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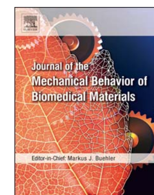

Interactions between rGO/TNT nanocomposites and cells: Regulation of cell morphology, uptake, cytotoxicity, adhesion and migration

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A B S T R A C T

Reduced graphene oxide/titanium dioxide nanotube (rGO/TNT) composites have superior properties, such as a large surface area, extraordinary mechanical strength, high carrier mobility, etc. However, the biosafety and biocompatibility of these composites, such as their influences on cell viability and cell functions, which are of paramount importance, are still not fully addressed. In this study, rGO/TNT nanocomposites were successfully synthesized through a modified hydrothermal treatment method. Then, the interactions between the rGO/TNT nanocomposites and Raw264.7 mouse monocyte-macrophage cells were further investigated. The results show that the rGO/TNT nanocomposites could be internalized by Raw264.7 cells and mainly gathered inside the cytoplasm. No rGO/TNT nanocomposites were observed in the nucleus. Moreover, the rGO/TNT nanocomposites exhibited low cytotoxicity toward Raw264.7 cells at a lower dose, though they may exhibit cytotoxicity to some extent at very high concentrations. In addition, the uptake of the nanocomposites influenced the cell cytoskeleton organization, while the cell adhesion and migration abilities were also impaired.

1. Introduction

With the development of nanotechnology, nanomaterials are increasingly used in our daily life due to their outstanding performance (Wang and Keddie, 2009; Christian et al., 2008). Titanium dioxide nanotubes (TNT), which exhibit superior properties, such as a large surface area, high porosity, low toxicity, chemical stability, etc. (Lee et al., 2010; Zhang et al., 2001; Mei et al., 2014; Moseke et al., 2012), have been used in various fields. Currently, TNT have become one of the ideal catalysts in environmental applications. For instance, they are reported to be used for the photocatalytic degradation of organic compounds (Carp et al., 2004; Gaya and Abdullah, 2008). In addition, TNT have great potential for application in the area of bionanotechnology and nanobiomedicine, such as nanocarriers for drug delivery, etc (Sarkar et al., 2012; Song et al., 2009).

Meanwhile, graphene, a two-dimensional layer of sp ^{2}-hybridized carbon atoms, has been widely used in sensors, electronics, drug delivery, supercapacitors and catalysis due to its unique electrical properties, such as a large specific surface area, high thermal conductivity, high carrier mobility and mechanical strength (Zhang et al., 2011; Kim et al., 2011; Geim, 2009; Lee et al., 2008). To date, a variety of graphene-based functional nanomaterials have been reported (Cheng et al., 2012; Li et al., 2011; Sreeprasad et al., 2011; Xie et al., 2013; Zhang et al., 2010a). Therefore, reduced graphene oxide (rGO) nanocomposites are one of the promising subjects for future research. For example, reduced graphene oxide/titanium dioxide nanotube (rGO/TNT) composites exhibit superior properties, which may have broad application prospects in many fields (Fan et al., 2016; Gu et al., 2014; Moon, 2014; Mou et al., 2014; Wang et al., 2014). However, the use of nanomaterials has raised safety concerns, as their small size facilitates accumulation in and interaction with biological tissues (Trouiller et al., 2009).

Currently, human are usually exposed to natural or industrial nanomaterials in the atmosphere through direct ingestion, breathing, penetrating skin and other ways (Berry et al., 1977; De Jong and Borm, 2008). Thus, the resulting effects of nanomaterials on the environment and human health are of increasing concern. However, the relationship between nanomaterials and organisms, e.g., the mechanism causing cytotoxicity, has not been clearly addressed. The relationships are of key significance for studying of the influences of nanomaterials on human health and survival, especially for nanotechnology's security applications in the field of biology and medicine (Fadeel and Garcia-Bennett, 2010; Nel et al., 2009; Oberdorster et al., 2000). Several groups found that the toxicity of nanomaterials mainly comes from two aspects: first, their nano size and surface chemical modification, which is likely to undermine cells in many biochemical processes; and second, the degradation of the nanomaterials and corresponding release of

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metal ions, which may affect the balance of free metal ions inside cells and the process of cell metabolism (Thompson et al., 2001; Sharma et al., 2009, 2011; Elder et al., 2006).

In recent years, there has been further research into TiO_2- and graphene-based nanomaterials causing cytotoxicity. Chen et al. studied the cytotoxicity of various TiO_2 nanostructure morphologies and indicated that the cell behavioral difference may be related to factors such as the differential uptake ability into cells of unmodified structures of varying geometries (Chen et al., 2010). Roslyn et al. found that serum protein adsorption would reduce the aggregate size distribution of TiO_2 nanoparticles and affect the particles' interactions with A549 and H1299 human lung cell lines (Tedja et al., 2012a). Zhang et al. believed the small GO nanosheets exhibited lower cytotoxicity and higher cellular uptake compared to random large GO nanosheets (Zhang et al., 2013). Mu et al. discovered that protein-coated large and small GO nanosheets are uptaken by cells predominantly through phagocytosis and clathrin-mediated endocytosis (Mu et al., 2012). But at present, there were no related studies to be reported about the influences of rGO/TNT nanocomposites on cell uptake, cell viability and cell functions.

Herein, we successfully prepared rGO/TNT nanocomposites and focused on the interaction between rGO/TNT nanocomposites and Raw264.7 mouse monocyte-macrophage cells, i.e., the regulation of cell morphology, uptake, cytotoxicity, migration and adhesion. This work is dedicated to revealing new insights regarding the biophysical impact of nanocomposites on exocytotic cell functions, thus illuminating details on how surviving cells are affected by rGO/TNT nanocomposites.

2. Materials and methods

2.1. Materials

TiO_2 nanoparticles (P25) were purchased from the Evonik Degussa (China) Co., Ltd. Graphene oxide (GO) with an average size of 5–8 μm was kindly provided by Tangushangxi Co., Ltd. Dulbecco's Minimum Essential Medium (DMEM) was purchased from Gibco. Fetal bovine serum (FBS) was purchased from Invitrogen. Penicillin-Streptomycin was purchased from Beyotime. Bovine serum albumin- fluorescein isothiocyanate conjugate (FITC-BSA), 4′6-diamidino-2-phenylindole (DAPI), 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), rhodamine-phalloidin and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich. All other reagents were of analytical grade and used as received. The water used in all experiments was purified by a Milli-Q water system (Millipore, USA).

2.2. Preparation of TNT and rGO/TNT nanocomposites

TNT was prepared through modified hydrothermal treatment as we previously reported (Wei et al., 2016a, 2015, 2016b). Briefly, 2 g of P25 were mixed thoroughly with 75 mL of 10 M NaOH solution and then transferred to a 100-mL Teflon-lined flask, which was held at 140 °C under atmospheric pressure for 36 h in the oven. After naturally cooling to room temperature, the precipitate was acidified with 0.1 M HCl solution until the pH value of the solution was 1.5 and kept stirring for 24 h. The crystals were collected by suction filtration and washed with deionized water repeatedly until the pH of the filter liquor became neutral. Finally, the crystals were dried at 80 °C in an oven for 24 h to obtain TNT. The rGO/TNT nanocomposites were prepared through a similar method, in which 2 g of P25 was substituted by 2 g of P25 and 0.02 g of graphene oxide (GO). The subsequent steps were the same as described above.

2.3. Labeling of TNT and rGO/TNT with FITC-BSA

TNT, rGO/TNT nanocomposites and FITC-BSA were dissolved in PBS solution with a final concentration of 5 mg/mL. Then, TNT and rGO/TNT nanocomposites were mixed with FITC-BSA solution at a mass ratio of 1:1. The mixture was incubated overnight at 37 °C and then centrifuged at 5000 rpm for 10 min. The precipitation was washed with PBS (0.1 M, pH = 7.4) and centrifuged at 5000 rpm for 10 min three times. The whole labeling process was protected against exposure to light.

2.4. Characterization of GO, TNT and rGO/TNT nanocomposites

The surface morphologies of GO, TNT and rGO/TNT nanocomposites were observed using transmission electron microscopy (TEM). GO, TNT and rGO/TNT nanocomposites were diluted in ethanol. Then a drop of solution was added to the copper grid. After being dried overnight, the samples were measured with TEM (JEM-1230EX, Japan). For the Fourier transform infrared spectroscopy (FTIR) analysis, the KBr pellet technique was used to prepare the samples, and then the analysis was performed using a spectrophotometer (Nicolet 5700, America). X-ray powder diffraction (XRD) patterns of all materials were measured with a Rigaku Ultima IV diffractometer (Cu Kα radiation) in the range of 10–80°. The surface compositions of GO, TNT and rGO/TNT nanocomposites were investigated via X-ray photoelectron spectroscopy (XPS). The samples were measured using a spectroscope (Perkin-Elmer PHI 5900) equipped with non-monochromatic source operating at 150 W. A dynamic light scattering (DLS) method was used to measure the sizes of nanomaterials in different solutions. Nanomaterials were first dispersed in PBS and DMEM containing 10% FBS, and then measured using a sub-micron scale particle analyzer (Delsa TM Nano, Beckman Coulter, USA) at room temperature (25 °C).

2.5. Cell culture

Raw264.7 mouse monocyte-macrophage cells were purchased from the Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China. Raw264.7 cells were cultured in DMEM containing 10% FBS and 1% Penicillin-Streptomycin and incubated in a 5% CO_2 incubator at 37 °C.

2.6. Intracellular distribution of TNT and rGO/TNT

Raw264.7 cells (~3×10^5 cells/well) were seeded in 6-well plates. After co-incubating with 100 μg/mL TNT or rGO/TNT for 12 h, Raw264.7 cells were washed three times with PBS (0.1 M, pH = 7.4) and then centrifuged at 1000 rpm for 4 min. The cells were prefixed with 2.5% glutaraldehyde h at 4 °C for 24 h and washed three times with PBS. Then, the cells were postfixed with 1% O_SO_4 aqueous solution at 4 °C for 1 h and washed three times with PBS. Next, the cells were dehydrated through a graded series of ethanol (30%, 50%, 75%, 90%, 95% and 100%), 15 min at each step. Afterward, the cells were incubated with a mixture of absolute acetone and the final Spurr resin mixture (v/v = 1:1) for 1 h at room temperature, transferred to the v/v = 1:3 mixture for another 3 h, and finally treated with the final Spurr resin mixture overnight. The as-prepared samples were placed in capsules containing embedding medium and heated at 70 °C overnight, followed by ultrathin sectioning with a Reichert ultra microtome. After stained by uranyl acetate and alkaline lead citrate for 15 min each, they were observed under a TEM (JEM-1230EX, Japan) at an accelerating voltage of 80 kV.

2.7. Cell viability assay

Raw264.7 cells (~5000 cells/well) were seeded in 96-well plates and incubated for 12 h. Then, the DMEM was replaced with fresh medium containing TNT or rGO/TNT of variable concentrations (50, 100, 200, 500 and 1000 μg/mL). After co-incubation for desired time periods (3, 6, 12, 24 and 48 h), 20 μL of 5 mg/mL MTT solution was added into each well of the 96-well plate, followed by incubation for

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