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# Cellular uptake, cytotoxicity, and ROS generation with silica/conducting polymer core/shell nanospheres

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ABSTRACT

The cellular response to conducting polymer (CP) nanospheres with similar physical properties was evaluated by *in vitro* cellular uptake and cytotoxicity in mouse macrophage RAW 264.7 and rat pheochromocytoma PC-12 cells. Four different CPs (polythiophene, poly(3,4-ethylenedioxythiophene), polyaniline, and polypyrrole) were deposited onto silica nanoparticles with a diameter of *ca.* 22 nm. Cellular uptake of silica/CP core/shell nanospheres in both cell lines was observed by transmission electron microscopy and they were internalized via phagocytosis and endocytosis. Cytotoxic effects were systemically assessed using live-cell microscopy, viability, oxidative stress, and lactate dehydrogenase assays. Silica/polythiophene core/shell nanospheres were the most toxic in both cell lines examined, because of the cellular effects of sulfur atoms. On the other hand, silica/polypyrrole core/shell nanospheres caused the lowest levels of toxicity in both cell lines. Furthermore, both rat and mouse cell viability was concentration-dependent with the nanospheres. These findings enhance nanotoxicological information regarding CP nanospheres when used with macrophage and neuronal cells, which may be useful in their application in bioelectronic and biomedical fields.


## 1. Introduction

Significant advances in materials science have enabled the fabrication of various nanomaterials with enhanced physicochemical properties due to the exponential increase in surface-to-volume ratio compared with their bulk counterparts [1,2]. Thus, nanomaterials with controlled sizes and shapes have prompted the development of biomedical applications such as drug delivery, bioimaging, and bioelectronics.

The unique physicochemical properties of nanomaterials may cause adverse effects to human organs [3–5]. For example, Nel et al. reported that the cellular response against nanomaterials was size-dependent, indicating that nanotoxicity was associated with endocytosis [6]. Additionally, the cytotoxicity and pro-inflammatory response of nanomaterials was previously shown to be size-, shape-, and surface functionality-dependent, indicating that the toxicity of nanomaterials was influenced by the interaction between the surface of the nanomaterials and cells [7]. For this reason, the systematic and accurate toxicity assessments of surface functionality should be implemented to ensure the safe application of nanomaterials.

To date, cytotoxicity studies have typically focused on various physicochemical properties of nanostructures, such as sizes, shapes, surface functionalities, crystallinities, and dopants [8,9]. On the other hand, comparing the cellular response of different nanomaterials has posed major obstacles because of the difficulty in fabricating nanostructures with similar physicochemical conditions (e.g., size, shape, surface properties). To the best of our knowledge, no relevant paper has been published concerning the precise and systematic nanotoxicological evaluation of different nanomaterials.

Among various nanomaterials, conducting polymer (CP) nanomaterials have received considerable attention, because of their useful properties, such as high conductivity [10,11], biocompatibility [12,13], and stability [14,15]. These nanomaterials can be used in various application fields including bioelectronic [15] and biomedical applications [16,17]. However, only a few papers have been published concerning the cytotoxicity of CP nanomaterials. Using nanomaterials with fixed sizes and shapes, nanotoxicity assessments can be made more precisely of each type of CP nanomaterial [18].

Macrophages are the first line of defense in the immune system and act as scavengers against foreign agents via phagocytosis [19–24], making them useful for nanotoxicity studies. Moreover, estimating cytotoxicity of CP nanomaterials should involve neuronal cell lines due to their bioelectronic importance [25,26].

Here, we investigated the cellular response of various CP nanoparticles that were fabricated via seeded polymerization on silica nanospheres (NSs; diameter = *ca.* 22 nm). Four different CPs (polythiophene, poly(3,4-ethylenedioxythiophene), polyaniline,

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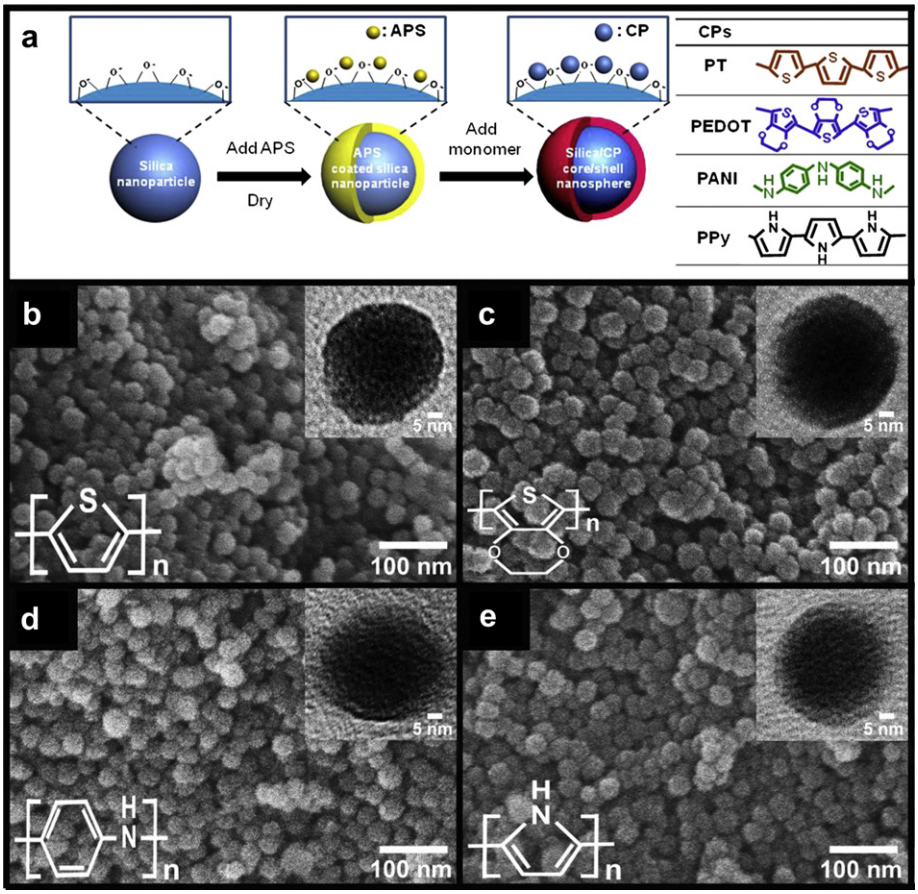


**Fig. 1.** (a) Schematic diagram for fabrication procedure of silica/CP core/shell NSs; SEM images of silica/CP core/shell NSs prepared via seeded polymerization (insets: TEM images of a corresponding single core/shell nanosphere. Scale bars = 5 nm); (b) PT, (c) PEDOT, (d) PANI and (e) PPy. Scale bars = 100 nm.

and polypyrrole) were used to fabricate silica/CP core/shell NSs and these were evaluated in terms of *in vitro* cellular uptake and cytotoxicity in mouse macrophage RAW 264.7 and rat pheochromocytoma PC-12 cells. The cellular internalization of the NS was analyzed by transmission electron microscopy. The cellular effect of the NS was estimated by adenosine triphosphate (ATP) production, propidium iodide (PI) staining, reactive oxygen species (ROS) detection, and lactate dehydrogenase (LDH) assays. In the present study, nanotoxicological assessment of the silica/CP core/shell NS consisted of the following: (1) an accurate comparison of cytotoxicity using different CP nanomaterials, (2) a simple procedure for fabricating uniform NS, and (3) a toxicological evaluation as a function of chemical composition.

## 2. Materials and methods

### 2.1. Materials

Ludox AS-40 aqueous colloidal solutions (diameter of *ca.* 22 nm) were purchased from Aldrich Chemical Co. and used without further purification. Monomers including thiophene (99%), 3,4-ethylenedioxythiophene (99%), aniline (99%), and pyrrole (99%) were obtained from Aldrich. Ammonium persulfate (APS) was used as an oxidant (Aldrich, 98%) and used without further purification.

### 2.2. Fabrication of silica/CP core/shell NSs

Silica/CP core/shell NSs were fabricated by seeded polymerization [12]. Oxidant coated silica NSs were prepared using 22 nm silica NSs. The silica NSs (10 mL) were added into 10 mL of distilled water. The mixture was stirred and APS solution (2 g) was added into the mixture. After 3 h, the mixture was dried at room temperature.

APS-coated 22 nm silica NSs (2 g) were dispersed in chloroform (10 mL). Then, 0.3 mL of each monomer (thiophene, 3,4-ethylenedioxythiophene, aniline, and pyrrole) was added into the reactor and the mixture was stirred vigorously for following conditions: 4 h at 3 °C. After polymerization, the products were washed several times with ethanol and then dried under vacuum oven for further experiments.

### 2.3. Characterization of silica/CP core/shell NSs

Field emission scanning electron microscopy (FE-SEM) images were taken with a JEOL JSM-840A microscope. Images of transmission electron microscopy (TEM) were obtained with a JEOL EM-2000 EX II microscope. The zeta potential of silica/CP core/shell NS was measured by Otsuka Electronics ELS-8000 (Osaka, Japan). Before measuring surface charge, the NSs were dispersed in distilled water at a concentration of 10 μg mL$^{-1}$, and then sonicated for 5 min. Fourier transform infrared (FT-IR) absorption spectra of the NSs were obtained by a Bomem MB 100 spectrometer (Quebec, Canada) at a resolution of 4 cm$^{-1}$ and with 64 scans. Element analysis was performed with Perkin–Elmer CHN Elemental Analyzer (model 2400).

### 2.4. Cell culture

Mouse macrophage RAW 264.7 and rat pheochromocytoma PC-12 cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). RAW 264.7 cells were cultured in Dulbecco's modified eagle medium (DMEM) with 10% fetal bovine serum, 1% penicillin–streptomycin solution, 4.5 g L$^{-1}$ D-glucose,

**Table 1**
Atomic and composition ratio of various silica/CP core/shell NSs.

| Shell materials | Atomic % | | | | Composition ratio | |
|---|---|---|---|---|---|---|
| | C | H | N | S | Theoretical value | Measured value |
| PT | 1.340 | 0.347 | | 0.867 | (S/C) 0.667 | (S/C) 0.647 |
| PEDOT | 1.109 | 1.114 | | 0.374 | (S/C) 0.444 | (S/C) 0.337 |
| PANI | 1.678 | 1.832 | 0.302 | | (N/C) 0.194 | (N/C) 0.180 |
| PPy | 0.682 | 1.169 | 0.219 | | (N/C) 0.292 | (N/C) 0.307 |