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Short Communication

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An Invitro Study on The Damage of Cell Membrane by Silica Oxide Nanoparticles

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Abstract

Nowadays, growing interest in the application of nanotechnology in biomedical and biotechnological fields has opened a new avenue to explore the nanoparticles-biological system interaction. Indeed, a clear gap is still in the cytotoxic effect of NPs on the biological systems. For this purpose, the interaction of the silica oxide nanoparticles (SiO2-NP) with the PC12 cell line, as a model of nervous system cell line, was examined by lactate dehydrogenase (LDH) assay. For LDH assay, PC12 cells were treated with varying concentration of SiO2-NP up to 100 μg/ml and all assays were conducted at 48h after SiO2-NP incubation. The results showed that the cytotoxicity of SiO2-NP approached to its highest level after 48 h. It was also revealed that as the concentration of SiO2-NP increases to higher amounts, the LDH absorbance enhances. Finally, it may be suggested that several factors such as nanoparticle concentration and exposure time could influence the type of interaction between NPs and the biological system. Thus, the type of interaction and cytotoxic effects of NPs can be mediated by considering these factors.

Keywords: PC12 cell, LDH, Silica Oxide, assay, nanoparticle, cytotoxicity

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Introduction

Nanotechnology has combined with different fields such as biotechnology and medicine (Abbasi et al., 2016). Nanoparticles (NPs) are one of the most potential candidates in medicinal and biotechnological applications for a wide range of purposes such as drug delivery and treatment (Wilczewska et al., 2012). Metallic NPs such as gold, silver, copper, iron, platinum, palladium and silica have been highly used in medicine due to their potential characteristics (Katrien et al., 2013). However, a profound knowledge is still absent in the cytotoxic effect of NPs on the biological systems (Kasemets et al., 2009). The toxicity and biodistribution of the NPs could be influenced by the type of NPs and cell, surface properties, and particle sizes (Hartung et al., 2009). Hence, toxicity and distribution investigation should be considered for evaluation of the potential side effect of NPs in medicinal fields.

Among the barrage of assays employed in the NPs toxicity assessment, the cell quantification after incubation with varying concentrations of the NPs relative to the control cell was widely used (Hillegass et al., 2010, Alarifi et al., 2013). These assays provide an advantage for quantifying the cell viability in comparison to untreated cells. For example, the integrity of viable cells can be quantified in comparison with control, indirectly reflecting the percentage of necrosis.

Lactate dehydrogenase (LDH) is an enzyme found in the cytoplasm which is released into the cell culture medium after disruption of the cell membrane integrity (Decker et al., 1998). Therefore, this assay can be employed to determine the membrane damage. Actually, this assay is based on the converting of lactate to pyruvate through oxidation by the LDH activity, subsequently, which can be examined by a spectrophotometer.

Silica oxide nanoparticle (SiO2-NP) shows a wide range of potential applications in biological fields such as antibacterial, antifungal, anticancer properties, and live cell imaging (Hemmerich et al., 2013). However, the integrity of cell membrane could be influenced by the SiO2-NP (Ambikanandan et al., 2003).

In this paper, the cytotoxic impact of SiO2-NP on the PC-12 cell as a nervous system model cell was carried out by lactate dehydrogenase (LDH) test. Indeed, this method can demonstrate the damage to the cell membrane and follow its leakage.

Materials and methods

Materials

SiO2-NP nanopowder with a particle size of 30 nm was purchased from US-nano Company (St. Louis, USA). Other reagents applied in this research were of analytical grade and purchased from Sigma–Aldrich Company (St. Louis, USA).

Methods

Cell culture

The effects of SiO2-NP on cell membrane were assessed using PC12 cell line (rat adrenal pheochromocytoma cells). The PC12 cell line was purchased from Pasteur Institute (Tehran, Iran). After thawing the cells, they were cultured in DMEM medium containing FBS (10%), penicillin (100 units/mL), and streptomycin (100 μ g/mL). The PC12 cells were incubated in a CO2 incubator (5%) at 37 °C. Trypsin was also used to detach the cells.

LDH assay

Lactate dehydrogenase (LDH) activity, as a result of membrane damage, was assessed to determine the toxic effect of varying concentrations of SiO2-NPs (0.1, 1, 5, 10 and 100 μg/mL) on PC12 cells. Treated and untreated samples were removed after 48 h of incubation,

and LDH activity was assessed spectrophotometrically (Expert 96, Asys Hitch, Ec Austria) to record the converting of lactate to pyruvate according to the manufacturer's protocols (Parsazmoon, Tehran, Iran). The percentage of LDH release into the cell culture medium to the total LDH activity was reported as the LDH release. All experiments were repeated in triplicates and data represented as the mean ± SD.

Results and discussion:

Cytotoxic effects of SiO2-NPs

The cytotoxicity of SiO2-NP against PC12 cell line was done by the LDH assay to calculate the cell membrane integrity. For LDH assay, the PC12 cell line was incubated with different concentrations of SiO2-NPs and assay was performed for 48h after SiO2-NP incubation. Figure 1, demonstrated that as the concentration of SiO2-NP increases to higher amounts, the LDH absorbance enhances. This result indicates that the membrane damage of the PC12 is induced by SiO2 NP. This data showed that the SiO2-NPs results in the membrane leakage and following LDH release. Utilization of the LDH assay, the doses-dependent effect of SiO2-NP was evaluated to stimulate and considerate possible in vitro effect.

Conclusion

There are wide variables to consider when working with NPs and these refer to the type of NPs and cell, their size, functional group, shape, dispersion potential, concentration and incubation time. By carefully designing the experimental procedures such as: in vitro safety, biodistribution, and the mechanism of NPs toxicity the application of NPs for in vivo system can develop. This can play an important role as a useful method to examine the impact of NPs. However, animal systems are needed to clarify the exact mechanism of NPs-biological systems interactions. Therefore, in vitro study can only play a considerable role to complement animal studies in assessing NPs safety.

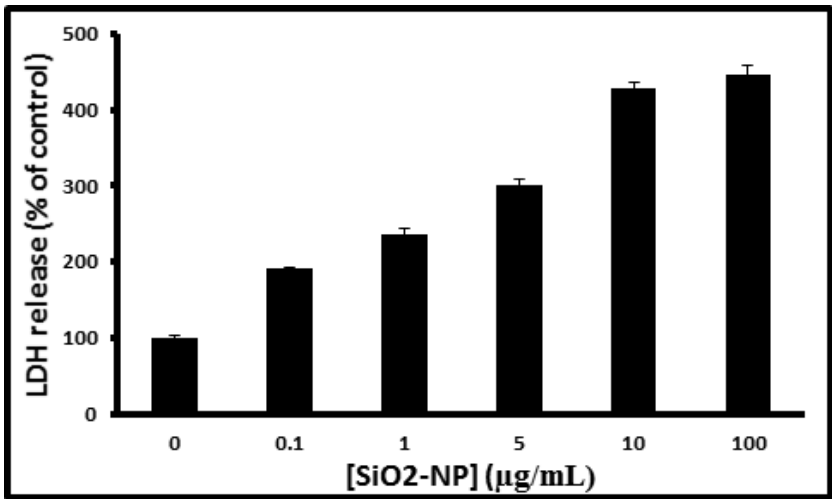

Figure 1: The effect of various concentrations of SiO2-NP on the LDH release of PC12 cell line after 48 h.

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Conflicts of Interest

None of the authors have any conflict of interest associated with this study.

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