Biosynthesized TiO$_2$ nanoparticles and their applications for the treatment of pediatric acute leukemia

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Abstract

The current study demonstrates the efficiency of Salvia Spinosa (S. Spinosa) leaf extract in the biofabrication of Titanium dioxide nanoparticles (TiO$_2$ NPs). An easy and green method was used to synthesize TiO$_2$ NPs, which involves the addition of Titanium hydroxide [Ti(OH)$_2$] solution and the leaf extract of S. Spinosa, followed by stirring results in the formation of a light green dispersion of TiO$_2$ NPs. Characterization of the formed TiO$_2$ NPs was performed using the spectroscopic techniques such as FTIR, XRD, EDS, SAED and TEM. The formation of polydisperse TiO$_2$ NPs with a mean particle size of 23 nm was revealed by TEM analysis. The biomolecules that are possibly involved in reducing TiO$_2$ ions are shown in the FTIR spectrum of the S. Spinosa plant leaf extract. Zeta potential results have confirmed the extreme negative charge of 19 mV for the formed TiO$_2$ NPs. The crystalline structure of the fabricated NPs was revealed by the analysis of XRD. In addition, the cytotoxicity results have confirmed that the cytotoxicity against acute lymphoblastic leukemia (Molt-4) cells depends on the dose, further signifying their potential for the advance of new materials and techniques for pediatric leukemia treatment.

1. Introduction

Cancer is the foremost problem in the world, among the existing diseases causing loss of lives which can be identified by abnormal cell proliferation. The standard methods that are being traditionally used in the cancer therapy are chemotherapy, radiotherapy and surgery. But, the usage of these methods is limited because of their side effects and cost effectiveness. Therefore, there is a high demand for low cost, non-toxic and effective treatments with less side effects, which can be easily accepted by people. Exceptional physicochemical characteristics of nanomaterials such as enhanced reactivity, high surface area, and their capability to easily enter into the cells may be helpful in treatment and diagnosis of cancer [1, 2]. Commercial applications of TiO$_2$ NPs includes their use in consumer goods like laundry additives, containers for food storage, water purifiers and room sprays [3–5].

TiO$_2$ as photocatalyst is applied widely as material for self-disinfecting and self-cleaning on various surface coating applications. On the other hand, TiO$_2$ also has been widely applied in cleaning environment, because of their antifogging effect, photo accelerated super hydrophobicity and specific non-toxicity [6, 7]. The advantages of preferring the use of inorganic TiO$_2$, when compared to the organic materials in biomedical applications includes their simplicity to fabricate a stable, heat resistant and less toxic nature [8–10]. TiO$_2$ NPs can be fabricated by using different techniques like sol–gel method [11, 12], hydrothermal method [13], and flame synthesis [14]. However, these methods involves the use of toxic and expensive chemicals which is a major disadvantage. On the other hand, the green fabrication of NPs has various benefits over the physical and
chemical preparations, which includes the low cost production, simple and environmental friendly ways without involvement of toxic chemicals [15].

The Current study showed the green fabrication of TiO₂ NPs using aqueous leaf extract of S. Spinosa. Further, the prepared TiO₂ NPs were studied for their cytotoxicity against acute lymphoblastic leukemia (Molt-4) cells to check their potential for use in future cancer therapy.

2. Materials and methods

2.1. Materials
Ti(OH)₂, 5-diphenyl tetrazoliumbromide, 3-(4, 5-dimethylthiazol-2-yl)-2 (MTT), KBr powder were purchased from Sigma-Aldrich, Shanghai. All the tests were carried out using Milli-Q water as solvent.

2.2. Preparation of plant extract
Fresh leaves of S. Spinosa plant were collected and washed thoroughly using tap water followed by cleaning with Milli-Q water. Leaves were weighed and about 20 g were added to Milli-Q water of 250 ml volume followed by boiling for 15 min at 60 °C and then filtered using Whatman filter paper to separate solid particles which are unwanted.

2.3. Green synthesis of TiO₂ NPs
For synthesizing TiO₂ NPs, about 100 ml of 10⁻¹ M aqueous Ti(OH)₂ solution was stirred at room temperature for 120 min and then 20 ml of leaf extract was added followed by continues stirring for a day till the color of the solution turns to light green.

2.4. Characterization of TiO₂ NPs
High resolution transmission electron microscope (TEM) (JEOL JEM 2100) was utilized at an acceleration voltage of 200 KV for the morphological analysis of TiO₂ NPs. For preparing the samples, nano colloid of about 1 ml was diluted with distilled water to 10 ml followed by ultra-sonication for 5 min. Then, a drop of the prepared solution was placed on a lacey copper grid on porous carbon film, which further allowed for vacuum drying. The completely dried grids were further utilized for the analysis of TEM along with a simultaneous measurement of selected area electron diffraction (SAED) analysis. The x-ray diffractometer (XRD) analysis for the sample of TiO₂ NPs was recorded by using D8 Advance XRD (BRUKER) with Cu Kα source (λ = 1.5406 Å) over the angle ranging from 10° to 80°. This instrument was operated with a step size of 0.02°, scanning rate of 4°/min and calibrated with lanthanum hexaboride (LaB₆) prior to the analysis of the sample. To obtain the information about the stability and size of the fabricated NPs, Zeta potential analysis was performed at 25 °C with the help of Malvern Zetasizer Nano ZS90 counter. A diluted dispersion of NPs was used to measure the zeta potential. Kratos Axis Ultra 165 x-ray spectrophotometers with A1 XPS instrument was used for x-ray photoelectron spectroscopy measurements. This instrument was operated at 150 W utilizing a 1486.6 eV non-monochromatic Al Kα radiation along with a 0.2 eV spectral resolution. Before performing the analysis, the sample was out-gassed overnight inside a UHV chamber (<5 × 10⁻⁸ Pa) at room temperature. The deconvolution of Cls was performed using the XPS Peak 4.1 software. In order to prepare the sample, the powder of nanoparticles was grounding in ceramic mortar followed by mixing with isopropyl alcohol to form a semisolid paste, which later was drop casted on a stainless-steel substrate. IR AFM (SHIMADZU) was used to record the Fourier transform- Infrared (FT-IR) spectrum to know the surface stabilization of prepared TiO₂ NPs. NPs powder was mixed with KBr which followed by grounding and made pellet using pellet maker. The prepared pellet was kept in FTIR machine and recorded FTIR spectrum.

2.5. Cell culture and MTT assay
Cell lines of Molt-4 were procured commercially from American Type Culture Collection (ATCC, Manassas, Virginia, USA). The culture of the cells was prepared by adding fetal bovine serum (FBS 10%) and penicillin/ streptomycin (1%) to the Roswell Park Memorial Institute 1640 medium (RPMI 1640). T 25 Cell culture flask was used to cultivate the cells (with a density of 1.5 × 10⁵ cells cm⁻²) at a temperature of 37 °C followed by incubating for a week with 5% carbon dioxide (CO₂) for 7 days in a humidified atmosphere. The medium under use was replaced with a fresh one for every 72–96 h. MTT assay was carried out to assess the cell viability. In this assay, the cells were seeded at 3 × 10⁴ cell well⁻¹ in a 96 well microtiter plate with 100 ml of culture medium comprising of different concentrations (0.1, 0.5, 1.0, 2.0 and 5.0 μg ml⁻¹) of TiO₂ NPs with <0.1 percent of DMSO followed by storing in an incubator for 3 days. Further, MTT (Methyl thiazol tetrazolium bromide) with 5 mg/ml⁻¹ as minimum concentration was added to each well followed by the incubation of the cells again for 240 min at a temperature of 37 °C. Later, 10 percent of sodium dodecyl sulphate (SDS)/10⁻² N HCl mixture was
added and incubated for the whole night for solubilization. Furthermore, the optical density was measured in all the wells by using ELISA plate reader (USA, BiotekELx 808) at a wavelength of 570 nm.

3. Results and discussion

3.1. Characterization of TiO₂ NPs

The preliminary confirmation of the formation of TiO₂ NPs was noticed by the change in color of the reaction mixture to light green. X-ray diffraction peaks of the green fabricated TiO₂ NPs were obtained by using the x-ray diffractometer (XRD) and are indicated in figure 1. The peaks found at 2θ of 62.60°, 55.06°, 48.05°, 37.82° and 25.49° confirmed the anatase phase and all the peaks are completely arranged in accordance with anatase phase of TiO₂ (JCPDS No: 99-201-6205). The obtained results were noticed to be in agreement with the previous report where TiO₂ NPs were prepared using C. tamala leaf extract [16].

It was observed from the TEM microscopic images that the fabricated NPs were existed in the size range of ∼23 nm (figures 2(A), (B)). Morphology of the NPs is observed to be irregular and are agglomerated in some areas. An organic layer, which is thin in texture is found in few locations in TEM images, which is due to the biomolecules existing in the extract of the S. Spinosa leaf (also confirmed from FTIR spectroscopy). Figure 2(C) showed the SAED pattern for the TiO₂ NPs. The bright rings are related to the standard polycrystalline rings of diffraction of the anatase phase. In addition, no signs related to the diffraction rings of other phases were observed that indicates the purity of NPs.

Zeta potential was calculated to further verify the stability of NPs and its value was noticed to be 19 mV (figure 3(A)) which specifies that in a given aqueous medium, the particles are discrete and stable. Deprivation of stability was not observed even after half a year at room temperature. Further, EDS spectrum of TiO₂ NPs showed the existence of peaks corresponding to Titanium and oxygen which further confirmed the synthesis of TiO₂ NPs (figure 3(B)).
Additionally, the analysis of the XPS was explored for the green fabricated TiO$_2$ NPs (figure 4). The XPS spectra showed the existence of O 1s and Ti 2p peaks of TiO$_2$. From the spectrum, it is obvious that the splitting photoelectrons of Ti 2p$_{3/2}$ spin-orbital are responsible for the separation energy found at 459.2 eV. In the same way, the signal of binding energy at 530.5 eV is related to the O 1s peak and each of these obtained results are finely in acceptance with an earlier reported study [17, 18], which verifies the formation of single phased and pure TiO$_2$ NPs.

The FTIR spectrum of S. Spinosa aqueous leaf extract exhibited characteristic bands that attributes to various functional groups (as shown in figure 5). The formation of IR peaks at about 3378, 1118, 1380, 1040, 1604, 1694, and 2848 cm$^{-1}$ are due the presence of functional groups like hydroxyl (–OH), aromatic amines (–C$_6$H$_2$NH$_2$), aliphatic amines (R-NH$_2$), carbonyl C=O (benzene), (>C=O), and C–H, respectively. The FTIR spectrum of synthesized NPs exhibited the formation of peaks at about 3388, 1391, 1018, 1685, 1607, and 2842 cm$^{-1}$, which are attributed to the functional groups of hydroxyl, aromatic, aliphatic amines, carbonyl, C=C of benzene and C–H, respectively (figure 5). Furthermore, these compounds having imino or hydroxyl functional groups that are capable of donating many free carboxylic or amino moieties and hydrogen atoms possess the ability of binding to the free gold surface. All the above results confirmed the capping of plant bioconstituents onto the surface of the synthesized TiO$_2$ NPs and these results were also noticed to be in accordance with earlier reports [19, 20].

In the recent times, improving the therapeutic efficacies and reducing the side effect of the drugs, which are in use for the cancer therapy are turning out to be the prominent encounters for enhancing the quality/standard of life in cancer patients [21]. In the present study, TiO$_2$ NPs have displayed the cytotoxicity with a broad range towards the cells of Molt–4. On the other hand, one of the significant drawback for using nanomaterials in chemotherapy is their inability to differentiate the tumor cells effectively. The cytotoxic effect of the TiO$_2$ NPs...
against cancer cells was still not been understood completely. However, the demand for the TiO₂ NPs in cancer therapy is increasing because of their specific cancer cell destruction ability. The specific cytotoxic effects of various NMs towards the tumor cells have been exhibited in different reports. The concentration dependent cytotoxicity of diastase stabilized AuNPs towards the cells of HCT 116 and A549 was shown in a study by Sireesh babu et al [22]. Similarly, the specific cytotoxic effects of the metal NPs towards the cells of HepG² and A549 was also reported in literature [23]. Additionally, the biocompatibility of diastase stabilized AgNPs, silk sericin and tyrosine stabilized graphene sheets was also described in the literature [24–26]. On the other hand, metal oxide nanoparticles such as Manganese-Doped Cerium Oxide Nanocomposite [27] and TEMPO-coated TiO₂ nanorods [28] are found to be effectively inhibiting the growth of MCF-7 cells. The cytotoxic effects of NPs are reliant on the external medium, shape and size, which significantly have an impact on their toxicity. But, the exact cytotoxic mechanism of the NPs is still not yet understood completely.

After treating for 72, 48 and 24 h, the cell viability of Molt-4 cells is inhibited by the TiO₂ NPs in a concentration/time dependent manner, as represented in figure 6. A decrease in the cell viability to 77.40, 51.73 and 11.20 percentage on the 1st, 2nd and 3rd day consecutively is observed at a concentration of 5 ppm when related with the group of control (figure 6). After 48 h, the cell viability of Molt-4 cells has reduced in control from 100 percent to 93.11, 84.78, 84.80.43 and 51.73 percentages, respectively. Moreover, the values of IC50 were 28.99, 6.53 and 1.285 μgml⁻¹ for treatment on the 1st, 2nd and 3rd day consecutively. The significant decrease in the cell viability in a dose and time reliant approach is shown in this data. Similarly, the values of IC50 obtained in this data revealed that concentration of the TiO₂ NPs, which is desirable for use in the in vivo studies is <28.9 ppm.
4. Conclusion

TiO\textsubscript{2} NPs were fabricated by an environmental-friendly method by utilizing the leaf extract of the S. Spinosa plant. XPS, XRD and TEM techniques were used to characterize the fabricated NPs, which further confirmed the formation of TiO\textsubscript{2} NPs. In addition, the cytotoxicity results and the corresponding microscopic images have confirmed that the cytotoxicity against Molt-4 cells depends on the dose, signifying their potential for the advance of new materials and techniques for pediatric leukemia treatment.

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Data availability statement

The data that support the findings of this study are available upon reasonable request from the authors.

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