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

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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.