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Synthesis and characterization of biocompatible zinc oxide nanorod doped-titanium dioxide nanosheet

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ABSTRACT

The titanium dioxide (TiO_2) and zinc oxide (ZnO) nanomaterials are widely used for several biomedical applications because of their semiconductor property. The present study demonstrates a strategy for synthesis of zinc oxide nanorod (ZnONR) doped titanium dioxide nanosheets (ZnONR@TiONS) via hydrothermal method. A series of characterization techniques indicated that TiONS exhibited band gap energy of 3.09 eV, while the ZnONR@TiONS showed 2.83 eV. XPS analysis confirmed the 4^+ oxidation state of TiONS and 2^+ oxidation state of ZnONR. *In vitro* cytotoxicity test indicated that the ZnONR@TiONS showed 99% of cell viability without any toxicity under 50 μg/mL of concentrations.

Introduction

The development of new types of biocompatible nanomaterials is emerged in various biomedical fields such as biosensors, tissue engineering, and drug-delivery [1–3]. Titanium dioxide (TiO_2) nanoparticles (NPs) have extensively been developed with various morphologies and particle sizes, and they exhibit wide range of electronic properties and photo catalytic activity [4–8]. The semiconductor nanomaterials such as TiO_2, zinc oxide (ZnO), nickel oxide, and cerium oxide find potential applications in biomedical fields because of their excellent optical properties [9–15]. The electronic property of TiO_2 NPs depends on their crystalline shape and size. TiO_2 is widely employed as a white pigment additive in the production of cosmetics, sunscreen agents, food products and pharmaceuticals. The TiO_2 NPs could find potential applications as antimicrobial agents in photochemical therapy for the decomposition of microorganisms and several environmental contaminants because of their excellent photo semiconductor properties [4,16,17].

The electron–hole pairs are created when TiO_2 is irradiated with the electromagnetic radiation of energy greater than its band gap energy of 3.2 eV, and the produced electron–hole pairs could cause redox reactions for the degradation of pollutants at TiO_2 surface. However, the disadvantages of pure TiO_2 are that the electronic excitation is possible only by irradiation with the UV light and the electron–hole pair recombination process take place within nanoseconds. Many types of metal and metal oxide NPs such as iron, copper, silver and ZnO NPs have been doped with TiO_2 NPs to enhance their visible light induced photocatalytic biological activity [18–22]. Zhao et al. have synthesized Zn doped TiO_2 NPs by hydrogen–oxygen diffusion flame method and found that the band gap energy of Zn doped TiO_2 nanoparticles was shifted to red region [23].

Various forms of TiO_2 and their hybrid nanostructures were synthesized by adopting different synthetic methods such as thermal plasma processing [24], electrochemical [25], pulsed laser ablation [26], hydrothermal [22,27], ball mill [28], sol–gel [29] and biosynthesis methods [30]. The TiO_2 nanosheets (TiONS) are interesting due to their high surface area and enhanced photo-catalytic performance. TiONS could be achieved by several synthetic approaches such as hydrothermal [31], chemical template [32] and sol–gel method [33]. The hydrothermal method

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is more prominent for the controlled synthesis of metal (or) metal oxide (or) metal sulfide NPs doped TiONS [34,35]. The hydrothermal method has also been widely employed for the synthesis of ZnO nanorods (ZnONR) [36]. Recently, Miles et al. achieved the ZnO nanowire core surrounded by TiONS by adopting hydrothermal method [37], while Wang et al. reported the growth of TiONS on ZnONR array by adopting the hydrothermal method [38]. However, the information about the band gap energy and biocompatibility of those materials were not clear.

Biocompatibility of the metal oxide nanomaterials is very important for their suitability in the field of cosmetics and biomedical applications. Recently, the biocompatibility of TiO_2 and ZnO NPs, and their applications in various biomedical fields were extensively studied. For example, Venkatasubbu et al. have revealed the biocompatibility and various mechanisms for the toxicity of TiO_2 and ZnO NPs against human pathogens *Salmonella typhi*, *Klebsiella pneumoniae* and *Shigella flexneri* [39]. Nica et al. have studied the interaction of TiO_2 photocatalyst with human dermal fibroblast, pulmonary fibroblasts and pathogenic microorganisms [22]. They indicated that the iron–nitrogen (Fe–N) doped TiO_2 NPs induced dose-dependent oxidative stress on lung and dermal fibroblast cells, and exhibited potential microbicidal activity. Recently, Stoyanova et al. have synthesized TiO_2/ZnO nanocomposites by non-hydrolytic sol–gel method for antibacterial activity [40]. They investigated that the anatase form in the TiO_2/ZnO nanocomposites killed the bacteria faster than the rutile form in TiO_2/ZnO nanocomposite. Hence, the phase purity also played a vital role in the biological activity of the TiO_2/ZnO nanohybrids. Since, the TiO_2/ZnO based nanomaterials exhibit photo-induced antibacterial activity and cell proliferation activity, they could find potential application in the field of tissue engineering [41,42]. Hence, the biocompatibility of TiO_2/ZnO nanohybrids must be studied on animal cell lines before using them in biomedical or cosmetic fields.

In the present study, TiONS has been synthesized by sol–gel method from titanium tetra *iso*-propoxide (TTIP), while the ZnONR doped TiONS (ZnONR@TiONS) have been derived by hydrothermal method. The ZnONR@TiONS nanohybrid materials have been characterized by FT-IR, Raman and diffused reflectance (DRS UV–visible) spectroscopic techniques, powder X-ray diffraction (XRD), transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS) and energy dispersive spectrum (EDS). The biocompatibility and cell proliferation activity of ZnONR@TiONS nanosheets have been investigated on adipose tissue-derived stem cells (ADSCs). *In vitro* cytotoxicity analysis was performed by WST-1 assay and live and dead (L/D) cell assay methods.

Materials and methods

Materials

Zinc acetate dihydrate (LR) (Fisher scientific chemicals), TTIP (Sigma–Aldrich), *iso*-propanol (98%) (Merck), absolute ethanol (AR 99.9%) (Jaingsu Huaxi International Trade Co., Ltd., China) were used without further purification.

Preparation of titanium dioxide nanosheet

The precursor solution was prepared by dissolving TTIP (0.8 g; 2.8 mmol) in 15 mL *iso*-propanol, and it was added to double distilled (DD) water (250 mL). The gel formation started when the pH of the solution was adjusted to 2 by using nitric acid under vigorous stirring. Hydrolysis of TTIP offered a turbid solution which was heated to 70 °C for 20 h. The colloidal solution was centrifuged at 23,062 RCF (×*g*). The residue was washed with ethanol and dried for 12 h at 70 °C under vacuum to obtain titanium hydroxide ($Ti(OH)_4$) and finally annealed at 400 °C for 2 h to obtain the TiONS.

Synthesis of ZnONR@TiONS

5 mL of 0.03 M zinc acetate in ethanol, 35 mL of 0.5 M ethanolic sodium hydroxide solution and 200 mg of TiONS were mixed together to form a suspension, and the mixture was stirred magnetically for 10 min. Then, the mixture was poured into a stainless steel autoclave and heated to 180 °C for 24 h. The residue was washed using double distilled water until the pH of the washings reached to 7. Finally, it was centrifuged at 23,062 RCF (×g) and dried at 70 °C for 12 h and then it was annealed at 400 °C for 3 h to obtain the ZnONR@TiONS.

Characterization methods

FTIR spectra of all samples were recorded on a Perkin-Elmer 8300 FTIR spectrometer using KBr pellets. TEM analysis was carried out by using a FEI TECNAI G2 (T-30) transmission electron microscope with an accelerating voltage of 250 kV. The crystalline nature of the nanoparticles was characterized by the powder X-ray diffraction using Brucker D8 advance diffractometer with monochromatic Cu-Kα_1 radiation ($\lambda = 1.5418$ Å). XPS analyses were carried out by XM1000 Omicron Nano Technology XPS system with Al-Kα monochromatic wavelength. The shift in XPS spectra due to surface charge density was calibrated to the C 1s core peak. DRS UV–visible spectra were recorded on a Perkin-Elmer lambda-650 DRS UV–vis spectrophotometer. The Raman spectral analyses were carried out on Laser Raman Microscope (Raman 11) (Nanophoton Corporation, Japan). The fluorescent microscopic images were captured on a Nikon fluorescent microscope.

In vitro *cytotoxicity*

Cell culture medium was prepared with Dulbecco's modified Eagle's medium (DMEM) (WISENT Inc.) (89% (v/v)), Penicillin–Streptomycin solution (WISENT Inc.) (1% (v/v)) and fetal bovine serum (FBS) (Corning) (10% (v/v)). ADSCs (Lonza: lot number — 0000421627; pass number: 4) were seeded in 96 well plates (5000 cell/well) and cultured for 24 h in humidified incubator at 37 °C with 5% CO_2. After 24 h of incubation, medium was carefully removed from the wells and the cells were washed by 1x Dulbecco's phosphate buffer saline (DPBS, WISENT Inc.) twice. Culture media containing ZnONR@TiONS with a series of final concentrations of 10, 25, 50, 100, and 200 μg/mL were applied to the ADSCs. After 24 h, cell proliferation was determined by WST-1 viability assay kit (EZ-Cytox, DAEIL Lab Service Co., Ltd.). After washing cells using 1x DPBS, WST-1 assay reagent was added to cells and incubated at 37 °C for 3 h under dark condition. After 3 h, optical density of the samples was measured at 440 nm by using UV–visible spectrophotometer (Thermo scientific). In addition, live/dead fluorescent staining solution (Thermo scientific) was prepared by mixing Calcein-AM (4 mM) and Ethidium homodimer-1 (2 mM) in 1x DPBS. After washing cells using 1x DPBS, L/D staining solution was added to each well and incubated at 37 °C for 30 min under dark condition. The stained cells were observed by fluorescent microscope (Nikon Ti-E).

Statistical analysis

Statistical analyses were performed using GraphPad PRISM software (GraphPad software Inc., San Diego, CA, USA). All experimental groups were performed in triplicates. Data are presented as mean ± standard deviation and analyzed by one-way

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