LETTER

Facile synthesis of multifunctional m-SiO$_2$@ZnO nanocomposite employing biocompatible polymers for potential theranostic applications

1 | INTRODUCTION

Mesoporous materials are a class of porous nanomaterials with pore size of 2 to 50 nm [1]. Specifically, mesoporous silica (m-SiO$_2$) is a popular research subject in biomedical applications due to its good biocompatibility, high drug-loading capacity, large pore density, size tunability, and ability for facile surface modification [2]. A variant of research on m-SiO$_2$ is its combinations with some functional nanoparticles (NPs), for example, iron oxide, ZnO, etc. ZnO/SiO$_2$ nanocomposites have been studied for different applications such as dye degradation [3], in oil recovery [4], in photocatalysis [5], and in optical and optoelectronic applications [6, 7]. Such structures have potential for theranostic applications as well. Unlike conventional imaging and therapy techniques, they are capable of simultaneously carrying therapeutic drugs along with diagnostic or imaging agents which can be helpful in magnetic resonance imaging (MRI) or photoluminescence imaging. They may also act as safe carriers for drugs which otherwise exhibit toxicity or instability if used on their own [8]. Moreover, their surfaces can be functionalized to achieve targeted drug delivery to tumours [9]. Efforts to successfully bring these materials to mainstream clinical medicine are still underway [8].

ZnO has a potential use in photoluminescence imaging due to its luminescence under UV rays [10]. ZnO is also reported to possess anti-cancer activity due to reactive oxygen species (ROS) generation [11]. So, combined potential of SiO$_2$@ZnO for photoluminescence and ultrasound imaging is an edge that may be used in the field of theranostics [12–14].

Various combinations of SiO$_2$ and ZnO have been explored for biomedical applications. Han et al. [15] prepared ZnO/silica core–shell composites and hollow silica particles and films, indicating their potential use in drug delivery. Generalov et al. studied the radiosensitizing effect of ZnO/silica nanocomposites [16]. They found that ZnO-silica core–shell NPs improve results of radiation therapy employed for certain cancer cell lines. Photoluminescence properties of ZnO/polymer have been explored in isolation by Pan et al. for in vivo imaging of mice [17]. ZnO@SiO$_2$ NPs show cytotoxicity as studied by Wiesmann et al. who advocated for further studies on the potential of ZnO NPs as anti-tumour agents [18].

It is evident from the literature that ZnO- and SiO$_2$-based nanocomposites have good properties for biomedical applications. However, an ‘all-in-one’ type of ZnO-SiO$_2$-based nanocomposites have not been reported which are synthesized through a facile, water-based synthesis. The present study aims to provide evidence for such a material where m-SiO$_2$@ZnO nanocomposites have been synthesized through a simple, water-based process. These nanocomposites are theranostic as m-SiO$_2$ allows drug loading whereas ZnO coating enables photoluminescence imaging. Polyethylene glycol (PEG) on ZnO NPs and chitosan on m-SiO$_2$ have been coated to enhance biocompatibility and provide two different varieties of surface functional groups.

2 | EXPERIMENTAL WORK

2.1 | Materials

Zinc sulphate heptahydrate (ZnSO$_4$·7H$_2$O) was purchased from Riedel. Sodium hydroxide (NaOH) and tetraethyl orthosilicate (TEOS) were procured from Merck. PEG (molecular mass of 4000) was obtained from BioChemica A1249. Cetyltrimethylammonium bromide (CTAB) was obtained from Avonchem. Chitosan (Molecular weight = 50,000–160,000) was purchased from Sigma-Aldrich. Ethanol (absolute 99.8%) was procured from AR - RCI Labscan. All the chemicals were of analytic grade and were used without any further purification.

2.2 | Synthesis of m-SiO$_2$ particles

m-SiO$_2$ particles were synthesized using CTAB via sol–gel method. In a typical experiment, 2.5 ml of TEOS, 65 ml of ethanol, and 137.5 ml of H$_2$O were magnetically stirred at room temperature and then ultrasonicated for 10 min. 0.4 g CTAB was then added in the resulting mixture along with water and
it was stirred for 10 min at the same temperature. Afterwards, 2.5 ml of 35% ammonia solution (35% NH₃ in water) was added in the reactants mixture consisting of TEOS, ethanol, H₂O, and CTAB, and the resultant mixture was magnetically stirred at 700 rpm for 3 h. The solution was centrifuged at 4500 rpm for 10 min. It was then washed and dried at 55°C. Finally, two step calcination (at 200°C for 6 h, then at 200°C for 6 h) was done to get m-SiO₂.

2.3 Synthesis of ZnO NPs

ZnO NPs were synthesized through a hydrothermal method reported in the literature [19] after some modification. 40 ml ZnSO₄·7H₂O (0.5 M) and 40 ml PEG (0.004 M) aqueous solution were magnetically stirred. NaOH was added to the mixture to adjust the pH value of the mixture to 12. The resulting mixture was then heated in an autoclave for 5 h at 150°C followed by oven cooling. The ZnO nanoparticles were obtained by centrifugation and repeatedly washed with water and ethanol. Finally, the PEG-coated ZnO NPs were dried at 80°C for 12 h.

2.4 Synthesis of chitosan-coated m-SiO₂@PEG-coated ZnO NPs

PEG-coated ZnO NPs were incorporated in the m-SiO₂ through physical absorption. First, 100 mg of SiO₂, 60 mg of chitosan and 40 ml of deionized H₂O were added in a beaker along with citric acid to adjust the pH value to 4. Stirring was done at room temperature for 24 h. PEG-coated ZnO NPs were then added in the resulting mixture along with 40 ml of deionized water. Finally, centrifugation and cleaning with water and ethanol was done, followed by drying at 50°C for 24 h. Schematic of the process is given in Figure 1.

2.5 Characterization

The crystal phases were identified using XRD (D8 Discover, Bruker), the morphology was measured using SEM along with EDX (Nova Nano SEM 430). FTIR was used to confirm the presence of polymers on particles surfaces. UV–vis spectroscopy was done by Spectro UV-VIS Double Beam UVD-3500 (Labomed, Inc.). PL Spectrophotometer was used for photoluminescence measurement. Finally, the biocompatibility measurement was done by MTT assay using RD cell lines. The cell viability was measured in triplets to evaluate error.

3 RESULTS AND DISCUSSION

After calcination at 600°C, m-SiO₂ particles were formed as indicated by the SEM image in Figure 2a. The size of the m-SiO₂ was found to be around 600 nm whereas the morphology can be seen to be spherical. The particles appear majorly monodisperse and some aggregation can be seen in the SEM image. The image is slightly blurred with uneven brightness at
some points due to charging effect during SEM. XRD of m-SiO$_2$ given in Figure 3d shows an amorphous band indicating the non-crystalline structure of the formed mesoporous silica. Elemental composition of m-SiO$_2$ was studied through EDS and their EDX spectrum is given in Figure 2b. Besides expected presence of Si and O which make up the m-SiO$_2$ particles, presence of C has also been indicated. This anomaly can be attributed to the use of carbon tape attached to the stub holding the SiO$_2$ powder sample. The mesoporosity can be indirectly inferred from the FTIR spectra of SiO$_2$ before and after calcination in Figure 2c. Characteristic FTIR bands for CTAB, which are visible in the FTIR spectrum for the uncalcined SiO$_2$, disappeared in the spectrum taken after calcination, indicating the removal of CTAB during the calcination process. As CTAB is removed, it leaves behind pores in the previously solid silica and results in the formation of m-SiO$_2$ [20, 21]. Figure 2d shows the standard FTIR spectrum for chitosan and the experimental FTIR spectrum obtained for m-SiO$_2$ after it was coated with chitosan. Overlapping bands of pure chitosan and of m-SiO$_2$ after the coating procedure indicate presence of chitosan on the m-SiO$_2$.

Figure 4a shows the SEM image of ZnO NPs prepared through hydrothermal method. The process yielded NPs with size ranging from 50 nm up to 500 nm. The product was majorly polydisperse and most particles had an aspect ratio near to 1 (Figure 4a,b). EDS results (Figure 4d) show the presence of Zn and O in the sample. XRD pattern of PEG-coated ZnO (Figure 4c) compared and matched well with the standard ZnO XRD pattern. The peaks were indexed as shown in the figure. The XRD pattern also shows that the prepared ZnO NPs are crystalline in nature. The crystallite size calculated through Scherrer’s equation was $\sim$13 nm. Peak broadening is observable in the XRD pattern of samples as compared to standard. In the UV–vis spectrum for ZnO NP shown in Figure 4e, two absorbance peaks were obtained: one at 258 nm and the other at 382 nm. The former is the exciton absorbing wavelength [22] whereas the latter is the characteristic absorbance peak of ZnO. The corresponding band gap to the characteristic peak was calculated to be 3.25 eV. After PEG coating on the surface of ZnO NPs, FTIR spectrum of the pure and coated samples were studied. Figure 4f contains the FTIR pattern of pure PEG and ZnO NPs after the PEG coating procedure. Presence of OH band and overlap of several peaks in the wavenumber range of about 2500-1700 cm$^{-1}$ strongly imply the presence of PEG on ZnO NPs. These bands have been reported with differing intensities in other studies regarding PEGylated ZnO NPs as well [23, 24].

Composite material based on ZnO NPs and m-SiO$_2$ was obtained as evidenced by Figure 3. Compositionally, the composites were found to contain Si, O, and Zn as shown in the
FIGURE 3 Synthesized nanocomposites based on chitosan-coated m-SiO$_2$@PEG-coated ZnO NPs. (a) EDX spectrum of ZnO@m-SiO$_2$ composite particles. (b) SEM image of ZnO@m-SiO$_2$ particles. (c) FTIR of polymers and ZnO@m-SiO$_2$ composite particles. (d) XRD patterns of silica, ZnO and ZnO@m-SiO$_2$ particles.

EDX spectrum of the samples (Figure 3a). SEM images of the m-SiO$_2$@ZnO NPs shown in Figure 3b indicate a sporadic coat on the substrate material (m-SiO$_2$) with ZnO NPs. The size difference between the two materials is clear in this image. The deposited ZnO NPs have a size range between 50 and 80 nm which is from the lower end of the size range in which the ZnO particles formed (Figure 4b). It appears that during mixing for composite formation, the smaller NPs were preferentially attached to the surface of m-SiO$_2$. Smaller particles may have acquired the bulk of PEG coating due to larger specific surface area. FTIR of PEGylated ZnO NPs (Figure 4d) shows that although many characteristic bands of PEG were found in it, some bands were missing or not as intense in the FTIR spectrum of PEG. This may mean that the quantity of PEG
was insufficient to provide a uniform coating on each particle. In this scenario, the available PEG chains could preferentially cover more of the smaller particles which later attached to SiO₂ surface. OH− end groups from PEG-coated ZnO nanoparticles may have caused chemical bonding to SiO₂ particles. The surface hydroxyl groups on SiO₂ particles can form a Si-O-C bond after elimination of a water molecule [25]. m-SiO₂ particles appear featureless in the SEM image which evidences their amorphous structure and supports the XRD result (Figure 3d). A comparative superimposition of FTIR spectra of pure PEG, pure chitosan, and the prepared m-SiO₂@ZnO NPs composite material is shown in Figure 3c. Presence of characteristic peaks of both chitosan and PEG in the prepared material indicate coating of these two polymers on silica and ZnO NPs. XRD pattern in Figure 3d shows the resultant XRD pattern of the prepared m-SiO₂@ZnO NPs composite material in comparison with XRD of crystalline ZnO and amorphous m-SiO₂. The sharp peaks at definite 2θ values come from the crystalline ZnO NPs whereas the hump below 2θ values of less than 30° is contributed by the amorphous SiO₂. A similar XRD pattern was reported by Chen et al. for ZnO@amorphous SiO₂ composite prepared through hydrothermal synthesis [26]. A comparative analysis reveals a mean crystallite size reduction of 1.38 nm for ZnO NPs after deposition as compared to as prepared ZnO NPs.

Photoluminescence spectrum of ZnO NPs is shown in Figure 5 (black) with two peaks. One sharp peak at 394 nm is due to the near-UV light violet emission from the ZnO excitons. Another broad peak centered at 564 nm has been attributed to oxygen defects in the ZnO [27]. The peak has shifted from the usual values of about 380 nm due to the PEG (polymer) coating on ZnO NPs [28]. When ZnO NPs were combined with m-SiO₂, the intensity of both peaks reduced (blue). This may be due to the slower recombination of excitons when ZnO is coated on m-SiO₂. Also, the peak for ZnO near-UV emission shifted towards 380 nm after being coated on the m-SiO₂, possibly due to the deposition of smaller ZnO nanoparticles on the final composite material. Blue-shift in PL spectra can be caused by smaller particle size due to band gap widening as evidenced by [29]. In present study, as the ZnO particles that deposited on the SiO₂ particles were of much smaller size, the blue-shift was observed in PL spectrum of the composite as compared to PL spectrum of only polydisperse ZnO NPs with larger particles as well.

Figure 6a,b shows the results for MTT assay on RDA cell lines. The prepared composite ZnO@m-SiO₂ material shows good biocompatibility with RD cancer cell lines (Figure 6b). All the tested conditions returned at least 80% viability. Figure 6a presents the biocompatibility of PEGylated ZnO NPs and chitosan-coated SiO₂ individually as well. SiO₂ particles
FIGURE 5  PL spectra of ZnO NPs and m-SiO$_2$@ZnO

FIGURE 6  MTT assay results of RD cells when incubated with: (a) ZnO nanoparticles and SiO$_2$ microparticles, (b) prepared nanocomposite under different conditions
exhibited 100% cell viability whereas ZnO NPs returned greater than 80% viability at 50 nM. This may also indicate the difference in weight percentage of PEG on ZnO NPs on their own and in the weight percentage of PEG on ZnO NPs that finally attach to the SiO2 particles. At 250 nM, cell viability of SiO2 increased slightly. Remarkable biocompatibility of ZnO NPs is facilitated by surface functionalization with PEG.

4 | CONCLUSION

A straightforward and water-based synthesis method has been used in this study to produce nanocomposites based on ZnO NPs of 50–80 nm diameter and submicron m-SiO2 of 600 nm diameter. Through photoluminescence spectroscopy, it was shown that ZnO shows its characteristic peak in the PL spectrum at 380 nm while present on the m-SiO2. Organic chitosan coating on m-SiO2 and PEG coating on ZnO NPs make the prepared nanoparticles biocompatible, but PEG concentration used in the method can be increased to impart even better biocompatibility. Sporadic, island-type coating of ZnO on silica makes it possible that two different types of functional groups (from chitosan and PEG) are available on the material surface. Such a multifunctional nanosystem with drug-loading capability, photoluminescence property, and biocompatible surface chemistry can be utilized in theranostic applications.

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REFERENCES

1. Sing, K.S.W.: Reporting physisorption data for gas/solid systems with special reference to the determination of surface area and porosity. Pure Appl. Chem. 54, (11), 2201–2218 (1982)
2. Meynen, V., Cool, P., Vanzant, E.F.: Verified syntheses of mesoporous materials. Microporous Mesoporous Mater. 125(3), 170–223 (2009)
3. Govindhan, P., Pragashishwaran, C.: Silver nanoparticle decorated on ZnO@SiO2 nanocomposite and application for photocatalytic dye degradation of methylene blue. Natl. Acad. Sci. Lett. 42, 323–326 (2019)
4. Ali, J.A.: Modification of LoSal water performance in reducing interfacial tension using green ZnO/SiO2 nanocomposite coated by xanthan. Appl. Nanosci. 9(3), 397–409 (2019)
5. Yang, H., et al.: Chemical precipitation synthesis and optical properties of ZnO/SiO2 nanocomposites. J. Am. Ceram. Soc. 91(5), 1591–1596 (2008)
6. Chakrabarti, S., Ganguli, D., Chaudhuri, S., Excitonic and defect related transitions in ZnO-SiO2 nanocomposites synthesized by sol-gel technique. Phys. Status Solidi Appl. Res. 201(9), 2134–2142 (2004)
7. Hagura, N., et al.: Enhanced photoluminescence of ZnO-SiO2 nanocomposite particles and the analyses of structure and composition. J. Lumin. 131(1), 138–146 (2011)
8. Dogra, P., et al.: Establishing the effects of mesoporous silica nanoparticle properties on in vivo disposition using imaging-based pharmacokinetics. Nat. Commun. 9, 4551 (2018)
9. Zhang, Y., et al.: A versatile theranostic nanoplatform based on mesoporous silica. Mater. Sci. Eng. C 98, 560–571 (2019)
10. Martinez-Carmona, M., Gun’Ko, Y., Vallet-Regí, M.: ZnO nanostructures for drug delivery and theranostic applications. Nanomaterials 8(4), 268 (2018)
11. Rasmussen, J.W., et al.: Zinc oxide nanoparticles for selective destruction of tumor cells and potential for drug delivery applications. Expert Opin. Drug Deliv. 7(9), 1063–1077 (2010)
12. Hu, H., et al.: Facile synthesis of amino-functionalized hollow silica microspheres and their potential application for ultrasonic imaging. J. Colloid Interface Sci. 358(2), 392–398 (2011)
13. Kumar, V.B., et al.: Synthesis of mesoporous SiO2–ZnO nanocapsules: Encapsulation of small biomolecules for drugs and “SiOZo-plex” for gene delivery. J. Nanoparticle Res. 15, 1904 (2013)
14. Raevskaya, A.E., et al.: Spectral and luminescent properties of ZnO-SiO2 core-shell nanoparticles with size-selected ZnO cores. RSC Adv. 4(108), 63393–63401 (2014)
15. Han, K., et al.: The sol-gel preparation of ZnO/silica core-shell composites and hollow silica structure. Mater. Lett. 61(2), 363–368 (2007)
16. Generalov, R., et al.: Radiosensitizing effect of zinc oxide and silica nanocomposites on cancer cells. Colloids Surf. B. 129, 79–86 (2015)
17. Pan, Z.Y., et al.: The application of ZnO luminescent nanoparticles in labeling mice. Contrast Media Mol. Imaging 6(4), 328–330 (2011)
18. Wiesmann, N., et al.: Zinc oxide nanoparticles as innovative anti-tumor agent. J. Trace Elem. Med. Biol. 51, 226–234 (2019)
19. Gupta, H., Paul, P., Kumar, N.: Synthesis and Characterization of DHA/ZnO/ZnFe2O4 nanostructures for biomedical imaging application. Procedia Mater. Sci. 5, 198–203 (2014)
20. Teng, Z., et al.: Preparation of hollow mesoporous silica spheres by a sol-gel/emulsion approach. Microporous Mesoporous Mater. 127(1–2), 67–72 (2010)
21. Puta, A.M., et al.: Pore ordering in mesoporous matrices induced by different directing agents. J. Porous Mater. 22, 321–331 (2015)
22. Talam, S., Karanunt, S.R., Gunnam, N.: Synthesis, Characterization, and spectroscopic properties of ZnO nanoparticles. ISRN Nanotechnol 2012, 1–6, (2012)
23. Liufu, S., Xiao, H., Li, Y.: Investigation of PEG adsorption on the surface of zinc oxide nanoparticles. Powder Technol. 145(1), 20–24 (2004)
24. Dehkoudi, B.R., Fatahian, S., Shahani, K.: Synthesis, characterization and renal toxicity of ZnO and polyethylene glycol coated ZnO nanoparticles. Nanomedicine J. 4(1), 55–60 (2017)
25. Wang, Y., Li, P., Kong, L.: Chitosan-modified PLGA nanoparticles with versatile surface for improved drug delivery. AAPS PharmSciTech 14, 585–592 (2013)
26. Chen, Y., Ding, H., Sun, S.: Preparation and characterization of ZnO nanoparticles supported on amorphous SiO$_2$. Nanomaterials 7(8), 217 (2017)

27. Vanheusden, K., et al.: Correlation between photoluminescence and oxygen vacancies in ZnO phosphors. Appl. Phys. Lett. 68(3), 403–405 (1995)

28. Guo, L., Yang, S., Highly monodisperse polymer-capped ZnO nanoparticles: Preparation and optical properties. Appl. Phys. Lett. 76(20), 2901–2903 (2000)

29. Gupta, P., Ramrakhiani, M.: Influence of the particle size on the optical properties of CdSe Nanoparticles. Open Nanosci. J. 3, 15–19 (2009)