Research Article

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Synthesis and characterization of Ce-doped TiO₂ nanoparticles and their enhanced anticancer activity in Y79 retinoblastoma cancer cells

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Abstract: Rare earth metal cerium-doped titania nanoparticles (titanium dioxide [TiO₂]) were produced utilizing a low-cost and straightforward sol–gel technique, and its enhanced photodynamic anticancer activity was tested on Y79 retinoblastoma cancer cells. The structural, optical, morphological, anticancer activity, and cytotoxicity of pure and cerium-doped TiO₂ (Ce-doped TiO₂) were investigated. In X-ray diffraction (XRD) measurements, apparent doping of cerium in TiO₂ was detected, with reported anatase patterns shifting toward a lower angle in the anatase structure. Raman spectra verify the presence of cerium doping in TiO₂ by revealing greater wave number shifting. The scanning electron microscope (SEM) and transmission electron microscope (TEM) analysis showed that the synthesized TiO₂ and Ce-doped TiO₂ nearly spherical. TiO₂ and Ce-doped TiO₂ were studied for their photodynamic anticancer activities, and the results suggest that cerium doping in TiO₂ improves anticancer activity.

Keywords: cerium, nanocomposites, TiO₂, TEM, cytotoxicity

1 Introduction

Due to their innovative biological applications, semiconductor nanostructures with diameters smaller than 100 nm have emerged as nanobiomaterials [1,2]. In recent years, a wide range of nanocomposite semiconductor materials has been created to increase the photocatalytic activity efficiency [3,4]. By covering metal or semiconductors nanoclusters with some other layer of appropriate materials, the functional characteristics of such materials can be substantially enhanced [5,6].

Titanium dioxide (TiO₂) has been extensively used and demonstrated as a critical perspective photosensitizer [7–9], photostability, low cost, and nontoxicity. According to a recent study, the particle size of TiO₂ has a significant impact on its photocatalytic activity [10]. The reduction in particle size suggests an increase in surface area and a high redox potential, resulting in a strong photocatalytic activity.

Several methods, including doping, surface modification with metal particles, and particle size reduction to the nanoscale, have been proposed to increase the effectiveness of photocatalytic reactions utilizing TiO₂ when exposed to visible light [11]. Chemically modified TiO₂ nanoparticles are used in several environmental applications due to their self-cleaning properties [12,13]. Because of their nontoxicity, excellent optical absorption, cheap cost, and good chemical stability, metal and metal oxide nanoparticles have been widely investigated [14–19]. Among them, the medical applications of TiO₂ are undeniably promising, with the potential to significantly enhance health care, notably cancer therapy. Doping of Ce in TiO₂ enhances the photocatalysis and photodegradation of TiO₂ [20].

Sol–gel, direct aqueous solution depositions, ultrasonic spray pyrolysis, and sputtering are some of the

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techniques used [21–27]. Among these techniques, the sol–gel process has several distinct benefits, including composition control, excellent homogeneity, reduced crystallization temperature, and, when dip coating is used, the capacity to create thin coatings on complex shapes [28].

This study uses the sol–gel technique to make TiO₂ and cerium-doped titanium dioxide (Ce-doped TiO₂) nanoparticles. XRD, SEM, TEM, and photodynamic anticancer activity analyses were used to characterize the created nanoparticles. Moreover, the anticancer effect of Ce-doped TiO₂ nanoparticles in Y79 retinoblastoma cancer cells was thoroughly addressed.

2 Experimental details

2.1 Synthesis and characterization of TiO₂ and Ce-doped TiO₂ nanoparticles

TiO₂ nanoparticles were created using a wet chemical (sol–gel) method [29,30]. TiO₂ sol was agitated for 3 h with a 0.1 M cerium nitrate hexahydrate solution. The white Ti(OH)₄ precipitate that forms is refluxed for 2 h before being agitated continuously for 12 h. The precipitate is centrifuged with deionized water and ethanol to eliminate contaminants. After centrifugation, the white precipitate is dried for 3 h in a hot air oven at 100°C to eliminate moisture and other solvents. Finally, the dry powder was crushed and calcined in an air tube furnace at 400°C for 2 h.

The crystalline phase construction of TiO₂ nanoparticles and Ce-TiO₂ nanocomposites was examined utilizing a D8 Advance X-beam diffraction meter (Bruker AXS, Germany) at room temperature. The structural identification of TiO₂ and Ce-TiO₂ nanocomposites was inspected utilizing an examining electron magnifying instruments SEM (Model JSM 6390LV, JOEL, USA) and TEM (JEOL-TEM 2100) [32].

2.2 Cellular incubation with TiO₂ nanoparticles and cell culture

2.2.1 Method of cell culture and counting

The Y79 cell line, a well-known cancer cell line, was used in this investigation. The cells were grown for 3 days in a 60 mm Petri plate at a concentration of around 3×10^5 . After the previous culture media was replaced with TiO₂ colloidal solution, the cells were recultured in an incubator for 24 h. After removing the colloidal solution, it was rinsed with phosphate-buffered saline (PBS, Invitrogen Corporation, Gibco). The cells were stained with trypan

blue after being cleaved by trypsin-ethylenediaminetetraacetic acid (Gibco; Nacalai Tesque, Kyoto, Japan). The cells were then counted using a hemocytometer under a 10× in-bright-field microscope. Cell survival was measured by the proportion of unstained (living) cells compared to control dish cells.

2.2.2 Anticancer activity of pure and Ce-doped TiO₂

A continuous wavelength UV lamp with a wavelength of 380 nm was utilized for the UV light irradiation experiment. The wavelength of the light was in the 380 nm region. After 3 days, the media was changed with colloidal TiO₂ solution, and the cells were incubated for another 24 h. The light control dish was photoirradiated without using a nanoparticle solution, whereas the other dishes were photoirradiated using TiO₂. The colloidal arrangement was then eliminated and washed with PBS before being stained with trypan blue to decide cell feasibility. Live cells were not stained blue; however, dead cells have gathered the color. The pictures were taken with an inverted magnifying instrument (Olympus, CKX41, Japan) equipped with a mathematical light field condenser that produces an exceptionally tight light emission from a tungsten light (sent light of 6V-30W halogen brightening) mounted on top of the examples. A similar system concerning Ce-doped TiO₂ nanoparticles was followed.

2.2.3 *In vitro* cytotoxicity and imaging of pure and Ce-doped TiO₂

The provided test sample was subjected to an *in vitro* cytotoxicity test technique by ISO 10993:5. The cell's culture media was changed with a new medium. The test sample was applied to the cells in triplicate [31].

The below formula calculated cytotoxicity and cell viability.

$$\text{Cytotoxicity} = [(\text{Control} - \text{Treated}) / \text{Control}] \times 100 \quad (1)$$

$$\text{Cell viability} = (\text{Treated} / \text{Control}) \times 100 \quad (2)$$

3 Results and discussion

3.1 XRD analysis of pure and Ce-doped TiO₂

XRD (Figure 1) analysis discovered that the peaks detected at 25.8°, 36.8°, 37.9°, 48°, and 54.5°, correspond to reflections 101, 103, 004, 112, and 200, demonstrate the

Figure 1: X-ray diffraction analysis of pure and Ce-doped TiO₂ nanoparticles.

existence of anatase phase in both samples. Using Scherrer's equation, the crystallite size of the nanoparticles was calculated to be 19 nm for TiO₂ and 15 nm for Ce-doped TiO₂, showing that Ce doping in TiO₂ inhibits nanoparticle grain development [33].

3.2 Raman analysis of pure and Ce-doped TiO₂

Raman analysis was used to detect surface structure changes before and after doping. The spectra demonstrate a blue shift in TiO₂, indicating that some small structural deformation happened in TiO₂. The observed bands at 150, 400, 510, and 640 cm⁻¹ correspond to the Raman active modes of Eg (1), B_{1g} (1), A_{1g} + B_{1g} (2), and Eg (2), respectively, and Eg (2) shows the presence of anatase phase (Figure 2) [34].

3.3 Microscopic (SEM and TEM) analysis of pure and Ce-doped TiO₂

Figure 3 depicts the SEM and TEM analyses of Ce-doped TiO₂ and TiO₂ nanoparticles. TiO₂ was shown to have an uneven shape, a nonuniform size distribution, and an aggregated character in SEM examination. However, Ce-doped TiO₂ has a spherical shape and a homogeneous distribution. Combining materials with high surface

Figure 2: Raman analysis of pure and Ce-doped TiO₂.

energy and a low number of surface states causes nanoparticle aggregation. Aggregation and nonuniform size distribution may be caused by the interaction between anion and cation in the synthesized material [35].

A TEM investigation was carried out to establish the precise size of the nanoparticles. The images indicate that all of the particles are on the nanoscale. According to the selected area electron diffraction (SAED) analysis, the more crystallites linked to the surface of the single particles, the more continuous ring patterns emerge from polycrystalline nature. The vivid ring patterns indicated the high density of crystallites in the materials [36].

3.4 Anticancer activity of pure and Ce-doped TiO₂

Pure and Ce-doped TiO₂ nanoparticle solutions were incorporated into well-grown Y79 retinoblastoma cancer cells, and the nanoparticle-containing cells were treated with UV radiation over various periods ranging from 1 to 6 h. The stage-by-stage irradiation and associated cell condition were documented using a microscopic picture. Figure 4 (TiO₂) and Figure 5 (Ce-TiO₂) show observed cell pictures; when exposed to UV radiation in the presence of nanoparticles, these cells die regularly. This was corroborated by the control picture, which was exposed to UV radiation for 2 h. The cytotoxicity data demonstrate that UV irradiation increases cytotoxicity and decreases viability in nanoparticle-incorporated cells compared to the control. The proportion of cytotoxicity increased with

Figure 3: SEM analysis of (a) TiO_2 and (b) Ce-doped TiO_2 . TEM and SAED analysis of (c) TiO_2 and (d) Ce-doped TiO_2 .

Figure 4: Anticancer activity of pure TiO_2 .

Figure 5: Anticancer activity of Ce-doped TiO₂.

Table 1: Anticancer activity of pure and Ce-doped TiO₂

Sample no.	Sample	Concentration (µg·mL ⁻¹)	Time period	Cytotoxicity	Viability	Reactivity
1	TiO ₂	200	1	49.6	50.4	Mild
			2	52.2	47.8	Moderate
			3	56.5	43.5	Moderate
			4	59.4	40.6	Moderate
			5	61.9	38.1	Moderate
			6	66.4	33.6	Moderate
2	Ce:TiO ₂	200	1	45.5	54.5	Mild
			2	48.1	51.9	Mild
			3	56.9	43.1	Moderate
			4	59.3	40.7	Moderate
			5	64.8	35.2	Moderate
			6	69.4	30.6	Moderate

increasing irradiation time. Pure TiO₂ exhibits 66.4% TiO₂ and 33.6% viability after 6 h of UV irradiation. The value was raised in Ce-TiO₂, which exhibits 69.4% cytotoxicity and 30.6% survivability. The results reveal that Ce-TiO₂ has a better anticancer activity than pure TiO₂ [37] (Table 1).

The anticancer action of Ce-doped TiO₂ may be attributed to cerium-doped into TiO₂ lattices, which has been

postulated to decrease the energy gap that may be activated by UV efficiently [38,39]. Due to its significant catalytic potential and light response extension, cerium has previously been doped into anatase structures to promote photochemical processes [40]. It can also prevent electron–hole pairs from recombination, extending their lifespan. Furthermore, cerium has a higher UV cross section than biological tissue components, resulting in a significant

UV interaction with host materials and subsequent reactive oxygen species production [41].

4 Conclusion

The sol–gel technique was successfully utilized to generate pure and rare earth metal Ce-doped TiO₂. The structure of the XRD patterns of pure and Ce-TiO₂ was anatase, with evident cerium doping in the TiO₂ matrix. Raman analysis was used to validate the structural purity and phase purity of pure and Ce-TiO₂. SEM and TEM examination verified the spherical shape and nanosize. Ce-doped TiO₂ nanoparticles show enhanced photodynamic anticancer activity.

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