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SHORT COMMUNICATIONS

Antimicrobial Activity of Biosynthesized Silver Nanoparticles Decorated Silica Nanoparticles

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Abstract The production of cheap and effective compound for medicinal application is a major challenge for scientific community. So, several biological materials have been explored for the possible application in material synthesis which are useful in biomedical uses. Here, biomolecules from green tea leaves were functionalized on the surface of silicon dioxide nanoparticles ($GSiO_2$ NPs). Next, the decoration silver (Ag) NPs on the surface of the $GSiO_2$ NPs was observed in very short time of incubation in aqueous $AgNO_3$. Ultraviolet–visible spectroscopy confirmed the formation of Ag NPs and the high-resolution transmission and scanning electron microscopies confirmed the decoration of spherical Ag NPs of 10 to 15 nm size on the surface of $GSiO_2$ NPs. The antimicrobial activity of the Ag–$GSiO_2$ NPs was determined against *Staphylococcus aureus* and *Escherichia coli*. The Ag–$GSiO_2$ NPs displayed sustainable antimicrobial activity compared to Ag ions. The results indicate the potential value of Ag–$GSiO_2$ NPs in surgical material and food processing.

Keywords Biosynthesis · Silver nanoparticles · Green tea · Biomolecules · Antimicrobial

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The various forms of the nanoparticles have been demonstrated for the different applications as the physical, chemical and biological properties of the nanoparticles varies with their shapes, size and surface properties [1–3]. Among the various nanomaterials, noble metals and noble metal-based nanomaterials have been not only synthesized and studied also extensively applied in various industrial and healthcare fields like medicinal, catalyst, energy, diagnostic, electronics, and other fields [4]. The platinum (Pt), palladium (Pd), silver (Ag) NPs, and gold (Au) NPs based nanomaterials have gained most interest due to their optical, physical and electrical properties over the other metal nanoparticles [5]. Among the various supporting materials, silicon dioxide (SiO_2) NPs have the most favored inorganic material, which is inert, thermally stable, carry negative change in neutral pH, and less interference in magnetic and optical fields [6–9]. Synthesis of Ag NPs to decorate the surface of SiO_2 NPs has been explored in the application of coating on antimicrobial materials. Several reported methods for formation of Ag–SiO_2 NPs have involved the use of hazardous chemicals like sodium borohydride [10], expensive instruments like microwave devices [11] and sonicators [12], and time-consuming microbial methods [13]. Thus, to date, the approaches to synthesize Ag–SiO_2 NPs have proven to be expensive and potentially hazardous. With the goal of devising a rapid, safe, and cost-effective method for Ag–$GSiO_2$ NP synthesis, active reducing molecules were used for the functionalization of SiO_2 NPs in this study. These molecules were then used for the reduction of Ag^+. The biosynthesized silver–silica composite was tested against *Staphylococcus aureus* and *Escherichia coli* to demonstrate the its effective antimicrobial activity. The Ag@$GSiO_2$ nanostructure formation was achieved as demonstrated in the schematics using biofunctionalized SiO_2 NPs (Fig. S1).

Briefly, the SiO_2 NPs were synthesized and functionalized with amine groups as previously described [10]. The alkaline ethanolic extract was prepared from the commercial green tea leaves as previously reported [14]. The amine-functionalized SiO_2 NPs were dispersed in the extract and incubated for 12 h. The alcoholic extract of green tea was enriched in polyphenols and displayed antioxidant activity [15]. During incubation, these active biomolecules of green tea covalently attached to the amine-functionalized SiO_2 NPs to form functionalized SiO_2 ($GSiO_2$) NPs. Deligiannakis *et al.* demonstrated the efficient functionalization of aminopropyl–SiO_2 NPs with gallic acid, which retained their antioxidant capacity [16]. The epicatechin and polyphenols present in green tea can reduce Ag^+ for the synthesis of Ag NPs [17]. The $GSiO_2$ NPs were dispersed in aqueous $AgNO_3$. After a 120-min reaction, the color change from greyish-white to brown was observed indicating Ag NPs synthesis on $GSiO_2$ NPs surface. The biomolecules present on the surface of the SiO_2 NPs reduced Ag^+ to Ag NPs and resulted in the formation of Ag–$GSiO_2$ NPs.

The synthesis of Ag NPs from aqueous $AgNO_3$ using $GSiO_2$ NPs was confirmed by the absorbance in the visible light region with UV–visible spectroscopy. In the UV–visible spectra for $GSiO_2$ and Ag–$GSiO_2$ NPs, $GSiO_2$ NPs did not show any absorbance in 300–700 nm range (Fig. 1a). In contrast, at 425 nm the Ag–$GSiO_2$ NPs was having absorbance of Ag NPs, which confirmed formation Ag NPs on the $GSiO_2$ NPs surface [18]. The XRD analysis of the Ag–$GSiO_2$ NPs demonstrated in Fig. S2 revealing its crystalline nature and phase conformation of the Ag NPs. The face centric cubic (FCC) crystal structure was obtained from the diffractogram with (111), (002), (022), and (113) planes of metallic silver corresponding to peaks at 2θ angles of 37.8°, 43.9°, 63.9°, and 76.8°, respectively (JCPDS No. 96-901-3048) [19]. The Ag–$GSiO_2$ NPs morphology was analyzed by the field emission scanning electron microscopy (FE-SEM). The rough surface of the spherical $GSiO_2$ NPs confirmed that the surface of $GSiO_2$ NPs was covered by Ag NPs (Fig. 1b). The elemental compositional analysis was determined using Energy-dispersive X-ray spectroscopy (EDS) to confirm the presence of elemental Ag at approximately 3 keV (Fig. 1c) [20]. The detailed morphology and size of the NPs were confirmed by high resolution transmission electron microscopy (Hr-TEM) (Fig. 2a). The $GSiO_2$ NPs were spherical in shape and agglomerated due to presence of the biomolecules. After reduction reaction, the Ag NPs became dispersed on the surface of the $GSiO_2$ NPs (Fig. 2b). The spherical Ag NPs varied in size ranging from 5 to 40 nm (Fig. 2c). The antimicrobial activity of the Ag–$GSiO_2$ NPs is due to the Ag NPs present on the surface. The antimicrobial activity of Ag–$GSiO_2$ NPs was confirmed using *S. aureus* and *E. coli*. Different concentrations of Ag–$GSiO_2$

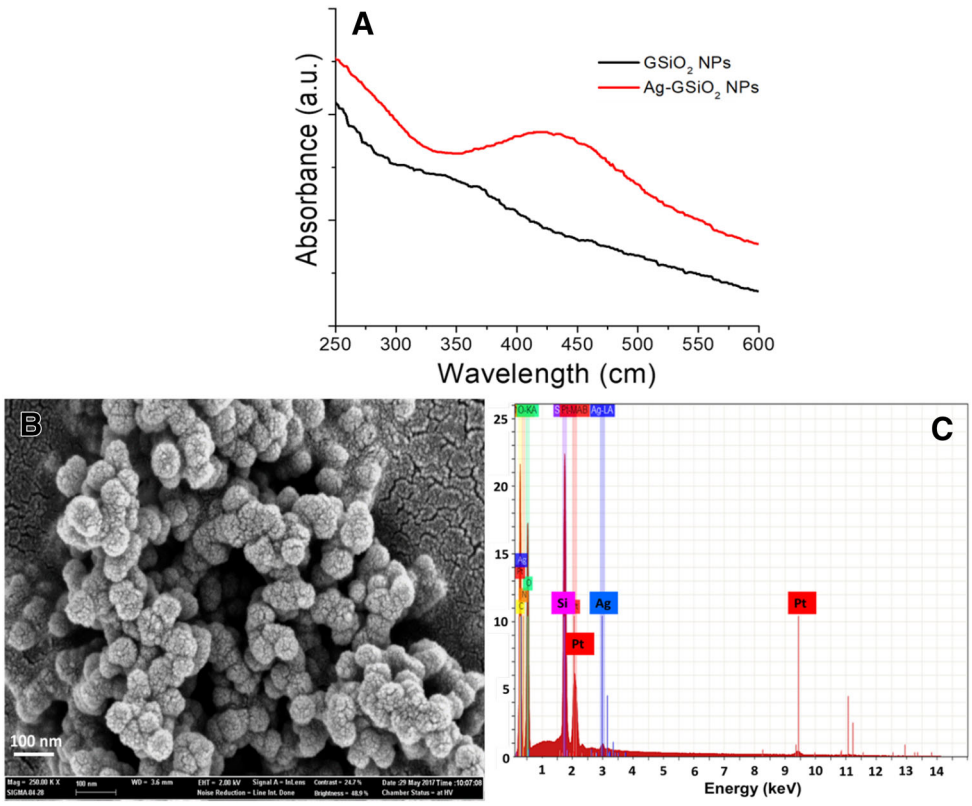

Fig. 1 Characterization of Ag–$GSiO_2$ nanoparticles **a** UV–visible spectra of $GSiO_2$ nanoparticles and Ag–$GSiO_2$ nanoparticles showing the characteristic absorbance at 425 nm, **b** X-ray diffraction pattern of the Ag–$GSiO_2$ nanoparticles, **c** field emission scanning electron microscopy to study the morphology of the Ag–$GSiO_2$ nanoparticles, **d** energy-dispersive X-ray spectra of the Ag–$GSiO_2$ nanoparticles

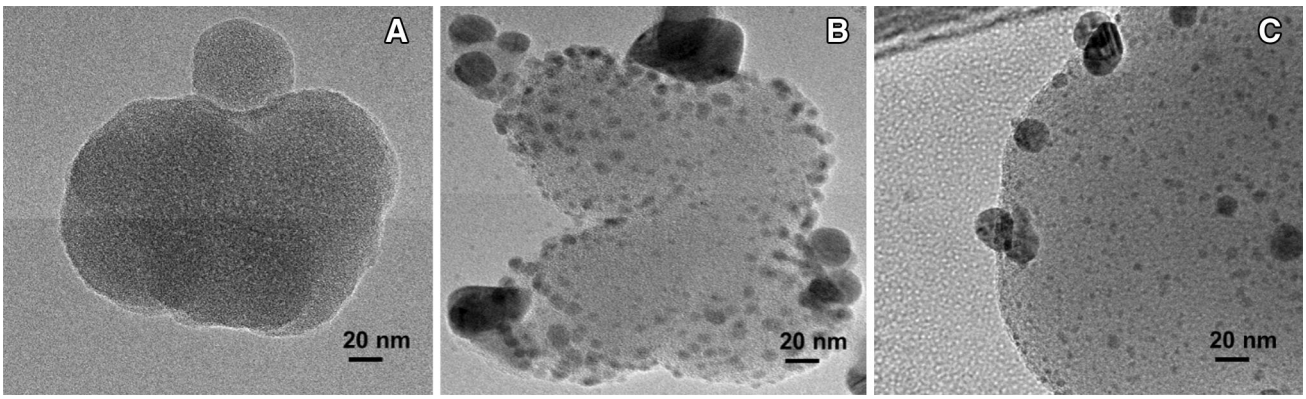

Fig. 2 Morphology and shape of the nanoparticles analyzed with high resolution transmission electron microscopy **a** $GSiO_2$ nanoparticles, **b**, **c** Ag–$GSiO_2$ nanoparticles

(0–30 mg L^{-1}) were added in 100 mL of LB broth containing 10^6–10^7 CFU mL^{-1} of *S. aureus* or *E. coli,* and these mixtures were incubated for 24 h at 37 °C. Serial dilutions were prepared, and 0.1 mL aliquots were dispensed in LB broth and incubated for 24 h. The colonies were enumerated and the numbers of viable bacteria calculated. Figure 3 displays representative colonies of *S. aureus* (Fig. 3a) and *E. coli* (Fig. 3f) developed without treatment of Ag–$GSiO_2$ NPs as control plates. As the concentration of the Ag–$GSiO_2$ NPs increased up to 30 mg L^{-1}, growth of both bacteria was adversely affected (Fig. 3b–d, g–i). At 30 mg mL^{-1}, Ag–$GSiO_2$ NPs completely inhibited the growth of *S. aureus* (Fig. 3e) with slight growth of *E. coli* evident (Fig. 3j). The LB broth medium was used to determine the minimum inhibitory concentration (MIC). The MIC of Ag–$GSiO_2$ NPs was 0.3 mg mL^{-1} for *S. aureus* and 0.35 mg mL^{-1} for *E. coli*. The results clearly demonstrated the antimicrobial efficiency of Ag–$GSiO_2$ NPs inhibiting gram-negative and gram-positive bacterial growth.

In summary, we developed a novel green tea leaf-based method which is rapid and easy for the synthesis silver–silica composite material. The active biomolecules present in the green tea extract functionalized SiO_2 NPs retained their capacity to reduce Ag^+, leading to formation of Ag NPs that decorated $GSiO_2$ NPs. Hr-TEM confirmed the synthesis of Ag NPs on $GSiO_2$ NPs surface. The Ag–$GSiO_2$ NPs displayed pronounced antimicrobial activity against *S. aureus* and *E. coli* after a short exposure time of 120 min. The green chemistry method allowed the rapid formation of nanohybrid structures, potentially useful in biomedical applications. However, further studies, including

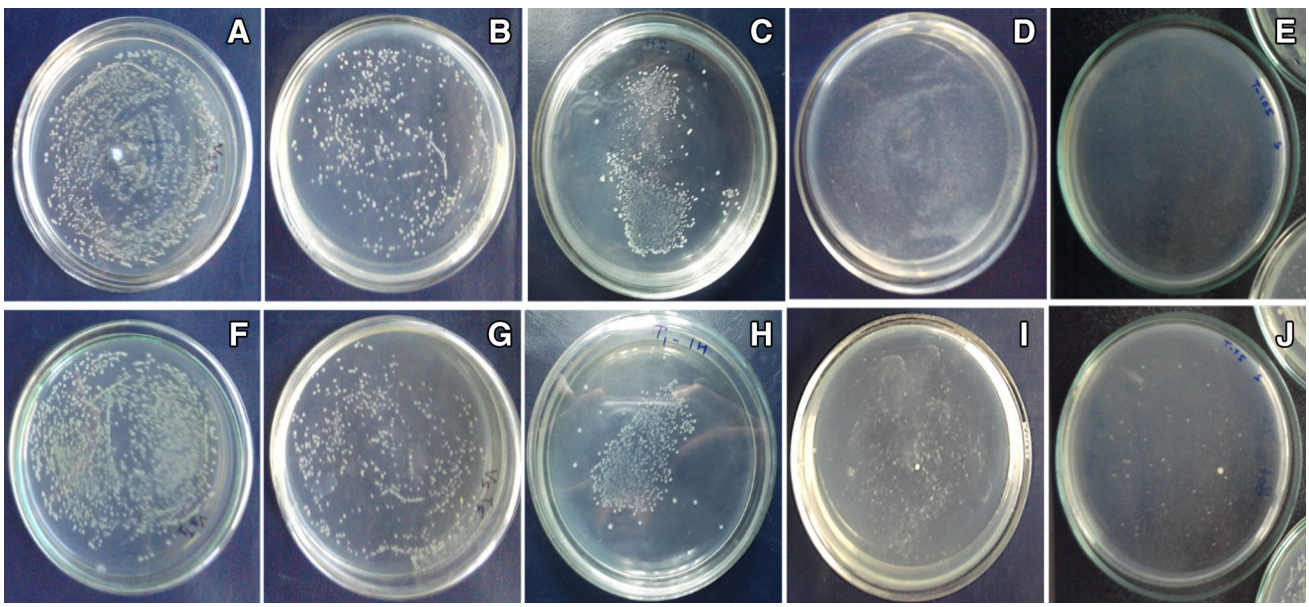

Fig. 3 Antimicrobial activity of Ag–$GSiO_2$ nanoparticles against *Staphylococcus aureus* (**a**–**e**) and *Escherichia coli* (**f**–**j**) exposed to concentrations of control (**a**, **d**), 15 mg L^{-1} (**b**, **g**), 20 mg L^{-1} (**c**, **h**), 25 mg L^{-1} (**d**, **i**) and 30 mg L^{-1} (**e**, **j**)

toxicological studies, are necessary to confirm the practical application of these Ag–GSiO$_2$ NPs.

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