

Effects of Silicon Dioxide Nanoparticles on Peripheral Nervous System Neural Cell Models

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ABSTRACT

Cell model systems *in vitro* facilitate the high throughput screening of putative toxicity of a variety of nanomaterials, including nanoparticles. However, most of such cell models consisted of non-neural cells. We have developed several central and peripheral neural cell models for the systematic investigation of cytotoxicity of nanomaterials. Our previous finding that the nanoparticles of several metallic and non-metallic oxides, including SiO₂, exert differential cytotoxic effects on central neural cells prompted us to investigate the effects of such nanoparticles

in peripheral neural cells. Results of our ongoing studies have revealed that SiO₂ nanoparticles exert differential cytotoxic effects on dorsal root ganglion (DRG) neurons and Schwann cells by lowering their survival. Moreover, they may have pathophysiological implications in how exposure to SiO₂ nanoparticles impacts on the structure and function of the PNS.

Keywords: Schwann cells, dorsal root ganglion neurons, SiO₂ nanoparticles, peripheral nervous system (PNS) and nanotoxicity

1 INTRODUCTION

As cautioned by the US Environmental Protection Agency, exposure to nanomaterials can occur during their manufacture or production: these industrial processes also may potentially give rise to environmental pollution of such materials. Thus, a case in point is the exposure to silicon dioxide (SiO₂) nanoparticles because SiO₂ – including a range of its particle sizes – is increasingly being used in cosmetics, food, and drug formulations. SiO₂ was generally regarded as a non-toxic substance in view of its numerous industrial applications. Nevertheless, there have been reports that SiO₂ particles are not as harmless as they were assumed to be. Consequently, there is increasing need to examine the biocompatibility of SiO₂ particles ranging from the micro- to the nanometer sizes.

Once they enter the body by inhalation or other routes, nanoparticles may be toxic to one or more organ systems [1]. Presence of nanoparticles in cosmetics and fabrics increase the contact of nanoparticles with the target tissue (i.e., skin) for a long period of time and can become a potential route for the uptake of nanoparticles by peripheral neural cells [1]. Dorsal root ganglion neurons internalize the nanoparticles and transport it to cell bodies by retrograde transport system [2]. However, the toxicity of nanoparticles in mammalian peripheral nervous system has not been reportedly studied although there is an increasing concern about the environmental and health impact of exposure to nanoparticles of different types. We have therefore initiated a series of studies to systematically elucidate the putative cytotoxicity of nanoparticles in peripheral nervous system cell types [3-4]. We developed cell culture models of peripheral nervous system using Schwann cells and DRG neurons to facilitate such systematic investigations [3-4].

Results from our previous studies [4] prompted us to hypothesize that silicon dioxide (SiO₂) particles exert differential effects on DRG neurons and Schwann cell. The present study was thereby initiated to investigate this hypothesis.

2 MATERIALS AND METHODS

2.1 Cells and Culture Conditions

Silicon dioxide (SiO₂) nanoparticles (STREM Chemicals, Newburyport, MA, USA; Cat. #93-1434; <12 nm particle size, 99+ % (flumed colloidal silica were dispersed in 100 mL of sterile saline in a sealed conical flask and the suspension stirred at ambient temperature overnight before being employed to be diluted to the specified concentrations for treatment of cells. The immortalized DRG neurons were a gift from Dr. Ahmet Hoke's Laboratory at Johns Hopkins University.

2.2 Cell Survival (MTT) Assay

DRG neurons or Schwann cells were seeded per well in a 48-well plate in DMEM with specified concentrations of SiO₂ nanoparticles. After incubation at 37°C for 36 hours, 10% of MTT (5 mg/ml in PBS) reagent was added to each well. After incubation for another 4 hours, the medium was removed gently and the cellular reaction product was solubilized in 200 µl DMSO. Then the optical density of the contents of each well was measured at 570 nm using a plate reader [5]. The absorbance corresponds to live cells present in each well [5].

2.3 Cellular Morphology

DRG neurons and Schwann cells were treated with SiO₂ nanoparticles at specified concentrations for 36 hours at 37°C as described above and bright field images were acquired by using a Leica light microscope (Leica DM IRB, Bannockburn, IL, USA) equipped with a digital camera (Leica DFC 300FX) [5].

2.4 Statistical Analysis of Results

Statistical significance of experimental results was analyzed with one-way ANOVA followed by Dunnett's post-hoc test with a minimum significance level set at $p < 0.05$ using the SPSS 17 software package.

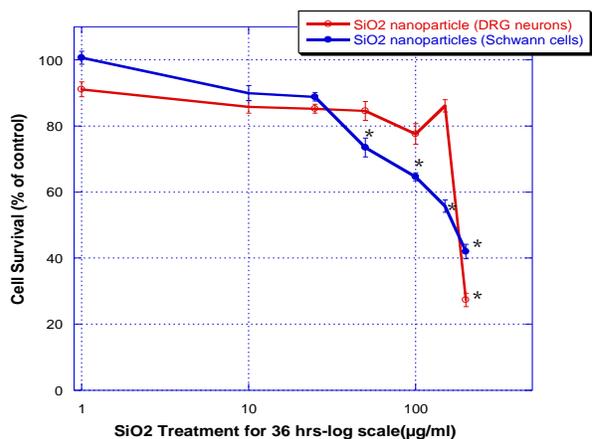


Figure 1: Effects of SiO₂ nanoparticles on DRG neurons and Schwann cells. Values are mean \pm SEM of 12 determinations and replicate experiments show the same trend. DRG neurons and Schwann cells treated with SiO₂ nanoparticles for 36 hours are marked red and blue, respectively.

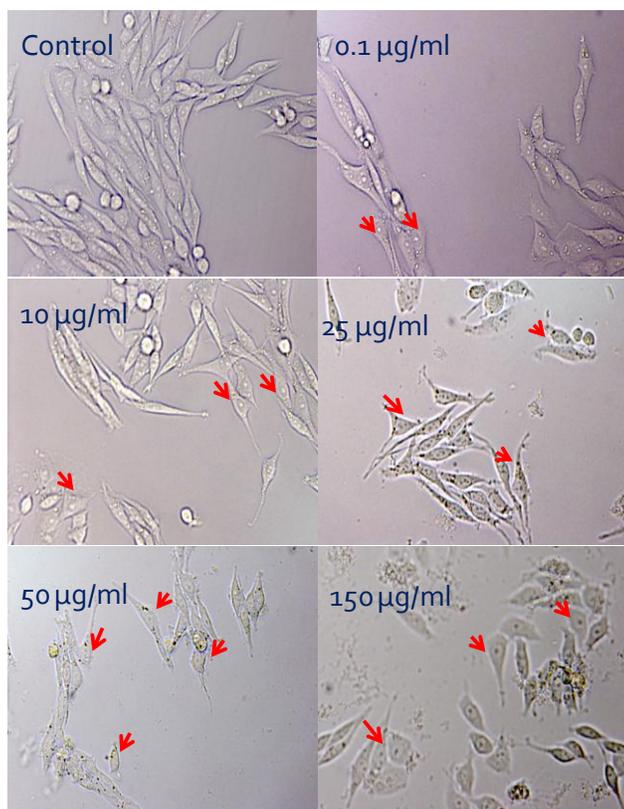


Figure 2: Effects of SiO₂ nanoparticles treatment on the morphology of Schwann cells. Schwann cells were treated with specified concentrations of SiO₂ nanoparticles for 36 hours. Then the morphology were assessed using bright field microscopy at 400x magnification. (Arrows indicate change in morphology)

3 RESULTS AND DISCUSSION

We have recently developed neural cell models based on neural cells derived from the PNS, namely dorsal root ganglion (DRG) neurons and Schwann cells. Our systematic studies have indicated that the two *in vitro* cell models are suitable not only for elucidating the pathophysiological mechanisms underlying diabetic neuropathy but also for nanotoxicology and tissue engineering studies [3-4].

Because the putative cytotoxic effects of metallic and non-metallic oxide nanoparticles in PNS neural cells are largely unknown, we have employed our *in vitro* models of DRG neurons and Schwann cells to systematically investigate the putative cytotoxicity of nanoparticles of several metallic and non-metallic oxides. Furthermore, employing flow cytometry, we noted the cytotoxicity of SiO₂ nanoparticles in the two cell models *in vitro* could be attributed, at least in part, to the uptake of the nanoparticles by the two cell types.

To our knowledge, we are the first to determine, in this study, the putative cytotoxic effects of SiO₂ nanoparticles

on neural cells of the peripheral nervous system (i.e., DRG neurons and Schwann cells).

As shown in Figure 1, treatment of Schwann cells (R3) with SiO₂ nanoparticles induced a dose-related decrease in their survival when we progressively increased the concentrations of the nanoparticles from 1 to 200 µM. The IC₅₀ for SiO₂ nanoparticles in lowering the survival of Schwann cells (R3) was ~150 µM and at the highest concentration employed, only ~40% of the cells survived (Figure 1).

Cellular uptake of SiO₂ nanoparticles were studied using FACSCalibur™ flow cytometer and dark field microscopy. The side scatters increase and forward scatters decrease sequentially, presumably due to substantial light reflection by the internalization of nanoparticles [6]. Schwann cell showed internalization of SiO₂ nanoparticles in a dose-related manner (data not shown).

Nanoparticles gradually sediments onto Schwann cells and Schwann cells appeared to internalize them. Once the nanoparticles entered a cell, they showed a tendency to aggregate around the nucleus (Figure 2). However, the nanoparticles did not appear to penetrate into the nucleus; rather, at higher treatment concentrations, the nuclei appeared to be swollen and their morphology changed. At higher treatment concentrations, the cell bodies of the Schwann cells appeared to be much more oval, presumably due to swelling: this altered morphology was distinctly different from the spindle-shaped cell bodies of untreated (i.e., control) Schwann cells (Figure 2). Thus, in general, the photomicrographs revealed that treatment with the nanoparticles induced dose-related changes in cellular morphology, a finding that appeared to correlate with consistent with the nanoparticle-induced, dose-related progressive decrease in cell survival of the treated cells.

When we exposed dorsal root ganglion (DRG) neurons to SiO₂ nanoparticles, we did not detect significant decreases in their survival at lower treatment concentrations (0.1-150 µg/ml) but their survival was significantly decreased only at the highest concentration employed (i.e., 200 µg/ml; Figure 1). Moreover, DRG neurons did not appear to internalize SiO₂ nanoparticles in large amounts (data not shown). Nevertheless, the DRG neurons treated with the nanoparticles did show some cytoplasmic swelling (Figure 3): indeed, a recent study suggests that these nanoparticles induce mitochondrial dysfunction in DRG neurons [7].

Results of our ongoing studies therefore strongly suggest that treatment with SiO₂ nanoparticles exert differential cytotoxic effects on PNS neural cells and that Schwann cells, which are prominent glial cells of the PNS, are more susceptible than DRG neurons to the effect of SiO₂ nanoparticles. That Schwann cells are susceptible to the cytotoxicity of SiO₂ nanoparticles is consistent with our previous finding that these nanoparticles also exert cytotoxicity on U87 astrocytoma (astrocytes-like) cells [5].

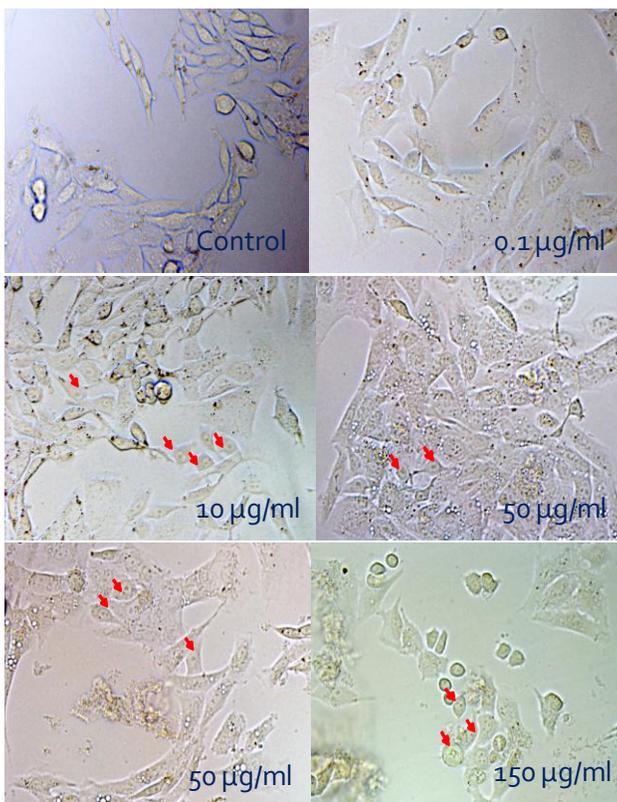


Figure 3: Effects of SiO₂ nanoparticles treatment on the morphology of DRG neurons. DRG neurons were treated with specified concentrations of SiO₂ nanoparticles for 36 hours. Then the morphology were assessed using bright field microscopy at 400x magnification. (Arrows indicate change in morphology)

4 CONCLUSIONS

The results from our studies to date have demonstrated that treatment with SiO₂ nanoparticles for 36 hrs lowered the survival of Schwann cells in a dose-related manner and that Schwann cells are more susceptible to the toxicity of SiO₂ nanoparticles DRG neurons. The effects of the nanoparticles in Schwann cells may be attributed, at least in part, to the fact that they internalize the nanoparticles.

The results of our ongoing studies strongly suggest that nanoparticles may exert differential cytotoxic effects on neural cell types of the peripheral nervous system. As such, our results may assume pathophysiological importance in the environmental health impact of nanoparticles. Obviously, this is an important area that deserves further study.

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