Green Synthesis and Anti-Cancer Activity of Multifunctional ZnO:Mn-Natural Biomolecule Quantum Dots System

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ABSTRACT

The present study investigates the anti-cancer activity of manganese doped zinc oxide (ZnO:Mn) quantum dots (QDs) and sucrose capped ZnO:Mn (ZnO:Mn/Sucrose) QDs synthesized by green synthesis method. The structural and optical properties of both the QDs were studied using X-ray diffraction (XRD), transmission electron microscopy (TEM) and Ultra-violet spectrometer (UV) techniques. In this study, a mixed cubic-hexagonal crystal structure and spherical shaped particles were found from the both QDs using XRD and TEM analysis. Finally, anti-cancer activities of both the QDs were tested on the human breast carcinoma cancer cells. The results of this study show a higher death rate of cancer cells caused by ZnO:Mn/Sucrose QDs when compared to ZnO:Mn QDs. The observed results suggests that the biomolecule sucrose capped ZnO:Mn QDs has the potential and can be suitable for cancer cell treatments. However, more detailed studies on using of various cancer cells to examine the effects of different types of ZnO QDs are needed.

Keywords: Zinc oxide, Green synthesis, Anti-cancer activity and Breast carcinoma cancer cells

1. INTRODUCTION

Recent researches on the semiconductor nanoparticles (NPs)/quantum dots (QDs) have become much important because of their greater potential in opto-electronic devices and biological applications [1-4]. More specifically, the NPs and QDs are making important contributions to the development of novel aims of drug delivery in cancer [5]. In this respect, many researchers suggested that the QDs are suitable for Bio-applications. The tunable optical properties of QDs are strongly depending on the inter-dot distance which in turn is due to the quantum size effect [6].

In general, zinc oxide (ZnO) is a well-known semiconductor with a wide bandgap (~3.37 eV) having unique optical and bio-compatible properties which make it a better candidate for bio-imaging than metal and chalcogenide nanoparticles [7]. Further,
the transition metal doped semiconducting nanocrystals plays an important role to providing the remarkable properties due to quantum confinement effect [8-10]. Mn doped ZnO (ZnO:Mn) nanostructures are well known promising material for many applications particularly opto-magnetic and bio-imaging applications [11-13]. Addition to this, the chemical capping method is now extensively used in the synthesis of NPs because the capping molecule controls the particles size as well as protection of the particles from coagulation [14]. Indeed, organic molecules capped inorganic nanostructure materials introduces a new type of photophysical and photochemical properties resulting from the combinatorial effects of organic/inorganic system [15-16]. In recent years, nanomedicine offers more targeted approach which promises significant improvements in various cancer treatments. In this case, much attention has been paid to the ZnO NPs for their implications in cancer treatment [17-19]. In this connection, the previous reports of Ostrovsy et al. [20] suggested that ZnO NPs may be employed as a selective cytotoxic agent for the eradication of cancer cells. Besides, Premanathan et al. [18] observed that ZnO NPs was exhibited a preferential ability to kill the leukemia cancerous cells. Sudhagar et al. [21] reported that the ZnO QDs can be used for cancer targeting and sensitive bioassays because of which is a non-toxic, biocompatible, and resistant to photobleaching. In addition, very recently, Fakhrouiean et al. [5] found that the surface modified ZnO QDs has a great potential and promising application as a breast and colon anti-cancer. In this regard, till date, there are no sufficient published data on the biomolecule capped ZnO:Mn QDs against cancer cells. Consequently, the present study is planned to prepare the ZnO:Mn QDs and the biomolecule, sucrose capped ZnO:Mn QDs by green synthesis method that method is considered as an environmental friendly one. The breast cancer in women is the most common type of cancer worldwide. Hence, we attempted to examine the potential effects of both the ZnO:Mn and ZnO:Mn/Sucrose QDs in breast carcinoma cancer cells. Further, the structural and optical properties both these prepared QDs are reported herein.

2. MATERIALS AND METHODS

2.1. Materials

All the chemicals were analytical grade reagent purchased from S-d fine chemicals and used without any further treatment, where double distilled water (DDW) as solvent. Zinc acetate Zn (CH$_3$COO)$_2$ is the starting material, manganese chloride (MnCl$_2$) is the metal dopant, sucrose is the capping agent and sodium hydroxide (NaOH) is the stabilizing agent.

2.2. Synthesis of QDs

For ZnO:Mn QDs, a Zn(CH$_3$COO)$_2$ solution was freshly prepared using DD water and kept under stirring which to a freshly prepared MnCl$_2$ was added. To this mixture, a freshly prepared NaOH solution was slowly added in drops till the pH reaches 10. Further, this solution was stirred vigorously and then a dark brownish color colloidal solution was formed which indicates the formation of ZnO:Mn crystals. Finally, sedimentation was obtained and it was washed to remove the unreacted compounds and then centrifuged to separate the particles from the solution and dried. Further, collected crystal sample was oxidized through annealing treatment and grinded well. For bio-molecule sucrose capped ZnO:Mn QDs (ZnO:Mn/Sucrose QDs), the same procedure was performed but the capping agent of sucrose was added in drops into the respective Zn(CH$_3$COO)$_2$+MnCl$_2$ mixture solution before changing the pH value. Further, both of the prepared samples were analyzed using various characterization techniques.

2.3. Cell proliferation assay

The cell proliferation assay was carried out using Cell Titer 96 aqueous one solution cell proliferation assay kit (Promega, USA). Briefly, upon reaching about 75-80% confluency, the human breast carcinoma (MDA-MB-231, obtained from American type culture collection) was plated in 96-well microplate in 100 µL media. After seeding for 72 hr, the cells were treated with 50 µM compound in triplicate. Doxorubicin (10 µM) was used as the positive control. At the end of the exposure period (72 hr), 20 µL CellTiter 96 aqueous solutions were added. The plate was returned to the incubator for 1 hr under a humidified atmosphere at 37°C. The absorbance of the formazan product was measured at 490 nm using a micro plate reader. The blank control was also recorded by measuring the absorbance at 490 nm with wells containing medium mixed with CellTiter 96 aqueous solution but no cells. Results were expressed as the percentage of the control (without compound set at 100%). The percentage of cell survival was calculated as OD value of cells treated with the test compound - OD value of culture medium / (OD value of control cell - OD value of culture medium) x 100%.

3. RESULTS AND DISCUSSION

3.1. Structural and Optical analysis
Figure 1 shows the XRD patterns of ZnO:Mn QDs (a) and ZnO:Mn/Sucrose QDs (b) and the crystal structure and approximate size of both the QDs. Both of the QDs showed a mixed cubic-hexagonal crystal structure due to the chance of superposition of crystal nature [22]. Debye-Scherrer’s formula was used to calculate the average particle size that was found to be approximately 19 nm for the ZnO:Mn QDs whereas 12 nm for the ZnO:Mn/Sucrose QDs. The surface morphology and accurate particle size were observed from the TEM images given in Figure 2 (a & b). The ZnO:Mn QDs (2a) shows spherical shaped particles with the size of ± 10 nm, ZnO:Mn/Sucrose QDs (2b) also showed spherical shaped particles in which an interesting cluster like surface morphology were observed. The reason for clustered morphology is the small size of spherical shaped ZnO:Mn particles were embedded or dispersed in the polymer like biomolecule (sucrose) matrix [23] and also it acts as a composite nature. The result of this study is an evidence for the interaction between QDs and biomolecules (sucrose).

The optical absorption behaviors of both the QDs are presented in the Figure 3 (a & b). The ZnO:Mn QDs shows a strong and broad absorption band which is maximum at 340 nm ($E_g$=3.65 eV), whereas the sucrose molecule capped QDs (ZnO:Mn/Sucrose QDs) has shown a strong enhanced absorption band at 347 nm (3.57 eV). The obtained bandgap energy value of Mn doped ZnO QDs is different when compared with our undoped QDs bandgap energy value (3.88 eV) [22] and it indicates the presence of Mn ions in the ZnO crystal lattice [24]. Further, the ZnO:Mn/Sucrose QDs bandgap energy value is red-shifted when compared with the ZnO:Mn QDs and this red-shift indicates the effect of sucrose molecule encapsulation around the ZnO:Mn QDs. Hence, these results shows an increased bandgap energy values when compared with bulk ZnO which can be explained by the Burstein-Moss effect [25]. Therefore, the red-shift is the evidence for the interaction between QDs and bio-molecule (Sucrose). Hence, these types of interaction and optical natures of QDs are suitable for bio-applications.

3.2. Anti-cancer activity

Figure 4 shows the cancer cell (MDA-MB-231) activity of Control (a), ZnO:Mn QDs (b) and ZnO:Mn/Sucrose QDs (c) against human breast carcinoma cancer cells. Their corresponding cell viability of survived cell rates was 100%, 98% and
81%, respectively. In this study, the ZnO:Mn QDs showed less cytotoxic effect on the breast carcinoma cancer cells. However, the reason for the role of Mn is not clear. Whereas, ZnO:Mn/Sucrose QDs caused higher death rate on cancer cells which may be due to the synergistic and adverse effects of Mn supplementation [26-27]. However, the results of Fakhroueian et al. [5] corroborate our findings on the higher death rate of cancer cells. In their study, they reported that the ZnO QDs can penetrate and diffuse into the cancer cells. In the present study, the similar mechanisms may be operated by the ZnO:Mn/Sucrose QDs. Typically, small sized particles play a significant role in the death of cancer cells. Indeed, different cytotoxic effects are relatively due to the formation of different size of QDs and also the generation of reactive oxygen species (ROS). Further, the results on cancer cell activity seem almost very similar to the results obtained in the anti-bacterial activity of ZnO:Mn QDs and ZnO:Mn/Sucrose QDs (not published), given for comparison purpose (shown in Figure 5). In our previous reports, we have discussed the reason for higher and lower killing effects of ZnO QDs [23].

**Figure 4.** Anti-cancer activity of Control (a), ZnO:Mn QDs (b) and ZnO:Mn/Sucrose QDs (c) against breast cancer cells (down-arrow indicates the increased cancer cell death rate from control).

**Figure 5.** Anti-bacterial activity of Control (a), ZnO:Mn QDs (b) and ZnO:Mn/Sucrose QDs (c) against *Staphylococi aureus* (up-arrows indicates the increased bacteria killing rate from control).

4. CONCLUSION

In the current study, ZnO:Mn QDs and ZnO:Mn/Sucrose QDs were successfully prepared using green synthesis method and their structural properties and optical behaviors were studied through XRD, TEM and UV techniques. For the bio-application purpose, anti-cancer activity of both the QDs was performed in the human breast carcinoma cells. Among these QDs, the ZnO:Mn/Sucrose QDs was produced an enhanced cytotoxic effects on the cancer cells (MDA-MB-231) when compared to the ZnO:Mn QDs. The results of the present investigation concluded that the ZnO:Mn/Sucrose QDs has the potential and can be suitable for cancer cell treatments. As an anti-cancer agent, the ZnO:Mn/Sucrose QDs can exhibit potential application in cancer therapy in the near future. Furthermore, the cancer cell activity of these QDs with various cancer cells need to be investigated in the future studies.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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