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Nanotechnologies — High throughput screening method for nanoparticles toxicity using 3D model cells

Nanotechnologies — Méthode de criblage à haut débit de la toxicité des nanoparticules utilisant des systèmes cellulaires 3D

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Foreword

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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).

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This document was prepared by Technical Committee ISO/TC 229, *Nanotechnologies*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

With an increasing number of nano-products including nanoparticles (NPs), potential exposure of consumers to NPs has increased. Therefore, the human and environmental impacts of NPs have recently emerged as an issue. High-throughput screening (HTS) approaches are often used for NPs toxicity screening. However, there are still limitations to provide the reproducible and reliable results based on a HTS method. To assess the potential toxicity of manufactured or engineered NPs, traditional in vitro toxicity studies have been performed using a surface attached two-dimensional (2D) culture system. 2D assays for cellular metabolic activity, cytotoxicity, or oxidative stress have been widely used in the first stage of hazard evaluation. However, several problems were encountered during assay validation, ranging from particle agglomeration in biological media to optical interference with the assay platform. There are ISO documents on the cytotoxic effects of NPs using cell viability assays and detection of reactive oxygen species (ROS) levels, but they can be applicable for a few classes of NPs that are well-dispersed in the media. Additionally, reagents used in the assays can interact with tested NPs or interfere with spectrophotometric reading.

This document describes a new assay platform, consisting of three-dimensional (3D) arrangement of cells on pillar inserts to evaluate cell viability and diminish artefacts arising from optical interferences and NP reactivity with assay components.

This document aims to overcome the optical interference of NPs and obtain reliable and reproducible cell viability results. The 3D-model cells are exposed to fresh cell viability reagent by simply transferring and immersing the pillar insert from one well to another well without optical interference from the NPs. In addition, 3D-model cell culture approaches facilitate cell-cell interactions and enhance cell-to-cell or cell-to-extracellular matrix (ECM) adhesion/signalling, ultimately leading to the expression of phenotypic proteins/genes and the formation of in vivo tissue-like morphology. It generates uniform cell-containing hydrogel droplets on the pillar insert and allows to easily change cell growth media or expose 3D-model cells to analytical reagents by immersing the tip of the pillar insert in different reaction plates.

Nanotechnologies — High throughput screening method for nanoparticles toxicity using 3D model cells

1 Scope

This document describes a method for high throughput evaluation of cytotoxic response of 3D model cells exposed to NPs without optical interference.

The method in this document is intended to be used in biological testing laboratories that are competent in the culture and growth of cells and the evaluation of cytotoxicity of NPs using 3D-model cells.

This method applies to materials that consist of nano-objects such as nanoparticles, nanopowders, nanofibres, nanotubes, and nanowires, as well as aggregates and agglomerates of these materials.

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/TS 80004-2, *Nanotechnologies — Vocabulary — Part 2: Nano-objects*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO/TS 80004-2 and the following apply.

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

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

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

3.1
agglomerate
collection of weakly bound particles or aggregates 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, modified — "weakly or medium strongly bound particles" has been replaced with " weakly bound particles or aggregates or mixtures of the two".]

3.2
dispersion
microscopic multi-phase system in which discontinuities of any state (solid, liquid or gas: discontinuous phase) are dispersed in a continuous phase of a different composition or state

Note 1 to entry: If solid particles are dispersed in a liquid, the dispersion is referred to as a suspension. If the dispersion consists of two or more liquid phases, it is termed an emulsion. A super emulsion consists of both solid and liquid phases dispersed in a continuous liquid phase.