Fabrication of protein-coated titanium dioxide nanoparticles for cellular uptake fluorescence imaging and treatment of colorectal cancer

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Abstract
Titanium dioxide (TiO₂) coated with different proteins has exhibited exceptional bio-compatibility, leading to various biomedical engineering applications. With the use of green and chemical reduction methods, we fabricated titanium dioxide nanoparticles that were protein-coated. Bovine serum albumin (BSA), lysozyme proteins, zein, and collagen have been used to coat titanium dioxide-aryl nanoparticles of the form TiO₂-NPs. However, in both cases, no catalysts or other stabilizing agents were used. These images of TiO₂-NPs fabricated using the green method show high crystallinity. It is a malignant colorectal tumour with dysfunctional cellular processes that cause colorectal cancer cells. It is hoped that studies employing SW1417 cells would give mechanistic ideas on the specifics of the amplification in cancers. This was done by flow cytometry utilizing and laser confocal fluorescence microscopy (LCFM) on the SW1417 colorectal cell line. Of the protein-coated Titanium dioxide nanoparticles fabricated green methods, BSA@TiO₂-NPs were the most readily absorbed. Of all TiO₂-NPs, lysozyme@TiO₂-NPs fabricated by the chemical reduction technique were the most effectively internalized by SW1417 cells out of TiO₂-NPs types. However, TiO₂-NPs fabricated by the green methodology were coated with zein and lysozyme and tiny. A hydrophobic covering is also on the two nanoparticles. There is a possibility that the variation in hydrophobicity and charge affected the internalization process. Colorectal diagnostic and therapeutic compounds might be synthesized from those coated nanoparticles that were effectively internalized.

1. Introduction

Titanium dioxide (TiO₂) is a naturally occurring mineral oxide that may be found in anatase, rutile, and brookite forms, among others. New inorganic chemistry and titanium dioxide nanoparticles (TiO₂-NPs) are widely employed in the cosmetics and pollution-treatment industries and the pharmaceutical and paint industries [1–4]. It has also been utilized in biomaterials due to its excellent stability and antimicrobial/anti-corrosive characteristics. As the number of nano-related goods prolife rates, so do the worries about their potential harm to humans and the planet. The International Agency for Research on Cancer has categorized TiO₂ as a category 2B carcinogen (probably harmful to humans by inhalation) (IRAC 2006) [5–7]. Large surface areas and tiny TiO₂ nanoparticles increase chemical reactivity, allowing them to penetrate living cells. Nanoparticles made of titanium dioxide (TiO₂) might penetrate human bodies and cause damage to organs such as the lung and brain [8]. TiO₂ nanoparticles might potentially harm cells such as HepG2, Raw 264.7, and HeLa. When it comes to
TiO₂’s entrance into the body, it can enter via inhalation, skin contact, ingesting, and parenteral interaction. For nanoparticles, the lungs are the primary target organ [9–11].

However, BSA’s hemocompatibility with Titanium dioxide nanoparticles has been well-documented in the literature as a reducing agent/capping agent for Titanium dioxide nanoparticle production. Fibrous collagen is employed extensively in nanotechnology research because of its many unique characteristics [12–14]. As the primary protein found in maize, Zein protein is employed in enzymes and drug delivery because of its superior coating ability, biocompatibility, and biodegradability [15]. Zein was also employed to synthesize TiO₂-NPs bioconjugates, and its green chemistry was investigated in the process. They also examined the biomineralization of TiO₂-NPs with lysozyme and its use in protein films [16].

According to recent research, the nanoparticle determines the absorption of nanoparticles by cells—protein bioconjugates, rather than the nanoparticles themselves [17]. This is due to various interfaces, such as other biological reactions and endocytosis [18]. According to Cheng et al, reports the corona serum protein surrounding the Titanium dioxide nanomaterials influences their internalization [19]. Another factor affecting nanoparticle internalization is the Titanium dioxide nanoparticle size. Researchers found that protein bioconjugations modify the Physico-chemical characteristics of nanomaterials surfaces, affecting their zeta potential, aggregation state, and cell uptake. Nanoparticles of Titanium dioxide are taken up and excreted by cells depending on their shape, size, and corona protein. Additionally, surface modifications of nanomaterials with specific functional groups such as carboxylic acid (COOH), and polyethene glycol (PEG) etc., create them more appropriate for biomedical applications [20]. The SW1417 colorectal cells were desired as their cell model for further examinations. SW1417 cells are selected in the investigation findings because they proliferate multiply than non-cancerous cancer cells. This investigation will deliver systematic ideas in the specifics of tumour development [21–25]. Consequently, the information acquired from SW1417 colorectal will benefit the therapeutics and diagnostics for colorectal cells [26–29].

The use readily reducible and water-soluble aryldiazonium titanium dioxide salts under ambient circumstances were used in our experiments to coat the titanium dioxide–carbon nanoparticles with BSA, lysozyme, zein and collagen. In this study, there are two sections. In this work, we focused on designing and characterizing protein-coated titanium dioxide–carbon nanoparticles using green and chemical techniques. When using protein bioconjugates to image SW1417 colorectal cells in the second phase of the investigation, we can (1) analyze the effects of bioconjugates on TiO₂-NPs internalization, and cytotoxicity and (2) improve intracellular uptake selectivity. To test the absorption of various nanoparticles, we used a variety of coatings and sizes.

### 2. Experimental section

#### 2.1. Materials

TBE buffer (0.045 M Tris–borate, 0.001 M EDTA; pH 8.3), lysozyme; 0.5 × , zein, type I rat tail collagen, lyophilized bovine serum albumin (98%), hydrochloric acid (37%), 0.5 M solution of 9-borabicyclo[3.3.1] nonane in THF were bought from Sigma-Aldrich Co. Ltd. (Shanghai, China). Roswell Park Memorial Institute-1640 medium (RPMI-1640, Corning), trypsin/EDTA (0.05%), penicillin/streptomycin, fluorescein isothiocyanate isomer (98%), trypan blue, fetal bovine serum (FBS), 4’,6-Diamidino-2-phenylindole (DAPI) were purchased from Beyotime Biotechnology (Shanghai, China). Phosphate buffered saline (PBS) with pH 7.4 was purchased from Roche.

#### 2.2. Characterization of nanoparticles

The micromorphology of BSA@TiO₂-NPs, Collagen@TiO₂-NPs, Zein@TiO₂-NPs, and Lysozyme@TiO₂-NPs was examined by a transmission electron microscope (Tecnai G2 F20 S-TWIN). The diluted samples were dropped onto the copper grid covered with nitrocellulose and dried at room temperature on a filter paper. Dynamic light scattering (DLS) measured the samples’ particle sizes, and zeta potentials were measured by dynamic light scattering (DLS) (Nano-ZS 90, Malvern Instruments, U.K.). The samples were diluted to 50 µg ml⁻¹ [50 µl of BSA@TiO₂-NPs, Collagen@TiO₂-NPs, Zein@TiO₂-NPs, and Lysozyme@TiO₂-NPs] for DLS measurement. The optical absorptions of BSA@TiO₂-NPs, Collagen@TiO₂-NPs, Zein@TiO₂-NPs, and Lysozyme@TiO₂-NPs were measured by a UV–visible spectrometer (UV-2600, Shimadzu, Japan). All samples were diluted to a specific concentration before the measurement. The wavelength range for the measurement was from 200 to 900 nm, and the scanning speed was 600 nm min⁻¹. The interactions between BSA@TiO₂-NPs, Collagen@TiO₂-NPs, Zein@TiO₂-NPs, and Lysozyme@TiO₂-NPs were characterized using an FTIR spectrometer (Bruker, Billerica, MA, USA).
2.3. Fabrication of nanoparticles by green reduction method

2.3.1. BSA@TiO$_2$-NPs
As well as the BSA (1 mM) solution, equivalent quantities of titanium chloride (TiCl$_4$) were added (10 mM). It was combined at room temperature, and the pH was adjusted to between 3.5 and 4 by adding 0.1 M NaOH. The pH of the solution was 2.6 at the beginning. It was then placed in a thermostatic water bath at 65 °C overnight under static settings until the reaction mixture was bright pink [30–32].

2.3.2. Collagen@TiO$_2$-NPs
The technique previously described was followed but with a few minor changes. This was done by mixing 1.5 ml of collagen solutions were prepared by 20 mg of collagen powder in 20 ml of acetic acid (0.2%) with 0.5% of ethanol, and the result was stunning. 100 ml of DD-water was then poured into the collagen–ethanol solution, then combined with 1 ml (10 mM) of titanium tetrachloride (TiCl$_4$) solution. As soon as the mixture reached boiling point, it was heated and swirled vigorously until cool. It was rapidly neutralized with 2 ml of NaOH (1%), and the heating up was turned off when the solutions began to boil. Upon reaching 7.0 pH, the fluid changed colour to purple [33–35].

2.3.3. Zein@TiO$_2$-NPs
We obtained 2.5 ml of zein (0.1%) in 25 mM sodium dodecyl sulphate (SDS) and 5 ml of titanium tetrachloride (TiCl$_4$) solution and adjusted the pH to 6 with 0.1 M NaOH. For 48 h, the combination was maintained at 65 °C in a thermostated water bath under static circumstances. Because of cysteine and other reducing amino acids in Zein decrease Titanium dioxide nanoparticles covered with Zein protein, subsequent in a shift from colourless to brownish-red [36–38].

2.3.4. Lysozyme@TiO$_2$-NPs
Lysozyme attached carboxyl functional groups to titanium dioxide-aryl nanoparticles by combining lysozyme in water with titanium chloride. The result was titanium dioxide-aryl nanoparticles containing carboxyl functional groups (TiCl$_4$). 0.1 M NaOH solution was used to alter the pH to 12.0. Further, 24 h at room temperature, the mixture was agitated, resulting in a deep cherry red colouration. We utilized a similar technique to the one we used during our earlier work to purify the samples [39–41].

2.4. Preparation of protein-coated TiO$_2$-NPs by chemical reduction method
Titanium oxide nanoparticles with protein coatings were synthesized with the aid of the reducing agent 9-BBN/NaBH$_4$. This was done by taking different solutions (1 mM BSA, Lysozyme, and 10 mM TiCl$_4$) and adding 10 ml of 10 mM TiCl$_4$. 30 min was allotted for them to mash, and then added drops of the 9-BBN were to both solutions until they became purple. A mixture of 4 ml of 2 mg ml$^{-1}$ collagen and 4 ml of TiCl$_4$ (10 mM) was used to make collagen-coated TiO$_2$-NPs, then dilute to 100 ml with DD-water. To this was added a 0.1 M NaBH$_4$ solution dropwise, stirred continuously until the solution became purple. A 0.25% protein solution was made by dissolving zein in ethanol and using it to coat TiO$_2$-NPs. An amount of titanium chloride (titanium tetrachloride) was added to the Zein solution (10 ml) (TiCl$_4$). After 30 min of stirring, 9-BBN was added drop by drop, resulting in a pink solution. It was then maintained overnight at room temperature with constant stirring. A refrigerator was used to store all the TiO$_2$-NPs that had been developed [42–45].

2.5. Cell culture experiments
Human SW1417 colorectal cells were cultured in RPMI-1640 medium supplemented with 10% (v/v) FBS, 0.03% L-glutamine, and 1% penicillin-streptomycin within a humidified atmosphere containing 5% CO$_2$ at 37 °C. The cell culture medium was replaced every two days.

2.6. Examination of MTT assay
8 × 10$^4$ cells were seeded onto 96-well plates with trypsin/EDTA, and the cells achieved 90% confluence and were incubated with eight various coated NPs and one uncoated TiO$_2$-NPs. Various TiO$_2$-NPs concentrations (50, 25, 12.5, 6.25, 3.13, 1.56, and 0.78%) were added to the cells for 24 h. 5 mg ml$^{-1}$ of MTT formazan stock solutions were produced. When using a full (RPMI-1640) medium, the working concentrations of 10% MTT solutions were attained. At 37 °C and 5% CO$_2$, SW1417 cells were exposed to the working solution for 4 h. After that, the cells were rinsed with MTT solution and DMSO (100 μl) was added to dissolve the formazan crystals. The optical density (OD) was read at 490 nm using a microplate reader (infinite M200, TECAN) [46–50].
2.7. Confocal microscopic imaging
FITC-tagged TiO$_2$-NPs were used to treat human SW1417 colorectal cells for 24 h. The cells were then rinsed in PBS, fixed in ice-cold formaldehyde (4%), and mounted on coverslips utilizing FITC with DAPI to visualize the nuclei. Further processing of the slides was done at a temperature of 4 °C. Following this, SW1417 cells were washed thrice with PBS for 5 min and fixed with 4% paraformaldehyde before CLSM observation. Fluorescence was examined under excitation at 425 nm for DAPI and 488 nm. Then, the fluorescence images of the cancer cells were taken by a fluorescence microscope (Olympus, Japan) [51–55].

2.8. Flow cytometry examination
TiO$_2$-NPs coated with FITC were used to measure cell uptake in human SW1417 colorectal cells for 24 h. PBS and trypsin/EDTA enzymes were used to enzymatically remove the cells, then centrifuged at 300g for 5 min. FACS tubes were filled with the density of 1 × 10$^5$ cells of cold buffer stain solution. TiO$_2$-NPs coated with proteins were used as a control. We used a flow cytometer (NovoCyte 2060R) to evaluate cells treated with 6.03 μg ml$^{-1}$ of these various TiO$_2$-NPs at each sample rate of 25,000 events. FlowJo software was used to analyze flow cytometric data. We calculated the proportion of SW1417 cell lines that absorbed TiO$_2$-NPs for each sample and controlled to analyze the data collected quantitatively.

3. Results and discussion

3.1. Synthesis and characterizations
To decrease water-soluble titanium dioxide, we employed four different proteins (figure 1). As part of the green synthesis, BSA@TiO$_2$-NPs at pH 3.5 and TiO$_2$-NPs at pH 4 were incubated for 12 h at 65 °C with an aryl diazonium titanium dioxide salt. After 12 h incubation time of the combination of the solutions at 70 °C, collagen@TiO$_2$-NPs showed a purple hue, whereas Zein@TiO$_2$-NPs showed a dark brown colour. At varied pH levels, lysozyme@TiO$_2$-NPs showed various shades from purple-pink to reddish-orange [56].

The protein-coated Titanium dioxide nanoparticles are extremely dispersible in an aqueous medium. Delicately reduced 9-BBN was used to fabricate bioconjugates. Researchers found that 9-BBN is relatively lower than NaBH$_4$ and produces fewer contaminants in nanoparticles fabrication. Though, the organic components of 9-BBN are challenging to remove from the nanoparticles’ exterior surface once they have been formed. We
conducted all chemical reductions without a phase-transfer agent, and all of them were successful. As a result, this approach may be less hazardous for human cells research than the greenway (*vide infra*).

### 3.2. Characterization of nanoparticles

Nanoparticles were further characterized with FTIR and UV–vis using protein-coated Titanium dioxide nanoparticles. In the colloidal solutions, the plasmon peaks were 500–600 nm in wavelength. Figure 2(A) shows the plasmon peak for BSA@TiO$_2$-NPs at pH 3.0 and 4.0 (figure 2(B)), and the redshift to 600–650 nm assessed to BSA@TiO$_2$-NPs at pH 4.0 (figure 2(C)). At more acidic conditions, the plasmon peak’s redshift and relative broadening suggest the formation of protein–TiO$_2$-NPs aggregates and a rise in their size-matched to prior nanoparticles at primary media. This confirms the complete reduction of aryldiazonium titanium dioxide solution to Titanium dioxide nanoparticles in the presence of proteins by measuring the plasmon peak for collagen@TiO$_2$-NPs at 540 nm and Zein@TiO$_2$-NPs at 560 nm. When the pH of lysozyme@TiO$_2$-NPs is changed, the plasmon peak changes. The acidic pH medium shifts the plasmon peaks to longer wavelengths due to potential aggregation, which is evident [57].

Further, green and chemically reduced protein@TiO$_2$-NPs were confirmed by the FT-IR spectral analysis. Each of these bioconjugates displayed wide absorption bands at 3250 cm$^{-1}$, which corresponded to OH stretching vibrations in the bioconjugates (BSA@TiO$_2$-NPs, collagen@TiO$_2$-NPs, Zein@TiO$_2$-NPs, and lysozyme@TiO$_2$-NPs, respectively). The carbonyl stretches at 1708 cm$^{-1}$ has a significant absorption band, suggesting the presence of carboxylate. It was found that protein–TiO$_2$-NPs displayed bands at 1300 cm$^{-1}$ and additional bands about 1000 cm$^{-1}$, which corresponded to the C=$+$C + H$+$, and C–OH groups in the protein. According to these results, the green and chemically fabricated TiO$_2$-NPs had the anticipated functional groups present in the IR spectra.

### 3.3. Characterization of size distributions and zeta (ζ) potential analysis

Light dynamic scattering (DLS) analysis determined the hydrodynamic parameters, and the size distribution of the proteins with TiO$_2$-NPs conjugates was determined by light dynamic scattering (DLS) analysis. All the experiments were repeated three times. The logarithm of the particle diameters determines the light scattering intensity (figures 3(A–D)). For green fabricated TiO$_2$-NPs, BSA@TiO$_2$-NPs showed bimodal particle distribution at pH 3.5 and 4, whereas collagen@TiO$_2$-NPs displayed a single peak. Large-sized BSA@TiO$_2$-NPs

![Figure 2. Characterization of protein-coated TiO$_2$-NPs (A) UV–vis absorption spectral analysis of chemical reduction fabrication of BSA@TiO$_2$-NPs, collagen@TiO$_2$-NPs, lysozyme@TiO$_2$-NPs, and zein@TiO$_2$-NPs. (B) Green fabrication of BSA@TiO$_2$-NPs, zein@TiO$_2$-NPs, and collagen@TiO$_2$-NPs. (D) UV–vis spectra of lysozyme@TiO$_2$-NPs synthesized at different pH values.](image-url)
were used, with sizes among 80 and 1500 nm. This is because the pH 3.5 size is bigger than the size in pH 4.0. For example, surface plasmon peak at pH 3.5 shift and widen, showing that cluster formation and aggregation occur in an acidic medium. The size of collagen@TiO$_2$-NPs and Zein@TiO$_2$-NPs varied from 10 to 60 nm, respectively. Moreover, their UV–vis absorptions results, which were at approximately a similar wavelength, corroborate the existence of small nanoparticles. Lysozyme@TiO$_2$-NPs had nanoparticles between 5 and 30 nm at pH 6.4–10, indicating smaller TiO$_2$-NPs in basic environments. Chemically reduced protein-coated TiO$_2$-NPs revealed a polymodal size distribution spanning 30 to 1600 nm, demonstrating the production of large and tiny TiO$_2$-NPs when reduced. Lysozyme@TiO$_2$-NPs were approximately 972 nm in size, whereas collagen@TiO$_2$-NPs and Zein@TiO$_2$-NPs were around 2 and 3 nm. A variety of parameters influence how nanoparticles are shaped and formed, including the concentration of the solution pH level, incubation period, temperature, and synthesis technique. Information on the stability of colloids and total surface charge in solutions may be gleaned from the ζ-potential values. The -potentials and sizes of all samples were measured concurrently using DLS at 25 °C. A three-times-repeated measurement’s mean and standard deviation (SD) is calculated. This is due to the existence of acidic conditions to fabrication TiO$_2$-NPs in BSA@TiO$_2$-NPs at pH 3.5 and 4, whereas negative range zeta-potential values were observed for collagen@TiO$_2$-NPs and zein@TiO$_2$-NPs. However, the NPs were less stable and tended to aggregate into bigger particles due to their low beta-potentials. According to our findings at pH 3.5, the ζ-potential value for BSA@TiO$_2$-NPs is more inferior to pH 4. In addition to UV–vis and DLS, this finding confirms the UV–vis conclusion. Since collagen@TiO$_2$-NPs and Zein@TiO$_2$-NPs have greater potentials, they must be more stable in solution, as evidenced by their higher prospects. For lysozyme@TiO$_2$-NPs, the ζ-potential exhibits negative values in the pH range of 6.4–10. Many nanoparticles were found in very acidic environments, and the ζ-potential was positive in these conditions. If the medium is acidic, the pace at which colloidal nanoparticles develop is slowed down.

### 3.4. Characterization of HR-TEM

Figure 4 shows TEM images of TiO$_2$-NPs coated with a variety of proteins. It is clear from the pictures that TiO$_2$-NPs are present, albeit in varied forms and sizes. Figure 4 shows that BSA@TiO$_2$-NPs at pH 3.5 are hexagonal and oval with well-organized a typical size of 105.25 ± 2.58 nm, whereas BSA@TiO$_2$-NPs at pH 4 are spherical with well-organized a regular size of 143.25 ± 3.40 nm. Intriguingly, amorphous carbon contained nanoparticles measuring 7.8 nm by 1.8 nm. For example, the titanium dioxide planes (200) and (111) are shown with lattice space groups of 0.205 and 0.236 nm, respectively. Collagen@TiO$_2$ nanoparticles are usually round or
Oval. Protein-reduced and chemically synthesized collagen@TiO2-NPs had particle sizes of 5.8 nm, 1.2 nm, and 11.3 nm, respectively. Titanium dioxide lattice space group of 0.205 and 0.236 nm indicates that the reagents used for the greener technique reduced Titanium dioxide ions effectively to develop well-controlled protein@TiO2-NPs in the (200) and (111) planes of Titanium dioxide. On the other hand, Zein@TiO2-NPs exhibited various shapes; while making TiO2-NPs out of zein, the average size of TiO2-NPs fell from 40.1 nm to 9.9 nm after chemical treatment. TiO2-NPs were effectively fabricated using the chemical approach as illustrated in figure 4, as evidenced by the observed lattice space of 0.205 nm for the plane (200).

### 3.5. Cell experiments

The desirable nanoparticles should not cause harm to the cells. In the latest study, the survival of both non-cancerous and cancer cells was not affected by native Titanium dioxide nanoparticles or lysozyme-conjugated Titanium dioxide nanoparticles, showing they are suitable for drug delivery. Native Titanium dioxide nanoparticles and BSA@TiO2-NPs showed no impact on red blood cells of hemolysis in a distinct investigation [58].

### 3.6. In vitro cytotoxicity examination

To determine if these conjugated TiO2-NPs may be utilized for therapeutic intervention or medication administration, they must be tested for cytotoxic effects first. Titanium dioxide nanoparticles (TiO2-NPs) were tested for cytotoxicity in SW1417 cells at varying concentrations (figure 5). Concentrations of TiO2-NPs tended to rise. However, all the TiO2-NPs were shown to be rather non-cytotoxic at low concentrations. The 50% concentration of Zein@TiO2-NPs and collagen@TiO2-NPs fabricated by both techniques was the most cytotoxic. TiO2-NPs fabricated using the green approach also had lower cytotoxicity than those made using the traditional method. 1.56% and 0.78% TiO2-NPs showed no or minimal cytotoxicity. In previous work, lysozyme@TiO2-NPs fabricated by the green technique were also evaluated for cytotoxicity, and we discovered that they had been non-toxic at minimal concentrations [59].

Chemical or green methods are used to fabricate these TiO2-NPs, which have various coatings. When coated, these TiO2-NPs developed an entirely new chemical composition. These changes would impair the SW1417 cells' ability to internalize these nanoparticles. As shown in figure 5, all of TiO2-NPs offers in a variety of sizes. Thus, part of the variances in internalization efficiencies may be attributable to variations in TiO2-NPs sizes. The internalization of nanoparticles is also affected by their net charges. To compensate for this, cell membranes often have a negative control. It follows that positive-charged nanoparticles are more likely to interact with cell membranes and become internalized than negative-charged nanoparticles.

On the other hand, nanoparticles having a negatively charged lipid can interact with the positive charged lipids sphere of the cells membrane and be internalized with cells. Using a reduction technique, 972 nm...
lysozyme@TiO$_2$-NPs exhibited the maximum internalization efficiency because of their positive charge. Synthesized lysozyme@TiO$_2$-NPs, 5–30 nm in size, have a negative charge. The net positive charge of these lysozymes@TiO$_2$-NPs may be responsible for their excellent internalization efficiency in the reduction procedure. They were around 70 nm in size and had an overall negative charge. Thus, SW1417 cells were able to internalize them with the third-highest efficiency. Collagen@TiO$_2$-NPs (2 nm) was fabricated by the chemical-reduction method. They have internalized another effect of all the nanoparticles tested. Because this collagen@TiO$_2$-NPs were positively charged and the smallest of all TiO$_2$-NPs, they may have been more readily internalized. Because they were so big, the BSA@TiO$_2$-NPs synthesized by the green technique had the highest internalization efficiency among all TiO$_2$-NPs fabricated by the green method. BSA@TiO$_2$-NPs synthesized by the chemical reduction technique did not internalize and those synthesized by the BSA@TiO$_2$-NPs method. These TiO$_2$-NPs appear to be internalized more efficiently because of the positive charge. These nanoparticles were between 10 and 60 nm, possessed a negative charge, and was the minimum effective in internalization. A positive charge was added to the chemical reduction synthesis of Zein@TiO$_2$-NPs, which resulted in smaller particle sizes and more effective internalization of SW1417 cells. Internalization may be less efficient because of their more significant toxicity at high concentrations (figure 5). Even functionalized jackets have an impact on this. For the green synthesis, all the coated TiO$_2$-NPs were internalized far less effective than the uncoated TiO$_2$-NPs. The internalization of TiO$_2$-NPs may have been influenced by coatings and net charge as a function.

3.7. Cellular uptake by SW1417 colorectal cells

Human SW1417 colorectal cells were first examined qualitatively using microscopic technique and flow cytometry to TiO$_2$-NPs coated with proteins. SW1417 cells were exposed to bioconjugates with nanoparticles and without nanoparticles with any conjugates linked to FITC fluorescents probes to investigate their uptake. A colorectal cancer cell line, SW1417, behaves similarly to mesenchymal stem cells in that it can develop into multiple cell types. However, SW1417 cells absorb TiO$_2$-NPs to various degrees, according to the results of the confocal microscope study.

![Figure 5](image.png)

Figure 5. The cytotoxicity study of the different TiO$_2$-NPs fabricated by chemical reduction method at various concentrations. The SW1417 cells were exposed to varying concentrations of the various TiO$_2$-NPs. All of the TiO$_2$-NPs displayed cytotoxicity at all concentrations compared to control. The cytotoxicity study of the TiO$_2$-NPs synthesized by green method at various concentrations. The SW1417 cells were exposed to different concentrations of the various TiO$_2$-NPs. The TiO$_2$-NPs synthesized by the green method does not display cytotoxicity at low concentrations. The collagen–coated nanoparticles were assessed at one time.
It was determined that the cells took up the protein bioconjugates by utilizing confocal microscopy (figure 6) to detect their intracellular distribution and subjectively evaluate their degree of internalization. Figure 6 shows the overlay of the two channels. Bioconjugate-treated cells were plated on coverslips for 24 h before images were taken. Because of the strong blue fluorescence at 425 nm and 475 nm excitation and emission wavelengths, the SW1417 cells contained the nanoparticles. The confocal pictures get increasingly bright when the nanoparticles are absorbed. Nanoparticles with different coat proteins may internalize at varying rates depending on the nature of their coatings. Nanoparticles synthesized using other synthesis techniques have changed internalization rates. A chemical reduction technique was used to produce the nanoparticles, which were coated with lysozyme and collagen. TiO₂-NPs fabricated by the green method, such as BSA@TiO₂-NPs, were readily absorbed by the SW1417 cells (figure 7).

Flow cytometry was used to quantify the proportion of SW1417 cells that absorbed these various TiO₂-NPs out of 20 000 events to determine the quantitative absorption of the TiO₂-NPs (figure 8). The green approach was the least efficient for Zein@TiO₂ nanoparticle production, with 87.1 and 91.9% of SW1417 cells internalizing the nanoparticles. Chemically reduced lysozyme@TiO₂-NPs were absorbed by the SW1417 cells, with the most remarkable percentage of 99.4% in SW1417 cells. Using the chemical reduction technique, collagen@TiO₂-NPs fabricated a 99.15% internalization rate of TiO₂-NPs in SW1417 cells. SW1417 cells absorbed 99.05% of the uncoated TiO₂-NPs, the third-highest % age of internalization. There were slight differences in the effectiveness of SW1417 cells to internalize TiO₂-NPs coated by the green method: collagen@TiO₂-NPs and BSA@TiO₂-NPs were internalized by 98.5 and 99% of SW1417 cells.

Compared with uncoated nanoparticles, SW1417 cells absorbed lysozyme@TiO₂-NPs and collagen@TiO₂-NPs fabricated by the chemical reduction technique more effectively (99.6 and 99.23%, respectively). SW1417 cell lines absorbed 97.1 and 97.65% of BSA@TiO₂-NPs and Zein@TiO₂-NPs fabricated by chemical reduction. The internalization of BSA@TiO₂-NPs fabricated by the greener technique and nanoparticles with uncoated conjugates were virtually identical in SW1417 cells (99.09% versus 99.55%). Comparatively, TiO₂-NPs generated using the green approach internalized SW1417 cells more effectively than TiO₂-NPs synthesized using the chemical reduction technique (figure 7). There are fewer TiO₂-NPs in the green method confocal pictures than in the uncoated TiO₂-NPs confocal images (figure 7).

4. Conclusions

It is possible to reduce the toxicity of nanoparticles by bioconjugation with biological molecules. A chemical reducing agent and biomimetic method were used to fabricate bioconjugates with BSA, lysozyme proteins, zein, and collagen. While all proteins-coated Titanium dioxide nanoparticles were consumed, the degree of absorption differed depending on which proteins were coated. SW1417 internalized lysozyme-coated TiO₂-NPs
generated by chemical reduction the most, followed by collagen-coated TiO$_2$-NPs made by chemical reduction. Zein coated TiO$_2$-NPs fabricated by green reduction had the least effective absorption. SW1417 cells absorbed the most TiO$_2$-NPs fabricated by the green technique coated with BSA. The internalization of these TiO$_2$-NPs...
appears to be positively affected by net positive charge as well, according to the study. At low concentrations, these various TiO$_2$-NPs showed negligible cytotoxicity. A comparison of cytotoxicity between the green method and reduction technique nanoparticles showed that the green approach fabricated less hazardous nanoparticles at any given concentration. As a result, more study is needed to determine how various protein coatings affect the absorption of TiO$_2$-NPs by cells, focusing on endocytosis. The size and form of TiO$_2$-NPs and functionalized groups linked to the nanoparticles impact the variance in uptake. For this reason, future research should focus on determining how and where the TiO$_2$-NPs go through human cells.

Data availability statement

No new data were created or analysed in this study.

Notes

The authors declare no competing financial interest

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