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# Synthesis and anti-fungal effect of silver nanoparticles–chitosan composite particles

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**Abstract:** Silver nanoparticles have been used in various fields, and several synthesis processes have been developed. The stability and dispersion of the synthesized nanoparticles is vital. The present article describes a novel approach for one-step synthesis of silver nanoparticles–embedded chitosan particles. The proposed approach was applied to simultaneously obtain and stabilize silver nanoparticles in a chitosan polymer matrix in-situ. The diameter of the synthesized chitosan composite particles ranged from 1.7  $\mu$ m to 2.5  $\mu$ m, and the embedded silver nanoparticles were measured to be  $15 \pm 3.3$  nm. Further, the analyses of ultraviolet-visible spectroscopy, energy dispersive spectroscopy, and X-ray diffraction were employed to characterize the prepared composites. The results show that the silver nanoparticles were distributed over the surface and interior of the chitosan spheres. The fabricated spheres had macroporous property, and could be used for many applications such as fungicidal agents in the future.

**Keywords:** silver, nanoparticles, chitosan, anti-fungal

## Introduction

Silver nanoparticles exhibit many unique electronic, catalytic, optical, and other physical and chemical properties that their bulk counterparts do not have.<sup>1</sup> Silver nanoparticles have been applied in many fields including photonics, micro-electronics, photocatalysis, lithography, and surface-enhanced Raman spectroscopy.<sup>2,3</sup> Several types of chemical and physical technologies, such as chemical reduction,<sup>4–6</sup> electrochemical reduction,<sup>7–9</sup> photochemical reduction,<sup>10–12</sup> and heat evaporation<sup>13,14</sup> have been developed for the synthesis of silver nanoparticles. In recent years, bio- or green-synthesis of silver nanoparticles has become an emerging science. For example, using plant extracts for reducing silver nitrite to obtain nanoparticles with antimicrobial activities has emerged as a cost-effective and eco-friendly approach.<sup>15,16</sup>

The fabrication of silver nanoparticles has been widely studied, and their stability and dispersion are crucial. Stabilizers such as surfactants, micelles, ligands, and polymers have been widely utilized in the literature.<sup>17–20</sup> Recently, incorporating metal nanoparticles into polymers has been proposed. Immobilizing metal nanoparticles on matrix polymers called nanometal polymer hybrids efficiently prevents nanoparticles' aggregation.<sup>21</sup> In addition, the biocompatibility can thus be improved.<sup>22</sup> Polymers can act as matrix materials to help metal nanoparticles' growth control and stabilization.<sup>23</sup> Various polymers have been employed for the synthesis of silver nanoparticles–polymer composites.<sup>24</sup> In addition, polymers [eg, chitosan, gelatin,<sup>25–27</sup> liposome,<sup>28</sup> poly(lactic acid),<sup>29</sup> polymethylmethacrylate,<sup>30</sup> poly(vinyl alcohol),<sup>31</sup> polyvinylpyrrolidone,<sup>32,33</sup> etc] can be used as reducing or stabilizing agents for the synthesis of silver nanoparticles, without other reducing agents.<sup>34,35</sup> For example, Bankura et al proposed a simple one-step synthesis of silver nanoparticles exhibiting multiple antimicrobial activities with

size range of 10–60 nm by reduction of silver nitrate ( $\text{AgNO}_3$ ) using aqueous dextran solution as both reducing and capping agent.<sup>36</sup> Recently, several strategies for silver nanoparticles polymer hybrids were proposed. For example, El-Rafie et al developed a green synthesis process for silver nanoparticles' generation by using polysaccharides extracted from marine macroalgae.<sup>37</sup> Wang et al fabricated uniform polystyrene spheres coated with silver nanoparticles by simultaneous photo-induced polymerization and reduction.<sup>38</sup> Abdel-Mohsen et al synthesized core-shell structures of silver/chitosan-*O*-methoxy polyethylene glycol nanoparticles.<sup>39</sup>

Spherical solid supports for silver nanoparticles such as microspheres are crucial for several fungicidal applications. For example, silver nanoparticles-embedded methacrylic acid copolymer microspheres were found to be effective bactericidal agents for water sterilization, because they were quite stable and were not washed away by water.<sup>40</sup> In addition, some hybrid materials with embedded silver nanoparticles were used against *Candida* spp., *Escherichia coli*, *Pseudomonas* sp., *Aspergillus niger* or *Penicillium* sp., and others.<sup>40–44</sup> In order to apply the silver nanoparticles composite materials to industry, developing a facile and low cost method for the synthesis of silver nanoparticles polymer composite particles becomes vital.

In this study, we would like to present a facile approach to produce uniform silver nanoparticles–chitosan composite spheres, and test the fungicidal properties of the prepared hybrid spheres. The fabricated spheres would be characterized by UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and energy dispersive spectroscopy (EDS).

## Materials and methods

### Materials

Chitosan (molecular weight: 150,000, 1.5% w/v),  $\text{AgNO}_3$ , glucose, and sodium hydroxide (NaOH) were purchased from Sigma-Aldrich Co. (St Louis, MO, USA), and used as received without further purification.

### Synthesis of the chitosan spheres

Chitosan (0.2 g, dissolved in 10 mL of 1% v/v acetic acid [ $\text{CH}_3\text{COOH}$ ] solution) was prepared and dropped into a 20% 25 mL NaOH solution using syringes (3 mL, Terumo®) with 24 gauge needle (24G  $\times$  1", Terumo®) and a pump. Chitosan spheres were obtained after 15 minutes. Spheres were collected and washed twice with 30 mL of dd- $\text{H}_2\text{O}$  (double distilled water) to remove residual alkali.

### Synthesis of the Ag nanoparticles-chitosan composite spheres

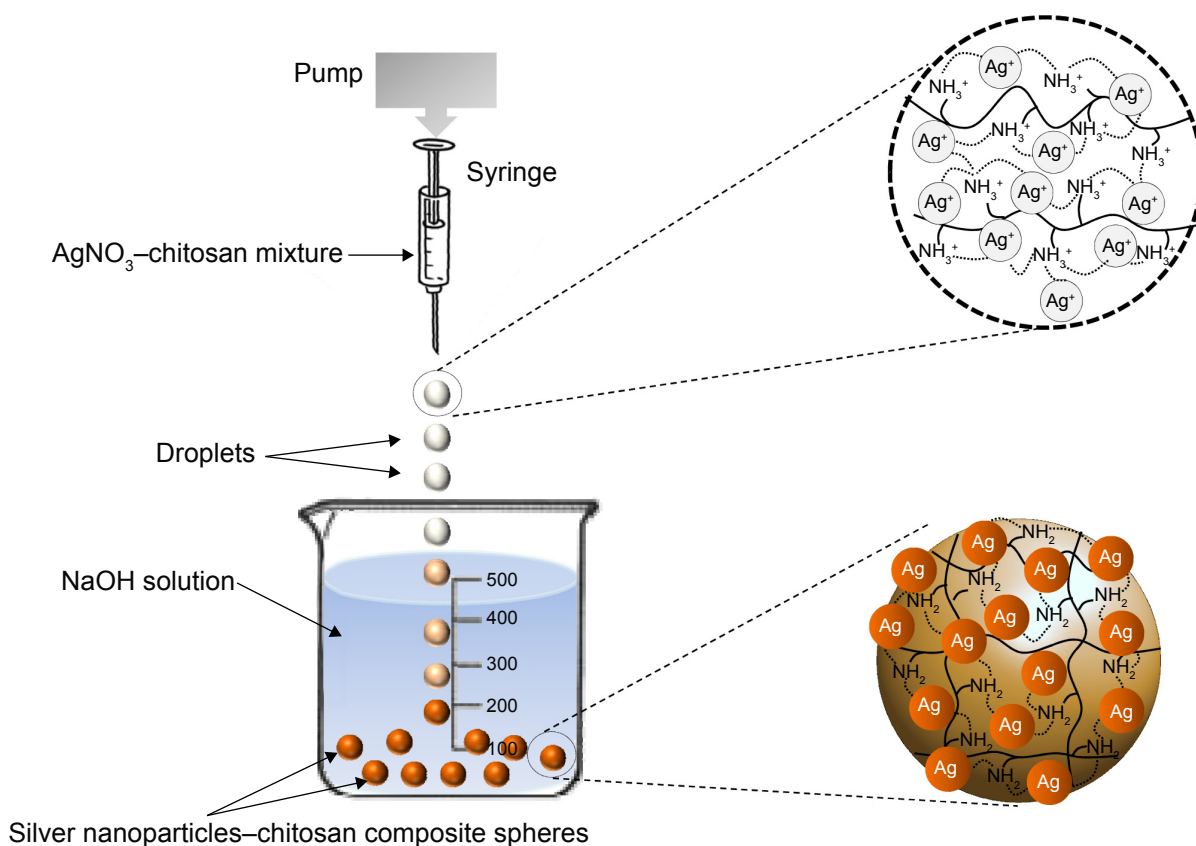
Based on our previous studies,<sup>41–44</sup> the one-step production of silver nanoparticles–chitosan composite spheres is shown in Figure 1. Chitosan (0.2 g, dissolved in 10 mL of 1% v/v  $\text{CH}_3\text{COOH}$  solution) and 2% 10 mL  $\text{AgNO}_3$  was mixed by constant stirring for 30 minutes, and then an  $\text{AgNO}_3$ –chitosan mixture solution was obtained. The  $\text{AgNO}_3$ –chitosan mixture solution was then dropped into a 20%, 30%, or 40%, 25 mL NaOH solution, respectively by using a syringe pump. After 15 minutes, yellow-brown color spheres (eg, silver nanoparticles–chitosan composite spheres) were yielded. Spheres were collected and washed twice with 30 mL of dd- $\text{H}_2\text{O}$  to remove residual alkali.

### Characterization

The average diameter of the spheres, expressed as mean  $\pm$  standard deviation, was obtained from the photographs taken by random sampling of approximately 50 individual particles to minimize selection bias. X-ray diffraction (XRD, D8 Advance, PANalytical X'PERT PRO) patterns were obtained at room temperature using Cu K- $\alpha$  radiation ( $\lambda=1.5406 \text{ \AA}$ ) with a range of  $2\theta=20^\circ$  to  $80^\circ$ , and a scanning rate of  $0.05 \text{ s}^{-1}$ . The FTIR spectra were recorded with a Spectrum RXI FTIR Spectrometer, using KBr pellets, in the range of 400 to  $4,000 \text{ cm}^{-1}$ , with a resolution of  $4 \text{ cm}^{-1}$ . The micromorphology of the silver nanoparticles–chitosan composite spheres was analyzed using a SEM (S-2700, Hitachi Ltd., Tolyo, Japan) equipped with an energy dispersive spectrometer. The characterization of the silver nanoparticles was carried out using TEM (FEI Tecnai G<sup>2</sup> 20 S-Twin) and a UV-Vis absorbance spectrophotometer (Thermo Scientific Spectrascan UV 2700). The silver nanoparticles solution was prepared by adding 20  $\mu\text{L}$   $\text{CH}_3\text{COOH}$  solution and 1 mL dd- $\text{H}_2\text{O}$  to ten silver nanoparticles–chitosan composite spheres, and then vortexing for 3–5 minutes. The silver nanoparticles–chitosan solution was dropped to grid by a micropipette for TEM analysis report.

### Anti-fungal effect test of *Cordyceps militaris* or *Antrodia cinnamomea*

To test anti-fungal effect, we used *C. militaris* (or Cm, Biore-source Collection and Research Center (BCRC) 32219) or *A. cinnamomea* (or Ac, BCRC 35716) in this study. We spread Cm or Ac on BCRC agar plate (malt Extract 2%, BD 218630; peptone 0.1%, BD 211677; glucose 2%, J.T.Baker 1916-05; agarose 1.5%) until these plates were fully covered by them. The silver nanoparticles–chitosan solution was prepared by adding 20  $\mu\text{L}$   $\text{CH}_3\text{COOH}$  solution and 1 mL dd- $\text{H}_2\text{O}$  to ten



**Figure 1** Schematic drawing of the silver nanoparticles–chitosan composite spheres synthesis.

**Notes:** Droplets were pushed out from pin tip of a pump-driven syringe and then dropped into an NaOH solution, which was used for silver nanoparticles' reduction and chitosan solidification. Silver nanoparticles–chitosan composite spheres were formed in 15 minutes. The distance between solidifying liquid surface and the tip was one centimeter. The diameter of the needle of the syringe was 8.73 mm.

**Abbreviations:** AgNO<sub>3</sub>, silver nitrate; NaOH, sodium hydroxide.

silver nanoparticles–chitosan composite spheres, and then vortex for 3–5 minutes. The chitosan solution was prepared by adding 20  $\mu$ L CH<sub>3</sub>COOH solution and 1 mL dd-H<sub>2</sub>O to ten chitosan spheres, and then vortexing for 3–5 minutes. Then we put a round filter paper (diameter 2 cm) with dissolved chitosan sphere or silver nanocomposite chitosan sphere in the middle of the plate and calculated the diameters of the inhibition zone on Day 0, Day 9, and Day 18, respectively. The analysis graph was drawn by GraphPad Prism and data were analyzed using the two-way analysis of variance (ANOVA). A *P*-value less than 0.05 was considered statistically significant.

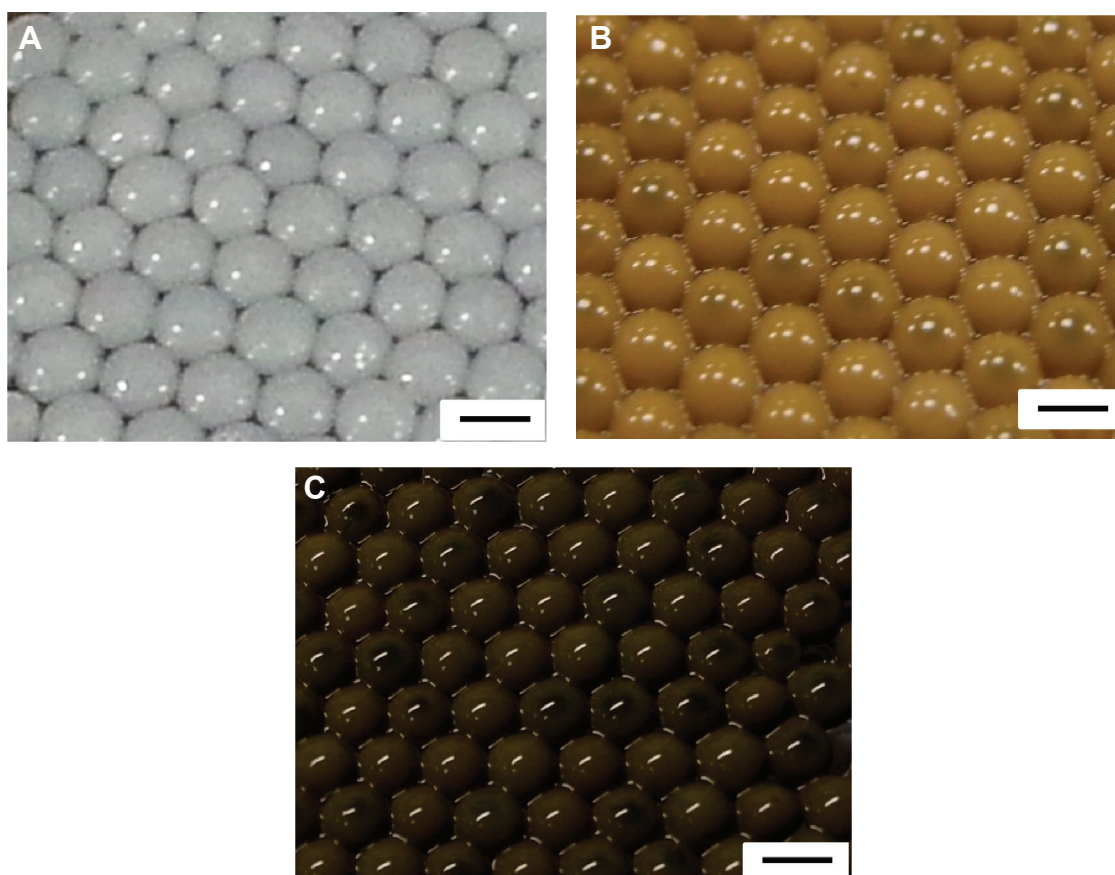
## Results and discussion

### Morphology of silver nanoparticles–chitosan composite spheres

Figure 2 shows that the silver nanoparticles–chitosan composite spheres are obtained by using various concentrations of AgNO<sub>3</sub>. The pure chitosan spheres showed milky-white (Figure 2A), whereas the fabricated spheres showed more gray when the concentration of AgNO<sub>3</sub> (Figure 2B) was

lower, and more khaki when the concentration of AgNO<sub>3</sub> was higher (Figure 2C). The color variation provided some clues to the formation of silver nanoparticles.<sup>45</sup> Highly coherent appearance of the prepared silver nanoparticles–chitosan composite spheres was obtained when various concentrations of AgNO<sub>3</sub> were used. However, the diameter of the silver nanoparticles seemed to decrease with an increasing concentration of AgNO<sub>3</sub> (Table 1). Most of the relative standard deviations of the sphere diameters observed at various concentrations of AgNO<sub>3</sub> were less than 10%, indicating that the manufactured spheres met the typical criterion for monodispersity.<sup>46</sup> Results shown in Figure 2 illustrate the composite spheres fabricated with low concentrations of AgNO<sub>3</sub> were slightly flattened in contact with the plate bottom, and thus they became slightly enlarged when measured by a top view. This phenomenon occurred because of fewer silver nanoparticles decreasing the mechanical strength of the spheres. In contrast, when the composite spheres were fabricated with high concentrations of AgNO<sub>3</sub> (there will be more silver nanoparticles formed), the composite spheres





**Figure 2** Photographs of the synthesized spheres.

**Notes:** (A) Chitosan spheres. (B) Silver nanoparticles–chitosan composite spheres synthesized with 1 mM  $\text{AgNO}_3$ . (C) Silver nanoparticles–chitosan composite spheres synthesized with 8 mM  $\text{AgNO}_3$ . The concentration of chitosan was 2%. All scale bars are 2 mm.

**Abbreviation:**  $\text{AgNO}_3$ , silver nitrate.

presented a superior mechanical strength, and thus exhibited properties such as easy spheroidization and aversion to deformation and fragmentation. The literature related to the fabrication of silver nanoparticles–chitosan composite films has mentioned that silver nanoparticles were uniformly dispersed and captured by the networks of amine ( $-\text{NH}_2$ ) and hydroxyl ( $-\text{OH}$ ) functional groups,<sup>47</sup> and thus the mechanical strength could be enhanced.<sup>48,49</sup> Those results were consistent

with our finding. We presume that the silver nanoparticles in silver nanoparticles–polymer composite play an identical role of cramp bar in architecture to reinforced concrete. Concrete (imagine as polymer) has relatively low tensile strength, while the presence of reinforcing bars (imagine as silver nanoparticles) shows higher tensile strength and/or ductility.

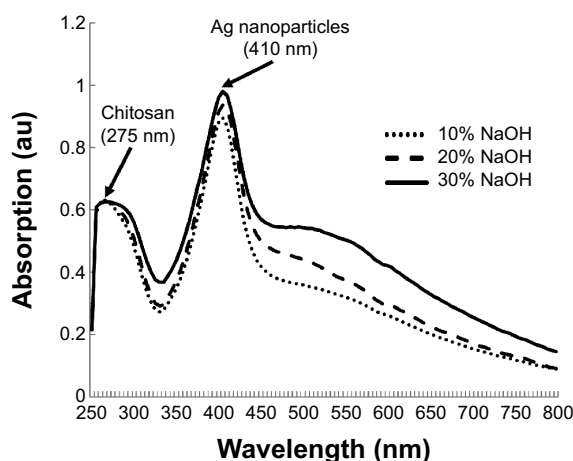
## Characterization of silver nanoparticles–chitosan composite spheres

Figure 3 shows the UV-Vis absorbance spectra of the synthesized silver nanoparticles–chitosan composite spheres at various concentrations of NaOH. Two significant peaks were observed: the peak at 275 nm is a characteristic peak of chitosan, whereas 410 nm is a characteristic peak of nano size silver particles.<sup>50</sup> Figure S1 shows the TEM photo of the synthesized silver nanoparticles. The particle size was measured to be  $15 \pm 3.3$  nm, which was consistent with the result of UV-Vis spectroscopy. The absorption peaks of the UV-V is spectra shifted to a longer wavelength when the particle

**Table 1** The relationship between average diameter of fabricated silver–chitosan composite spheres and the concentration of silver nitrate ( $\text{AgNO}_3$ )

$\text{AgNO}_3$ concentration (mM)	Sphere diameter (mm)	SD (mm)	RSD (%)
0.25	2.10	0.13	6.21
0.5	2.48	0.18	7.28
1	1.97	0.24	12.02
2	1.66	0.18	10.6
4	1.79	0.18	9.8
8	1.76	0.14	8.14

**Abbreviations:** SD, standard deviation; RSD, relative standard deviation.



**Figure 3** The UV-Vis absorbance spectra of the synthesized silver-chitosan composite spheres at various concentrations of  $\text{AgNO}_3$  solution.

**Abbreviations:**  $\text{AgNO}_3$ , silver nitrate; NaOH, sodium hydroxide; au, arbitrary unit; UV-Vis, ultraviolet-visible spectroscopy.

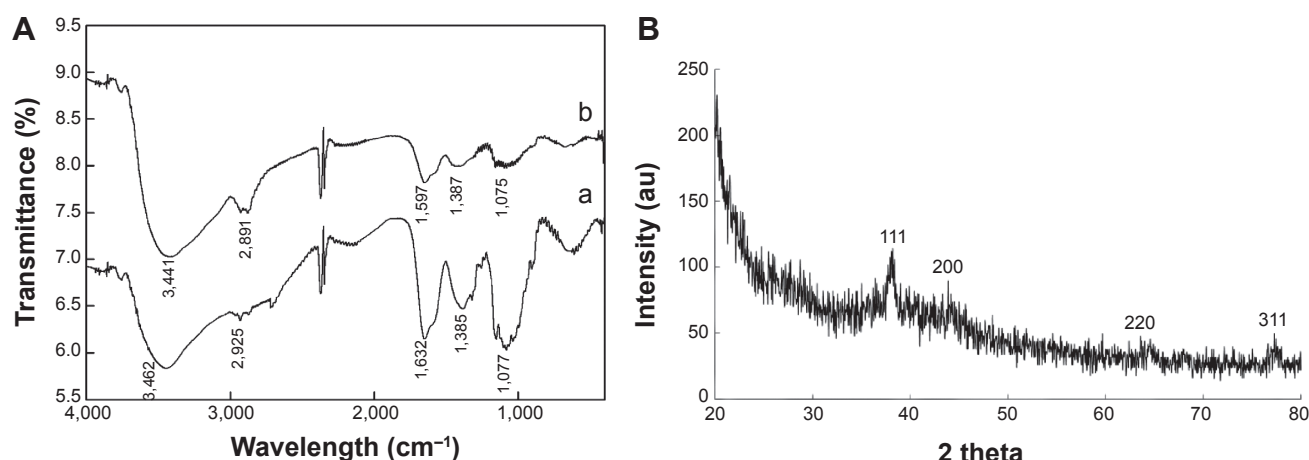
diameters were increased.<sup>50</sup> Nevertheless, the results shown in Figure 3 presume that there is nearly no change in the peaks ( $\sim 410$  nm) at the various concentrations of NaOH, indicating that the concentration of NaOH has no significant influence on the diameters of the synthesized silver particles.

Figure 4A shows the FTIR spectra of the silver nanoparticles-chitosan composite spheres. The bands between  $3,462\text{ cm}^{-1}$  and  $3,441\text{ cm}^{-1}$  were related to the stretching vibrations of amino groups from NH-amine. The bands between  $2,925\text{ cm}^{-1}$  and  $2,891\text{ cm}^{-1}$  corresponded to the alkane C-H-stretching lipids. The bands between  $1,632\text{ cm}^{-1}$  and  $1,597\text{ cm}^{-1}$  corresponded to the amino groups of amide. The bands between  $1,385\text{ cm}^{-1}$  and  $1,387\text{ cm}^{-1}$  were associated with the C=C stretching of aromatic amine groups. The bands between  $1,077\text{ cm}^{-1}$  and  $1,075\text{ cm}^{-1}$  were related to the

carbonyl stretch in proteins. The silver nanoparticles were bonded by protein, which served as a stabilizing agent, either through free amine groups or cysteine residues.<sup>51,52</sup> These proteins were present as enzymes that could reduce  $\text{AgNO}_3$  ions to form silver nanoparticles.<sup>52</sup>

Figure 4B shows the XRD patterns of the silver nanoparticles-chitosan composite spheres. Four characteristic peaks of silver nanoparticles corresponding to (111), (200), (220) and (311) are consistent with those found in the Joint Committee on Powder Diffraction Standards (JCPDS) database (PDF No. 65-3107). The results show that the synthesized nanoparticles were silver nanoparticles, because the position and relative intensity of all the diffraction peaks of the samples were consistent with the crystalline pattern of silver.<sup>53</sup> Furthermore, the chitosan being used for the synthesis process did not cause the formation of silver oxides. The shape of each peak was broadened as a result of the presence of the chitosan polymer.<sup>54</sup> Line broadening in the pattern was quantitatively evaluated by using the Debye-Scherrer equation  $d = (k\lambda/\beta \cos \theta)$ , which showed the relationship between peak broadening in the XRD and particle size.<sup>55</sup>

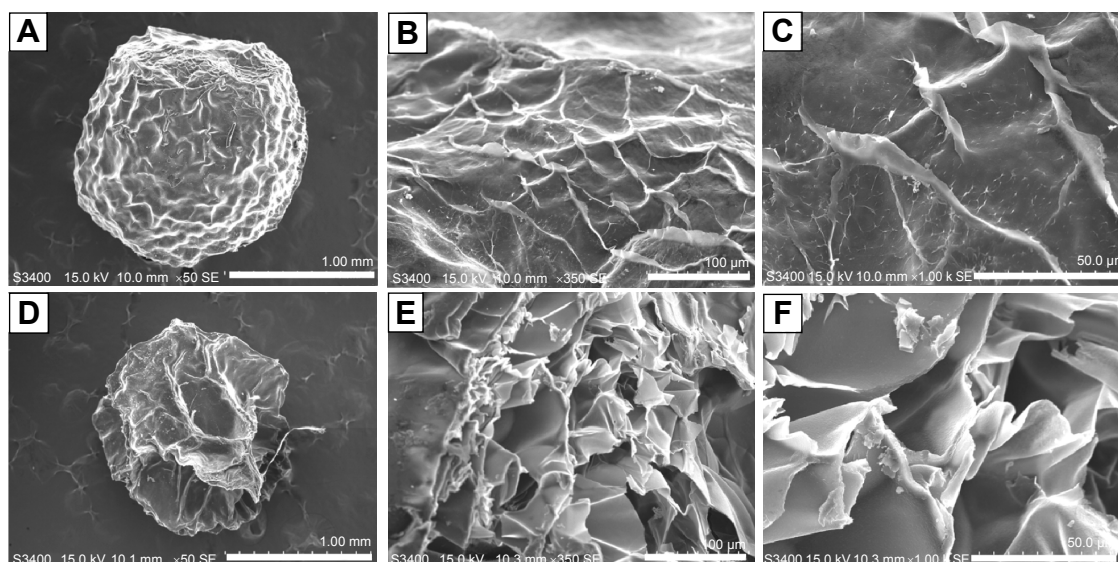
Figure 5 shows the SEM photographs of the fabricated silver nanoparticles-chitosan composite spheres (using 1 mM  $\text{AgNO}_3$  and 20% NaOH). Figure 5A–C shows the SEM graphs of the surface and the “zoom-in” of their intact sphere counterparts. Figure 5D–F represent the SEM graphs of the morphology, and the “zoom-in” of their sectional sphere counterparts. The results show that the spheres possessed many irregular macro pores, particularly in their interior. Figure S2 shows the morphology of the chitosan spheres, which have relatively smooth structures compared with the silver nanoparticles-chitosan composite spheres. Vimala et al



**Figure 4** Characterization of silver nanoparticles-chitosan composite spheres by FTIR and XRD.

**Notes:** (A) The FTIR spectrum, a represents Ag@chitosan, b represents chitosan and (B) the XRD graphs of the fabricated silver nanoparticles-chitosan composite spheres.

**Abbreviations:** FTIR, Fourier transform infrared spectroscopy; XRD, X-ray diffraction; au, arbitrary unit.



**Figure 5** The SEM photographs of the synthesized silver nanoparticles–chitosan composite spheres.

**Notes:** Panel (A) is the SEM photograph of a whole sphere. Panels (B) and (C) are the “zoom-in” counterparts of (A). Panel (D) is the SEM photograph of a sectioned sphere. Panels (E) and (F) are the “zoom-in” counterparts of (D). The scale bars are 1 mm (A and D), 100  $\mu$ m (B and E), and 50  $\mu$ m (C and F), respectively.

**Abbreviation:** SEM, scanning electron microscopy.

synthesized porous chitosan–silver nanocomposite films that had interdisciplinary applications in wound dressing, antibacterial agents, and water purification.<sup>49</sup> In our study, the silver nanoparticles–chitosan composite spheres also possessed porous structures, and could be used in multiple fields.

Figure S3 shows the EDS of the silver nanoparticles deposited on the outer surface relative to Figure 5A, B, C (Figure S3A), as well as the EDS of the inside of the synthesized silver nanoparticles–chitosan composite spheres relative to Figure 5D, E, F (Figure S3B). The intensity of the silver signal of the outer surface was higher than that inside the spheres, indicating that more silver nanoparticles were embedded in the interior of the spheres than deposited on the outer surface. The SEM-EDS mapping images presented in Figures S3C and D show the silver nanoparticles deposited on the outer surface and interior of the synthesized silver nanoparticles–chitosan composite spheres, respectively. The green dots represent the elemental map of the silver nanoparticles, which were considerably less on the outer surface than inside the spheres. The results of Figure S3C and D were consistent with those in Figure S3A and B.

### Anti-fungal effect test of silver nanoparticles–chitosan composite spheres

Figure 6 shows the anti-fungal effect. We found that Cm is inhibited by dissolved chitosan and silver nanoparticles–chitosan composite spheres (Ag@chitosan) on Day 9 (the

inhibition ring diameter of chitosan is  $3.2 \pm 0.1$  cm and Ag@chitosan is  $3.533 \pm 0.217$  cm) and Day 18 (the inhibition ring diameter of chitosan is  $2.7 \pm 0.2$  cm and Ag@chitosan is  $3.2 \pm 0.058$  cm) but not Ac. This finding indicates that Ag@chitosan and chitosan can both inhibit the growth of Cm but the activity of Ag@chitosan is better than chitosan (on Day 9,  $P < 0.05$ ; on Day 18,  $P < 0.01$ ). It is interesting to note that no inhibition ring is formed in Ac group, indicating that Ac is not affected by Ag@chitosan and chitosan. It is worth mentioning that on Day 9 we found not only chitosan but also Ag@chitosan show the best anti-fungal effects. We speculate that the reduction of anti-fungal effect might be caused by the degradation of chitosan and Ag@chitosan. As widely known, both chitosan and Ag@chitosan can inhibit many kinds of microorganisms. In this study, we found that Ag@chitosan or chitosan can inhibit the growth of Cm but not Ac. Taken all together, we thought chitosan and Ag@chitosan can act as a potent reagent to enhance Ac production by inhibiting other microorganism growth.

Bacterial infections still remain a major issue within health care industry and medical research. Therefore, it is urgently needed to develop novel antibiotics with high biocompatibility and without limitations of drug-resistance. Among diverse investigated antimicrobial agents, silver is one of the most promising because of its intrinsic properties of high thermal stability, low toxicity to mammalian cells and tissues,<sup>56,57</sup> versatile and long-term activity.<sup>58</sup> Synthesis of silver nanoparticles or silver-based antimicrobial



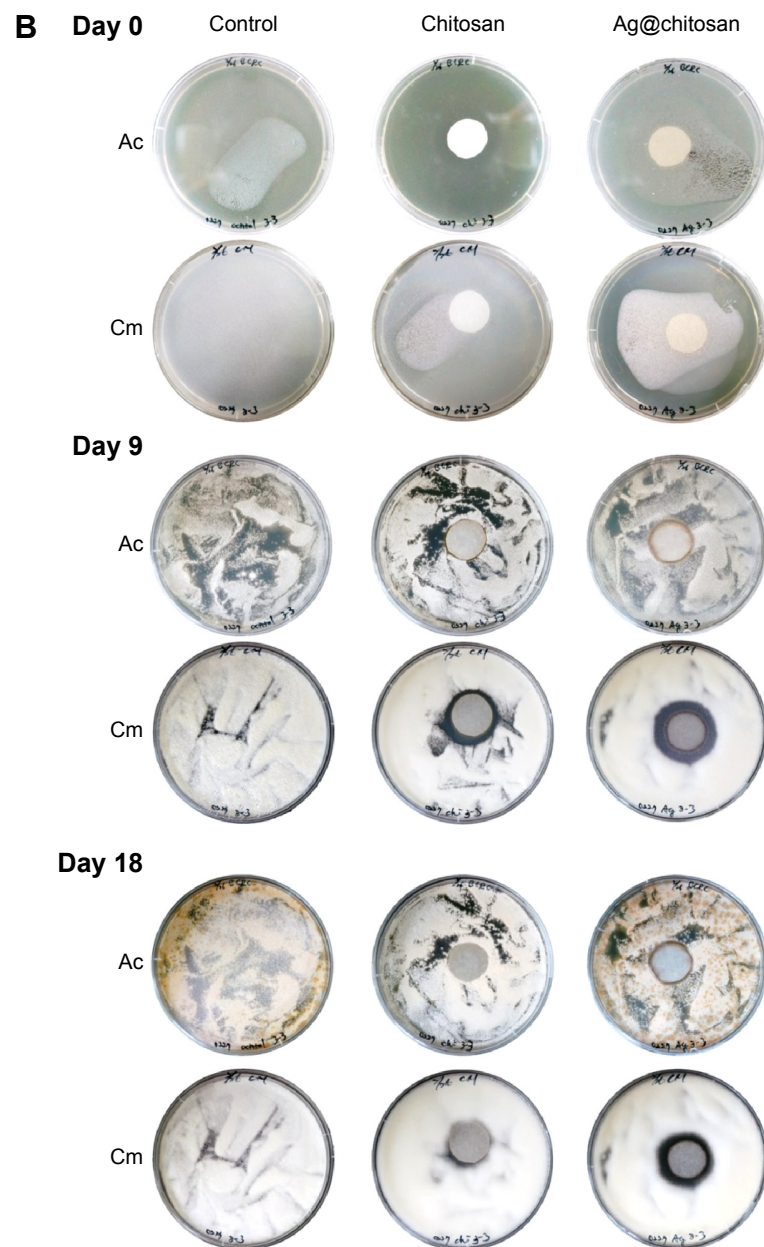
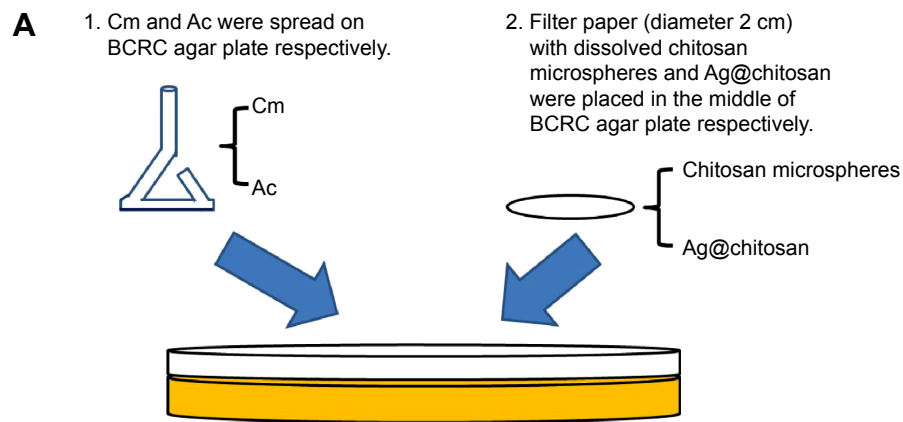
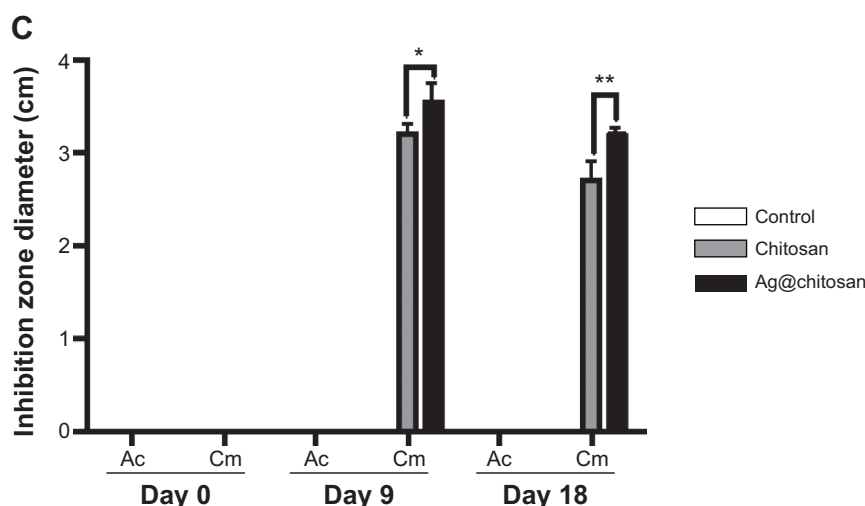


Figure 6 (Continued)





**Figure 6** Ag@chitosan anti-fungal effect.

**Notes:** (A) Flow chart of silver nanoparticles' anti-fungal effect. (B) and (C) the anti-fungal effect of chitosan microspheres and Ag@chitosan. \* $P < 0.05$ , \*\* $P < 0.01$ .

**Abbreviations:** Ag@chitosan, silver nanoparticles–chitosan composite spheres; Cm, *C. militaris*; Ac, *A. cinnamomea*; BCRC, Bioresource Collection and Research Center.

nanocomposite was well established by miscellaneous methods, for example employed  $\text{AgNO}_3$  as starting material to obtain silver nanoparticles by chemical reduction with some natural reductant (such as chitosan, dextran, sodium citrate, ascorbate and other reducing biomolecules like polypeptides, flavones, and alkaloids).<sup>59–61</sup> A number of literatures have reported that silver nanoparticles incorporated with chitosan-based matrix present a broad spectrum of antimicrobial activity to inhibit both Gram-positive and Gram-negative bacteria including multidrug-resistant pathogens such as *P. aeruginosa*, ampicillin-resistant *E. coli*, erythromycin-resistant *Streptococcus pyogenes*, methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *S. aureus* (VRSA).<sup>62–69</sup> However, the application of silver nanocomposite and chitosan as antifungal effect in Cm and Ac is rare. This study shows a selective inhibition by chitosan and silver nanoparticles–chitosan composite, suggesting that the cellular wall composition or modification of Cm and Ac is distinct. With the unveiling of antifungal ability of chitosan and silver nanoparticles–chitosan composite, it could offer an alternative to conventional antibiotics and a great opportunity to develop next-generation antibiotics.

## Conclusion

We proposed a facile approach for manufacturing silver nanoparticles–chitosan composite spheres in a one-step process. The diameters of the fabricated spheres ranged from 1.5 to 2.5  $\mu\text{m}$ , but their particle size could be reduced by using droplet microfluidic technology. The successful

formation of silver nanoparticles was evaluated using FTIR, XRD, and EDS mapping. The TEM image shows that the diameter of the fabricated silver nanoparticles was  $15 \pm 3.3$  nm. The UV-Vis spectroscopy shows a characteristic peak at approximately 410 nm, confirming the size of silver particle is within the nanoscale range. The peak wavelength does not substantially change even when various concentrations of NaOH are used. Compared with other approaches, the main advantages of this approach were: i) uniform-sized spheres could be continuously fabricated in a one step process, ii) silver nanoparticles could be obtained and stabilized simultaneously in a chitosan matrix, and iii) spherical shapes could have a wider range of applications compared with film structures. Because of the properties of silver nanoparticles, the synthesized spheres could become potential bactericidal agents for water disinfection, anti-pathogens, or surface plasma resonance enhancers. In addition, in this study we also found that chitosan and Ag@chitosan can inhibit the growth of Cm but not Ac, suggesting that chitosan and Ag@chitosan may be a potential reagent to enhance Ac production by preventing other microorganism growth.

## Acknowledgments

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## Disclosure

The authors have no conflicts of interest to disclose.

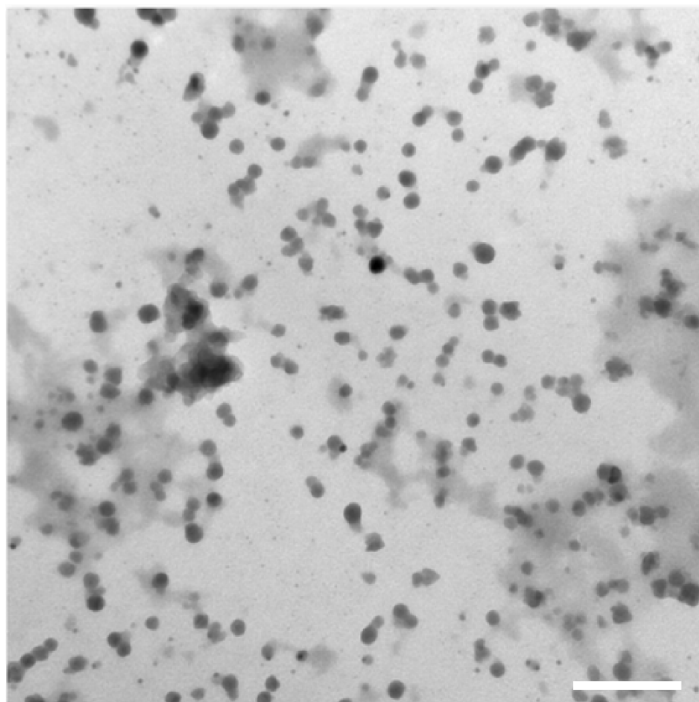
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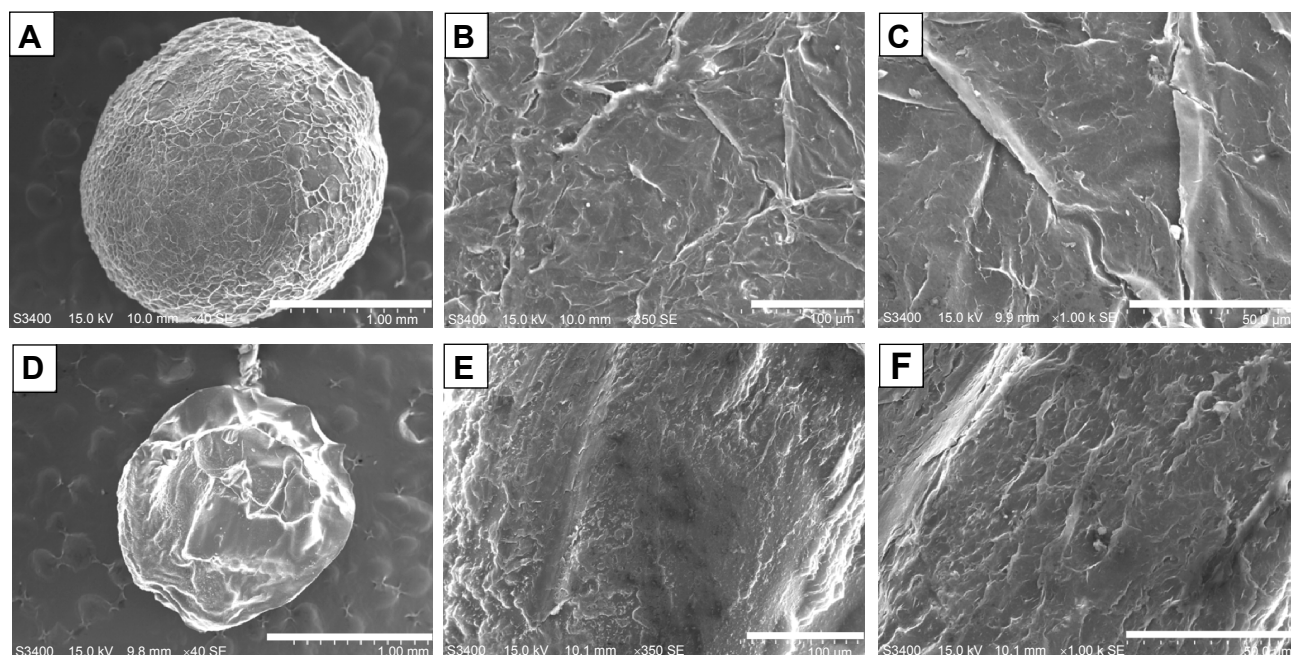


## Supplementary materials



**Figure S1** The transmission electron microscopy photograph of the synthesized silver nanoparticles.

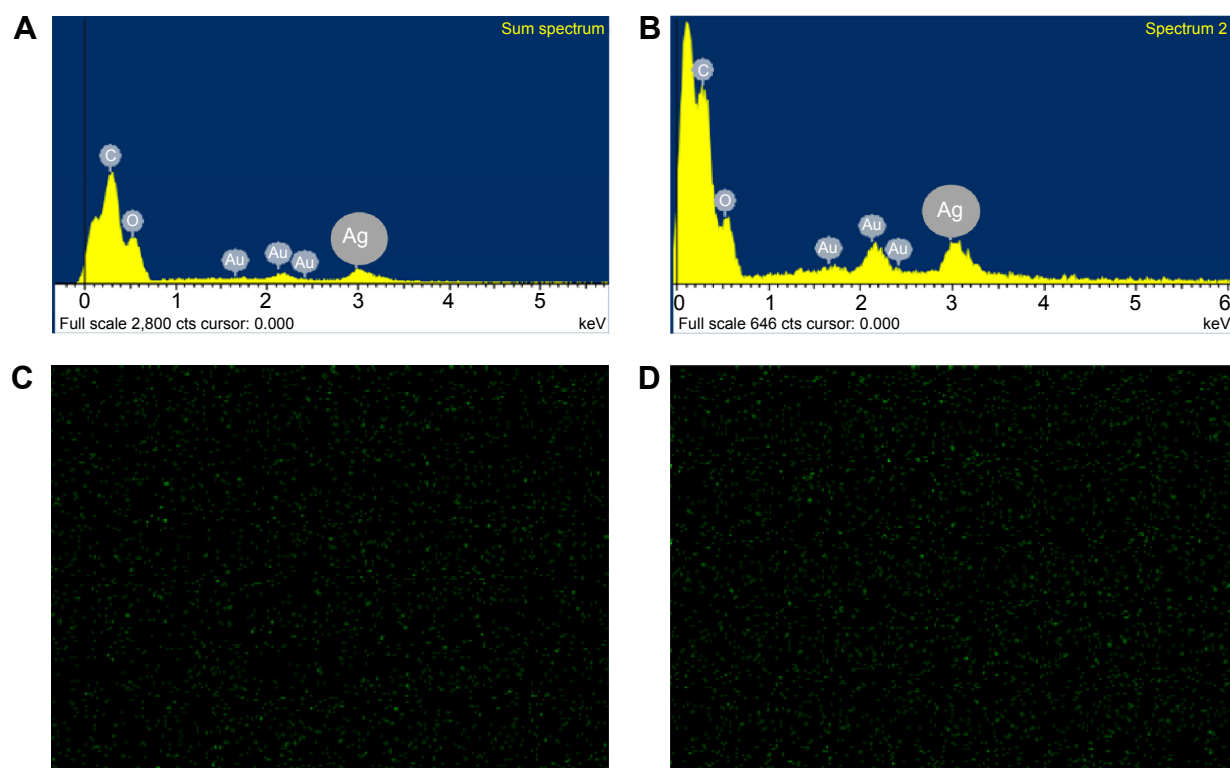
**Notes:** The silver nanoparticles–chitosan composite sphere was granulated, uniformly dispersed in water. The scale bar is 100 nm.



**Figure S2** The transmission electron microscopy (SEM) photographs of the synthesized chitosan spheres.

**Notes:** Panel (A) is an SEM photograph of a whole chitosan sphere. Panels (B) and (C) are the “zoom-in” counterparts of (A). Panel (D) is an SEM photograph of a sectioned chitosan sphere. Panels (E) and (F) are the “zoom-in” counterparts of (D). The scale bars are 1 mm for (A) and (D), 100  $\mu$ m for (B) and (E), and 50  $\mu$ m for (C) and (F), respectively.





**Figure S3** The SEM-EDS mappings of silver nanoparticles deposited on the outer surface (**A, C**) and inside (**B, D**) of the synthesized silver nanoparticles–chitosan composite sphere.

**Abbreviation:** SEM-EDS, scanning electron microscopy–energy dispersive spectroscopy.

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