM), confocal laser scanning microscopy (CLSM)] should be used as indicated in several guidance documents[57] [58] [59]. A report was published by the EU-JRC describing possibilities and pitfalls on nano-object size measurements[62].

Because characterization of nanomaterials is often scientifically and technically challenging, a quality assurance programme and laboratory best practices should be considered. The selection of techniques, analysis of nanomaterials and interpretation of nanomaterial properties should be performed by professionals appropriately qualified by training and experience. In performing the analyses, careful consideration should be given to sample preparation procedures to ensure that the data obtained are representative of the material used in the device. All aspects of the characterization process should be carefully documented to ensure transparency and reliability of results. Methodology used should be demonstrated to be appropriate for the nanomaterials investigated.

Table 2 — Key physicochemical characteristics and examples of measurement methods

Characteristic	Measurand	Example measurement methods	Relevant ISO guidance on methodology
Chemical composition and purity	The number and identity of elements alone or in molecules (can be expressed as a chemical formula Level or concentration of unintended constituents (impurities)	X-ray fluorescence X-ray photoelectron spectroscopy Auger electron spectroscopy Fourier X-ray diffraction (XRD transform infrared spectroscopy) (FTIR), Raman and other molecular spectroscopies Thermogravimetric analysis UV/visible spectrometry Scanning electron microscopy + XRD or energy dispersive x-ray spectroscopy (EDS) Nuclear magnetic resonance (Single particle) Inductively coupled plasma- mass spectrometry (spICP-MS)	ISO 22309 ISO 22489 ISO 24173 ISO 13084 ISO 18144

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Table 2 *(continued)*

Characteristic	Measurand	Example measurement methods	Relevant ISO guidance on methodology
Particle size and particle size distribution	**Particle size**: Equivalent spherical diameter for particles displaying a regular geometry Length of one or several specific aspects of the particle geometry **Particle size distribution**: Graphical representation, e.g. histogram, and/or values for statistical parameters such as mean, median, and/or mode	Dynamic light scattering Small angle X-ray scattering Size exclusion chromatography Analysis of images of scanning electron microscopy (SEM), transmission electron microscopy (TEM), or scanning probe microscopy (SPM) Differential mobility analysis Centrifugal liquid sedimentation Nanoparticle tracking analysis Raman spectroscopy Laser-induced incandescence Confocal laser scanning microscope (CLSM) Single particle ICP-MS Tangential flow filtration for nanomaterial separation followed by appropriate detection, e.g. ICP-MS (ISO 10993-14)	 ISO 9276 series ISO 9277 ISO 13318 series ISO 13320 ISO 22412 ISO 13322 series ISO 14488 ISO 14887 ISO 15900 ISO 16700 ISO/TS 19590 ISO 20998-1 ISO 21501 series ISO 22412
Aggregation/ agglomeration state	Particle size Number of aggregate/ agglomerate particles in comparison to the total number of primary particles Number of primary particles in the aggregate/ agglomerate Distribution of number of primary particles per aggregate/agglomerate	Analysis of (cryo-)SEM or (cryo-)TEM image Angle dependent scattering at different wavelengths Static light scattering Small angle X-ray scattering X-ray diffraction (XRD) X-ray absorption spectroscopy (XAS) Small angle neutron scattering Rheology methods Centrifugal liquid sedimentation Laser diffraction Nanoparticle tracking analysis	See guidance for particle size. ISO/TR 13097 ISO/TS 12025 ISO 13322-1
Shape	Size-independent descriptors of shape Distribution of values of the size-independent shape descriptors	Analysis of SEM, TEM, atomic force microscopy (AFM), or SPM images Scattering techniques spICP-MS	ISO 16700 ISO 13322-1
Surface area	Volume- and/or mass-specific surface area	Methods based on gas or liquid adsorption isotherms [Brunauer-Emmett-Teller (BET) theory] Liquid porosimetry Image analysis Laser-induced incandescence	ISO 15901-1 ISO 15901-2 ISO 15901-3 ISO 18757 ISO 13322-1 ISO 9277

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Table 2 *(continued)*

Characteristic	Measurand	Example measurement methods	Relevant ISO guidance on methodology
Surface nanostructures	Size and geometry	Interferometry Reflectometry Scanning probe microscopy (SPM) and atomic force microscopy (AFM) Scanning tunnelling microscopy (STM) Contact profilometry Non-contact profilometry	ISO 25178
Surface chemistry	Elemental and molecular abundance Reactivity (chemical reaction rate)	Auger electron spectroscopy X-ray photoelectron spectroscopy (XPS) Secondary ion mass spectrometry (SIMS) 3D atom probe tomography Energy dispersive X-ray spectrometry Electron energy loss spectrometry (EELS) Low energy ion spectroscopy Raman and other molecular spectroscopies	 ISO/TR 14187 ISO 18115 ISO 24236 ISO 15471 ISO 18118 ISO/TR 19319 ISO 17973
Surface charge	Net number of positive and negative charges per unit particle surface area Zeta potential	Isoelectric point Electrophoretic light scattering Electrophoresis Electro-osmosis Electric sonic amplitude Colloidal vibration current	 ISO 20998 ISO 13099
Solubility/ dispersibility	**Solubility:** Maximum mass or concentration of the solute that can be dissolved in a unit mass or volume of a solvent at specified (or standard) temperature or pressure **Dispersibility:** Maximum mass or concentration of the dispersed phase present in a unit mass of the dispersing medium (solvent) or unit volume of the dispersion (solvent plus dispersed phase) at specified (or standard) temperature and pressure	There are no specific methods for assessing the solubility of nano-objects. Tangential flow filtration for nanomaterial separation followed by appropriate detection, e.g. ICP-MS (ISO 10993-14) Methods to assess dispersibility of nano-objects are based on particle size/ size distribution and aggregation/ agglomeration state (see particle size above).	ISO 20998 ISO 13099

The information given in Table 2 is based on ISO/TR 13014. There are other documents available providing recommendations on nanomaterial/nano-object characterization (e.g. ISO/TS 17200).

Most of these parameters and methods listed are relevant for the characterization of nanoparticles. It should be noted that other forms/shapes of nanomaterials can be used, e.g. nanofibres and nanoplates.

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for the production of medical devices. Some parameters or methods listed might not be applicable to other forms/shapes of nanomaterials.

The methods listed can each have their limitations; therefore, the methodology used should be demonstrated to be appropriate for the nanomaterials investigated.

For certain nanomaterials (e.g. nanosilver, nanoscale zinc oxide), dissolution or ion shedding can occur. The dissolution of nanomaterials in different physiological environments can be assessed using tangential flow filtration which is a method to separate nanomaterials from their solubilized counterparts. The separated fractions can then be quantified using inductively coupled plasma mass spectrometry (ICP-MS)[63].

5.3 Use of reference materials

The availability of positive and negative control samples of nanomaterials is of key importance for reproducibility and reliability of nanomaterial safety testing. However, positive and negative control samples for nanomaterials are lacking. Therefore, the use of "representative test materials" has been proposed[65]. ISO/TC 229 has defined a representative test material as "material, which is sufficiently homogenous and stable with respect to one or more specified properties, and is implicitly assumed to be fit for its intended use in the development of measurement and test methods that target properties other than those for which homogeneity and stability have been demonstrated" (ISO/TS 16195). This approach is currently applied in the OECD Working Party on Manufactured Nanomaterials collaborative project for nanomaterial safety testing using materials from the European Commission Joint Research Centre repository of representative nanomaterials. Guidance on generic requirements for representative test materials for development of methods for characteristic testing, performance testing and safety testing of nanoparticle and nanofibre powders is provided in ISO/TS 16195.

General guidance for the use and preparation of reference materials in the biological evaluation of medical devices is provided in ISO 10993-12. However, there are currently few, reference materials available for nanomaterial properties other than size. Reference materials for nanomaterial particle size assessments are increasingly available from national and community agencies, including the National Institute of Standards and Technology (NIST, United States), the Joint Research Centre of the European Commission (JRC, European Union) and the Federal Institute for Materials Research and Testing (BAM; Germany). BAM, in cooperation with ISO/TC 229, maintains a database of all currently available nanoscale reference materials worldwide, available at http://www.nano-refmat.bam.de/en/. Currently available nanoscaled reference materials include titanium dioxide, colloidal silica, gold and single walled carbon nanotubes. A number of new reference materials are reported to be under development by these organizations, as well as commercial sources, but the initial focus of development has been on nanoscale reference materials for calibration of instrumentation, rather than on development of reference materials for benchmarking biological responses.

It should be noted that the use of appropriate standard reference materials (SRM) is significant only in the context of standardized protocols for size and shape metrology. Protocols should work across a range of imaging technologies and require high statistical certainty with minimal human bias. In addition, reference materials can also be used as internal and/or external reference. For example external standards can be used to calibrate the length scale of imaging tools for subsequent imaging, while internal standards allow the inclusion of a size standard within the unknown.

Development of a set of commonly accepted reference materials, including consensus on suitable positive and negative control nanoparticles for different testing systems, has been identified as a critical need for the risk assessment of nanomaterials[65]. Although the need for such reference materials is well recognized, development continues to be slow as a result of practical difficulties including lack of consensus on which materials and properties to characterize, significant technical challenges, and economic factors[64] [65] [66].

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