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Up to Date *In-vitro* Artefacts in the Detection of Nanoparticles Toxicity: Short Review

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Abstract

Nanoparticle toxicology is an emergent field that focuses on establishing the hazards of human exposure to nanoparticles and their potential risk. Accurate assessments of nanoparticles risk involve the investigation of multiple factors such as the nanoparticles parameters, the test system and the cell type. Some nanoparticles may interfere with the toxicity detection assays or the enzymatic activity of the cell type. Thus, this lead to inaccurate obtained data which could mislead researches. In this short review, we provided up to date assessment on the cause of nanoparticles toxicity artefacts. Coating nanoparticle recently has been shown to hinder the interference with cell viability assays; however, this was found to be cell type and concentration dependent. Therefore, researchers suggest adding more washing steps to minimize the bound of nanoparticle with proteins or membranes. We suggest that conducting an interference test for each nanoparticle prior toxicity assessment to avoid any flaws.

Keywords: NPs toxicity; Interference; Cell viability assay; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Lactate dehydrogenase; Parameters

Introduction

The use of nanomaterials (NMs) in medicine, biology and industry applications has increased rapidly in the past decade [1]. NMs have been a crucial substance in the production of many therapeutic agents. However, the safety use of NMs has been a concern for many scientists [2]. Many conflicting reports on the potential toxicity of NMs have made the estimation of their biological effect complicated [3,4]. One of the main issues affecting the assessment of NMs toxicity to human and environment is the use of biochemical assays that could be affected by the NMs themselves and provides a false data or subsequent incongruent prediction of toxicity [5]. An inconsistent and/or inaccurate data will made it complicated to establish guidelines for the safety use and production of NMs. Common assays used in the detection of NMs toxicity are lactate dehydrogenase (LDH) cytotoxicity assay, alamar blue, tetrazolium based assays (e.g. MTS and MTT) and crystal violet assay [6,7]. These assays have been reported to be affected by NMs artefacts data. In addition, more of *in-vivo* and *in-vitro* nanotoxicology assays have given false positive results due to the presence of a variety of NMs [8]. Studies reported the interference to be from NMs binding to the proteins or dyes and then alter their structure or their functions; thus could be a common reason in every toxicity assay [9,10]. Other study reported that the presence of NMs in toxicity detection assay may adversely interrupts the cellular reaction and causes significant changes in enzymatic activity, fluorescence and absorbance values of indicator molecules [11]. If live cells were used in the detection of nanoparticles (NPs) interference as analyte, it would be complicated to distinguish differences between assays and cells interference [5]. Carbonaceous NPs have shown to bind to coomassie blue, alamar blue, neutral red MTT dye and WST-1 dye, and thus interfere with assays using these indicators [12,13]. Vertegel identified the secondary structure of a chicken egg lysozyme adsorbed onto silica NPs with various diameters. They discovered a change in the protein structure upon adsorption, with major loss in R-helix content caused by particles with larger diameter [14]. In another study, varying sizes of silica NPs were tested onto the adsorption of human carbonic anhydrase variants [15]. They observed a larger disturbance of protein secondary structure from the particles with larger diameters. Smaller NPs seems to promote the retention of native protein structure and function comparing to larger NPs. A study on the interruption of silica NPs to two different proteins in shape and size, bovine serum albumin and fibrinogen, showed that bovine serum albumin less ordered on larger size silica particles while fibrinogen denatured from smaller size silica particles [16]. NPs optical properties (created from varying size, shape, composition, surface modality and inter-particle interaction) can interfere with the endpoint measurement of absorbance or fluorescence in toxicity assays. For instance, the absorbance spectrum of gold NPs interfere with the absorbance range measured in haemolysis assay, which led to false results [17].

Previously we tested the interference of eight nanoparticles (NPs) with and without the presence of HaCaT cell line using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and crystal violet assays [6]. The presence of trisilanol phenyl POSS and trisilanol isooctyl POSS have shifted the optical density measurements of MTT assay. Similarly, gold NPs interfered with the crystal violet dye leading to significantly less OD values compared to the control. In our study, the size of the eight NPs test was in between 20-100 nm [6]. NPs parameters investigation has been a research focus to avoid interference with common toxicity assays *in-vitro* and *in-vivo* [18]. These parameters include NPs morphology, crystal structure, purity, mass concentration, size and size distribution, surface area/charge, chemical composition, surface stability under experiment conditions, degree of aggregation. These characterizations are in a particular importance not only for *in-vivo* studies, but more for the correct interpretation when these NMs performed under realistic environmental conditions [19]. The toxicity studies under environmental conditions will be influenced by the dispersion and

adsorption of various molecules on the surface of NPs also an additional toxicity due to a change in the accumulation of heavy metals in the existence of metal oxide NPs [20]. Since then studies have been focused on achieving a well dispersed suspension by the addition of surfactant or additives which could control the NPs agglomeration [21]. A recent study investigated if the interference of NPs is based on the surface characteristic of metallic NPs by studying the effect of different surface coatings of Silver (AgNPs) and maghemite NPs (γ-Fe_2O_3NPs) on classical *in-vitro* assays targeting two of the main cytotoxic points which are cell viability and oxidative stress response [22]. The cell viability assays were MTT, MTS, and WST-8 and assays utilizing fluorescent dyes as markers for the production of reactive oxygen species such as DCFH-DA, DHE and glutathione level. The results concluded that the NPs affected all of the investigated assays giving a false interpretation of the obtained data [22]. The range and the type of interference were dependent on the surface coating of NPs, their stability in biological media, concentration, and particle and assay dependence [22].

In conclusion, we recommend more stringent control for nanotoxicological studies to minimize the potential of NPs interaction with assays. Concentrations ≥ 10 mg/ml have shown to interfere with the assay function and the use of this concentration is not rare in nanotoxicological studies. Thus, NPs concentration should be completely limited, knowing that even with multiple washes and/or centrifugation NPs are able to remain within the cells or attached to membranes. However, multiple centrifugations to remove NPs bounded to the assay components can lead to remove dyes and proteins important in obtaining an accurate reading. Finally, each *in-vitro* test system has to be evaluated for every NPs type to avoid flaws and gives an accurate assessment of the safety of NPs toxicity.

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