Antibacterial Activity and Anticancer Activity of Ag Doped TiO$_2$@SiO$_2$ Nanocomposite

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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 (MSNP) 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 spectroscopy (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$_2$ with Ag NPs.

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].

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$_4$OH, NaOH, HCl was received from Merck chemicals. Citytrimethyl ammonium bromide (CTAB), tetrathyl 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 characterized 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)

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 characterization show the prepared nanoparticle maintaining the surface
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 TiO₂@SiO₂-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 TiO₂@SiO₂-Ag nanocomposite (25, 50, 75, and 100 µg/mL). The growth kinetics were measured (OD) at 500 nm regular intervals of 3 hrs up to 24 hrs. It is noticeable from the graph that the number of bacterial cells decreased with increasing TiO₂@SiO₂-Ag nanocomposite concentration and with time of exposure. Furthermore, there is no growth inhibition in bacterial culture alone without TiO₂@SiO₂-Ag nanocomposite treatment and the cells survived till reach decline phase. From this investigation, it has been found that TiO₂@SiO₂-Ag nanocomposite being smaller in size easily penetrates the cell wall and subsequently penetrate it, causing structural changes like perforation, which results in the leakages of intracellular components and also TiO₂@SiO₂-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 TiO₂@SiO₂-Ag nanocomposite, which might be due to the electrostatic attraction between the negative charge on the cell membrane and weak positive charge of the TiO₂@SiO₂-Ag. The TiO₂@SiO₂-Ag may also serve as a vehicle to delivery Ag⁺ more effectively to bacteria whose proton motive force would decrease the local pH and enhance Ag⁺ release. Additionally, TiO₂@SiO₂-Ag nanocomposite interacts with a disulide 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 TiO₂@SiO₂-Ag Nanocomposite

The bactericidal activity of TiO₂@SiO₂-Ag nanocomposite was performed against Staphylococcus aureus, Klebsiella pneumonia, E. coli, Pseudomonas aeruginosa, A. Nigere, Trichophyton, Fusarium, and Mucor around 10⁶ colony forming units of bacterial and fungal isolates. They were treated with various concentrations of TiO₂@SiO₂-Ag nanocomposite (25, 50, 75 and 100 µg/mL) and grown MHA plates are shown in Table 1.

![Fig. 2 Bacterial and fungal activity assay by TiO₂@SiO₂-Ag nanocomposite](Image)

Table 1 Bactericidal and fungal activity assay of TiO₂@SiO₂-Ag nanocomposite

<table>
<thead>
<tr>
<th>Organism</th>
<th>TiO₂@SiO₂ Ag added (µg/mL)</th>
<th>Zone of inhibition (mm/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>25, 50, 75, 100 µg/mL</td>
<td>12, 13, 14, 16, 20</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>14, 18, 26</td>
<td></td>
</tr>
<tr>
<td>E.coli</td>
<td>14, 17, 19</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>11, 14, 15, 16</td>
<td></td>
</tr>
<tr>
<td>A.niger</td>
<td>14, 18, 20</td>
<td></td>
</tr>
<tr>
<td>Trichophyton</td>
<td>14, 15, 16</td>
<td></td>
</tr>
<tr>
<td>Fusarium</td>
<td>14, 15, 16</td>
<td></td>
</tr>
<tr>
<td>Mucor</td>
<td>14, 15, 16</td>
<td></td>
</tr>
</tbody>
</table>

After treatment, a higher reduction in the number of colonies were observed, respectively in MRSA and cons with 100 and 75 µg/mL of TiO₂@SiO₂-Ag nanocomposite. It was noted that there was a noteworthy decrease in the number of colonies when supplemented even with a low concentration of TiO₂@SiO₂-Ag nanocomposite. Our result supported by other researchers [16], the bacterial activity increased with increase concentration of TiO₂@SiO₂-Ag nanocomposite and higher concentrations were able to bring about complete inhibition of cells and TiO₂@SiO₂-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 TiO₂@SiO₂-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 TiO₂@SiO₂-Ag significant effect on MCF-7, Hep-G2, and Caco-2. Particularly 50 mg/mL TiO₂@SiO₂-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 TiO₂@SiO₂-Ag [17]. Brest adenocarcinoma (MCF-7), liver carcinoma (Hep-G2) and intestinal adenocarcinoma (Caco-2) cell lines represented as cellvibility percentage ± standard deviation.

![Fig. 3 Anticancer activity of TiO₂@SiO₂-Ag nanocomposite](Image)

Table 2 Anticancer activity of TiO₂@SiO₂-Ag nanocomposite

<table>
<thead>
<tr>
<th>Concentration mg/mL</th>
<th>MCF-7</th>
<th>Hep-G2</th>
<th>Caco-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>77.4 ±0.85</td>
<td>76.2 ±0.11</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>70.9 ±0.85</td>
<td>44.0 ±0.26</td>
</tr>
<tr>
<td>12.5</td>
<td>50</td>
<td>56.6 ±0.31</td>
<td>48.1 ±0.91</td>
</tr>
<tr>
<td>25</td>
<td>100</td>
<td>52.7 ±0.16</td>
<td>49.6 ±0.56</td>
</tr>
<tr>
<td>50</td>
<td>100</td>
<td>56.6 ±0.31</td>
<td>48.1 ±0.91</td>
</tr>
</tbody>
</table>

4. Conclusion

Antibacterial activity increased with increase concentration of TiO₂@SiO₂-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 TiO₂@SiO₂-Ag and the composite with MCF-7 was the most resistant cell line towards the cytotoxicity activities.

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