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Antibacterial Activity and Anticancer Activity of Ag Doped $TiO_2@SiO_2$ Nanocomposite

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ABSTRACT

The syntheses of TiO_2 nanomaterials have attracted great interest due to their importance of wide area application. In this study a novel synthesis of TiO_2 NPs and mesoporous silica nanoparticle (MSNPs) sol-gel method has employed. The $TiO_2@SiO_2$ nanocomposites were modified and decorated with Ag NPs by the chemical reduction method. The nanocomposites were characterized by scanning electron microscope (SEM) and energy dispersive X-ray spectroscope (EDAX). Antibacterial activities were determined by using *Pseudomonas aeruginosa* bacteria and Trichophyton fungi for higher inhibition efficiency. The significant of nanocomposite's anti-cancer activity were analysed and shows that the significant anticancer activity with 50 mg/mL for $TiO_2@SiO_2$-Ag on MCF-7, Hep-G2, and Caco-2.

1. Introduction

Nanoscience is constantly exists imagination biologists and biotechnology. The nanomaterials particularly provide interesting opportunities for the biotechnological development. Since nanoparticles are unique structures and properties [1, 2]. Titanium dioxide (TiO_2) shows a really weak or no dark toxicity in vitro and in vivo study [3]. Captivatingly, the photo catalytic properties of TiO_2 mediated toxicity have been shown to destroy cancer cells [4]. It is now well standard that TiO_2 nanoparticles, on coverage to ultraviolet (UV) light, reproduce electrons and holes important afterwards to the development of reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl radical, and superoxide radicals [5]. These oxygen species are highly reactive with cell membranes and the cell interior, with damaged areas depending on particle location upon excitation. The oxidative reactions affect cell rigidity and the chemical arrangement of surface structures, leading to cell toxicity [6]. Despite promising outcomes in killing cancer cells, such treatments would be difficult to implement in clinical settings for the following reasons. First, UV light cannot penetrate deeply into human tissues, thus limiting this technique to superficial tumors. Second, UV-mediated assembly of reactive oxygen species (ROS) has a very short life span and thus would not be able to provide a continuous prolonged cancer-killing effect [7, 8].

Silver containing nanomaterials have been long well-known as an effective antibacterial activity. Silver ion exerts most biocidal efficiency of bacterial, fungal and viral study. Metallic silver nanoparticles attract great interest in recent years on explanation of their exception antimicrobial capability [9]. Among Ag nanocomposite contain metal oxides, TiO_2, SiO_2, Fe_3O_4 and Al_2O_3 have been investigated broadly due to their antibacterial activity and stability [10]. This work explains antibacterial activity determination on *Pseudomonas aeruginosa* bacteria and Trichophyton fungi for higher inhibition efficiency.

2. Experimental Methods

2.1 Materials

All the chemicals purchased from analytical grade in Sigma Aldrich Mumbai, India Titanium (IV) chloride, (sigma Aldrich 99.9 %), and absolute methanol, EtOH, NH_4OH, NaOH, HCl, was received from Merck chemicals. Cityltrimethyl ammonium bromide (CTAB), tetraethyl orthosilicate (TEOS) (Sigma Aldrich, 99.99%) and silver nitrate ($AgNO_3$) (Sigma–Aldrich, 99.9%). All the other chemicals used in this work were of analytical grade. Unless otherwise mentioned double distilled water was used for the preparation of aqueous solutions and washings.

2.2 Synthesis of TiO_2/SiO_2-Ag Nanocomposite

The successfully synthesis of TiO_2/SiO_2-Ag nanocomposite was using a solgel method according to our previous reported article [11].

2.3 Characterization

The preparation of TiO_2/SiO_2-Ag nanocomposite was characterizated by field emission scanning electron microscope (SEM, JSM-7600F Japan) with energy dispersive spectrum (EDX) of elemental analysis. The nanocomposite used to analyse antimicrobial activity and anticancer activity.

3. Results and Discussion

3.1 Surface Morphology Analysis (SEM)

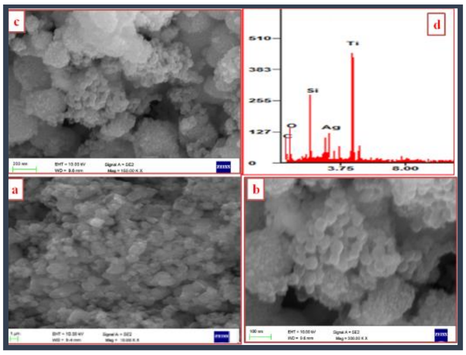

Fig. 1 SEM image for TiO_2 (a) $TiO_2@SiO_2$-Ag (b, c) and (d) EDX- Spectrum of elemental analysis

The SEM (Figs. 1a-c) images are showing the morphological observations of TiO_2 and $TiO_2@SiO_2$-Ag. They were mostly spherical structure and uniformly formed in size. The surface structure characterizations show the prepared nanoparticle maintaining the surface

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nanostructure. The dielectric constant led to the nature of particles shapes as spherical which is shown in the Fig. 1a. Hence it is clear that the formed nanocomposite for Ag NPs are uniformly deposited on the surface with spherical structure. These TiO_2@SiO_2-Ag composite was characterized for EDX element analysis and this EDX spectrum has presented in Fig. 1d.

3.2 Antimicrobial Activity

The bacterial growth kinetics were examined with different concentrations of TiO_2@SiO_2-Ag nanocomposite (25, 50, 75, and 100 μg/mL). The growth kinetics were measured (OD) at 500 nm regular intervals of 3 hrs upto 24 hrs. It is noticeable from the graph that the number of bacterial cells decreased with increasing TiO_2@SiO_2-Ag nanocomposite concentration and with time of exposure. Furthermore, there is no growth inhibition in bacterial culture alone without TiO_2@SiO_2-Ag nanocomposite treatment and the cells survived till reach decline phase. From this investigation, it has been found that TiO_2@SiO_2-Ag nanocomposite being smaller in size easily penetrate the cell wall and subsequently penetrate it, causing structural changes like perforation, which results in the leakages of intracellular components and also TiO_2@SiO_2-Ag nanocomposite release Ag^+ ion on reaching the interior and generate reactive oxygen species which interact with the membrane proteins affecting the electron transport chain. Some research has recommended probable mechanism for the antibacterial activity of TiO_2@SiO_2-Ag nanocomposite, which might be due to the electrostatic attraction between the negative charge on the cell membrane and weak positive charge of the TiO_2@SiO_2-Ag. The TiO_2@SiO_2-Ag may also serve as vehicle to delivery Ag^+ more effectively to bacteria whose proton motive force would decrease the local pH and enhance Ag^+ release. Additionally TiO_2@SiO_2-Ag nanocomposite interacts with a disulfide group of the intracellular enzymes that lead to inhibition metabolic processes with numerous metallic nanoparticles acting antimicrobial agents.

3.3 Bactericidal and Fungal Activity Assay Tio$_2$@Sio$_2$-Ag Nanocomposite

The bactericidal activity of TiO_2@SiO_2-Ag nanocomposite was performed against for *Staphylococcus aureus*, *Klebsiella, pneumonia*, *E. coli*, *Pseudomonas aeruginosa*, *A. Niger*, Trichophyton, Fusarium, and Mucor around 10^6 colony forming units of bacterial and fungal isolates. They were treated with various concentrations of TiO_2@SiO_2-Ag nanocomposite (25, 50, 75 and 100 μg/mL) and grown MHA plates are shown in Table 1.

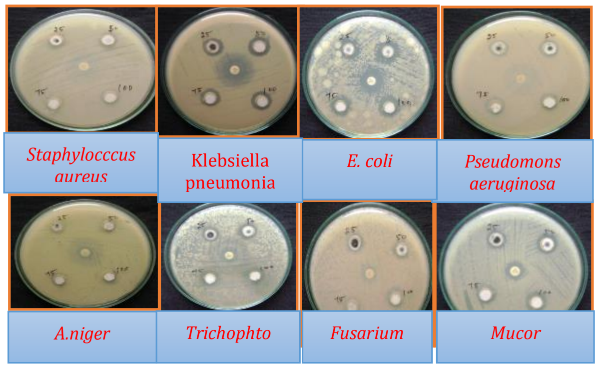

Fig. 2 Bactericidal and fungal activity assay by TiO_2@SiO_2-Ag nanocomposite

Table 1 Bactericidal and fungal activity assay of TiO_2@SiO_2-Ag nanocomposite

Organism	TiO_2@SiO_2-Ag added and Zone of inhibition (mm/mL)				
	25 μL	50 μL	75 μL	100 μL	Control
Staphylococcus aureus	12	13	14	16	20
Klebsiella pneumonia	14	18	16	18	26
E.coli	14	15	16	17	28
Pseudomonas aeruginosa	15	17	19	21	22
A.niger	11	12	13	14	22
Trichophyton	14	16	18	20	28
Fusarium	12	14	15	16	13
Mucor	12	14	15	16	15

After treatment, a higher reduction in the number of colonies were observed, respectively in MRSA and cons with 100 and 75 μg/mL of TiO_2@SiO_2-Ag nanocomposite. It was noted that there was a noteworthy decrease in the number of colonies when supplemented even with a low concentration of TiO_2@SiO_2-Ag nanocomposite. Our result supported by other researchers [16], the bacterial activity increased with increase concentration of TiO_2@SiO_2-Ag nanocomposite and higher concentrations were able to bring about complete inhibition of cells and TiO_2@SiO_2-Ag nanocomposite when subjected to bacterial cells and fungal cells were found to anchor the cells at several sites and make perforation in the membrane which could result analysis.

3.4 Anticancer Activity

The cytotoxicity of the TiO_2@SiO_2-Ag was studied in vitro against MCF-7, Hep-G2, and Caco-2 cancer cell lines at different concentration (0.5, 12.5, 25 and 50 mg/mL). The results obtained from MTT assay after 48 hours of incubation show that TiO_2@SiO_2-Ag significant effect on MCF-7, Hep-G2, and Caco-2. Particularly 50 mg/mL TiO_2@SiO_2-Ag was effective against Hep-G2 and Caco-2 cancer cell line, as it led to inhibition in cell growth which are represented in Table 2. The MCF-7, Hep-G2 and Caco-2 cell lines were the most sensitive cell lines towards the cytotoxicity activities of the tested TiO_2@SiO_2-Ag [17]. Brest adinocarcinoma (MCF-7), liver carcinoma Hep-G2) and intestinal adinocarcinoma (Caco-2) cell lines represented as cell viability percentage (±) standard deviation.

Table 2 Anticancer activity of TiO_2@SiO_2-Ag nanocomposite

Concentration mg/mL	0	5	12.5	25	50
MCF-7	100	80 ±0.22	70.9 ±0.85	56.6 ±0.31	44.0±0.26
Hep-G2	100	77.4±0.73	60.1±0.82	52.7 ±0.16	48.1 ±0.91
Caco-2	100	80.1±0.26	71.3 ±0.35	63.2 ±0.11	49.6 ±0.56

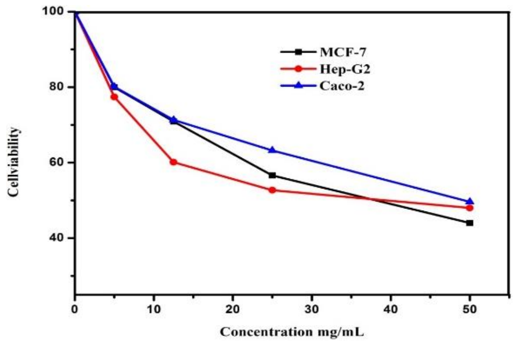

Fig. 3 Anticancer activity of TiO_2@SiO_2-Ag nanocomposite

4. Conclusion

Antibacterial activity increased with increase concentration of TiO_2@SiO_2-Ag nanocomposite and the higher concentrations were able to bring about complete inhibition of bacterial cells. The Hep-G2 and Caco-2 cell lines were the most sensitive cell lines towards the cytotoxicity activities of the tested TiO_2@SiO_2-Ag and the composite with MCF-7 was the most resistant cell line towards the cytotoxicity activities.

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