Development of bio fabricated gold nanoparticles for disinfectant applications in impregnated medical gloves to improve the personal protection during the nursing care

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Abstract
The present study involves the use of Gnidia glauca (G. glauca) leaf extract in the fabrication of gold nanoparticles (Au NPs), dip-coated onto the surface of gloves and their antimicrobial properties were studied for use in nursing care. The prepared Au NPs were studied by various spectroscopic and microscopic techniques. Au NPs were Purple in color with a characteristic plasmon resonance peak on the surface at a wavelength of 532 nm. The images of transmission electron microscopy (TEM) showed the Au NPs with size ranging from 22–35 nm. The crystalline size and nature of Au NPs was demonstrated by x-ray diffraction (XRD) and Energy dispersive x-ray spectroscopy (EDS) analysis. Further, antimicrobial results showed that the AuNPs-coated gloves demonstrated good antibacterial effect, notably against multidrug-resistant bacteria. Hence, the highly effective AuNPs-coated gloves may be ideal candidates for preventing or minimising indirect microbial transmission and cross-contamination in nursing care.

Introduction
The constant threat to hospitals and other medical organizations is a big worry because of Infectious diseases triggered by a number of adaptive bacteria are common. Gram-negative bacteria that are resistant to antibiotics, as well as multidrug-resistant infections like vancomycin-resistant enterococci and methicillin-resistant S. aureus are reasons for the healthcare-associated infections. The interaction of gloves with objects after touch with a pathogenic origin has been identified as a risk factor to healthcare related infections. On the other hand, the WHO gloving and Hand hygiene recommendations have been established by the organisation, which is a key focus of many healthcare and infection-prevention programmes [1].

Pathogens can spread to the external area, and excess usage of gloves can contaminate objects that are in direct interaction with other patients. Gloves are frequently left on between patients and health care staff. On the other hand, the misuse of gloves is common among patients or between interactions with several areas on an individual patient, are frequently found in all medical facilities worldwide, allowing the spread of disease to be facilitated.

To reduce cross-contamination and disease spread via gloves onto surroundings, effort has been directed on antibacterial glove developments. Antimicrobial-coated gloves have been shown to be successful in suppressing the transmission of pathogens. Gloves have been coated with chlorhexidine and vivid green dye [2]. Similarly, gloves have been treated on their external surface with substance such as polyhexamethylene biguanide hydrochloride [3]. Fluorinated silica nanoparticles were used to enhance the preventative effectiveness of protective gloves towards microbial infection [4]. Although antibacterial gloves did suppress microbial
contamination, there are indeed a few difficulties which need to be addressed such as inefficacy towards antibiotic-resistant microorganisms and long-term antibacterial ineffectiveness.

On the other hand, Plasmonic photo-thermal treatment is a non-invasive, drug-free treatment utilizing the properties of plasmonic metal nanoparticles that may transform radiation energy into heat. For instance, when gold nanorods were subjected to NIR radiation, a considerable decrease in bacterial cell viability was discovered using the photothermal-driven method [5]. Using gold nanorods, it was feasible to specifically target and eliminate a harmful Gram-negative bacterium, _Pseudomonas aeruginosa_ [6] and _E. coli_ [7]. Gold nanorods have been exhibited comparable bactericidal and antibiofilm activity against oral bacteria using plasmonic photothermal treatment achieved at 67 °C [8]. Similarly, Santos _et al_ have shown a rapid photothermal bacterial inactivation by irradiating bacterial cell wall using NIR light utilising nano gold discs [9].

Owing to its simplicity for quick production upon reduction process of metal salt, the environmentally benign route utilising natural plant extract for the biosynthetic pathway of AuNPs has raised interest in past few years over traditional approaches of AuNP production [10]. Its usefulness is demonstrated by its economic feasibility and environmental friendliness. Furthermore, compared to chemically manufactured NPs, such green synthesised NPs are harmless for clinical research [11]. Literature have already established the use of pharmaceutical plant extract with antioxidant potential for AuNPs production [12]. Similarly, significant therapeutic potential fruits solid wastes such as banana peels are used to make AuNPs [13]. On the other hand, several biomolecules such as silk sericin [14], casein [15], tyrosine [16] and plant extracts [17] have been used for the synthesis of various nanomaterials.

Various biomolecules such as polyphenols, proteins, flavonoids, polysaccharides, alkaloids, cellulose, and secondary metabolites are employed in the fabrication of NPs [18]. These may comprise the bioreduction of metal ions to corresponding NPs and function as stabilising agents [19]. Plant extracts comprise proteins with functionalized amino groups (–NH2) that may engage effectively in the AuNP formation process [20]. Functional groups (–C–O–, –C–O–C–, –C=O– and –C=C–) contained in phytochemicals such as polyphenols, flavones, anthracenes and alkaloids contribute to the formation of AuNPs. Different phytochemicals operate as reducing and capping agents for the extracellilular production of AuNP, substituting the hazardous compounds such as NaBH₄ [21] and hence the prepared NPs are highly suitable for biological applications.

The present study involves the use of _Gnidia glauca_ ( _G. glauca_ ) leaf extract in the fabrication of gold nanoparticles (AuNPs) and dip-coated onto the surface of gloves and their antimicrobial properties were studied for use in nursing care. The prepared Au NPs were studied by various spectroscopic and microscopic techniques.

**Materials and methods**

**Materials**

Chloro auric acid (HAuCl₄), Potassium bromide (KBr) and other chemicals were purchased from sigma aldrich, Shanghai. Deionised water was used for all experiments in this study.

**Preparation of gold nanoparticles (Au NPs)**

_ _G. glauca_ extract was prepared as described earlier. Milli-Q water of 50ml volume was used to dissolve freeze dried (lyophilized) aqueous _G. glauca_ extract weighing 50mg and passed through Whatman filter paper (number 41) for filtration. 0.0001 M HAuCl₄ (Sigma Aldrich) was added to _G. glauca_ extract of 50 ml by continuous stirring at 60 °C. No change in color of solution mixture was observed after 15 min indicating that the formation of Au NPs was not significantly influenced either by 15 min of heating or by room light. Later, purple color change in solution mixture was observed instantly after the addition of 10 µM Sodium Borohydride (NaBH₄) indicating the formation of Au NPs. Reproducible ability (reproducibility) was confirmed after repeating the experiment thrice.

**Dip-coating of NPs onto surface of gloves**

Dip-coating method was performed to deposit AuNPs (3 g ml⁻¹) onto the surface of glove. Initially, the latex gloves of size 2.5 cm × 2.5 cm pieces were prepared and then immersed into the AuNP’s dispersion, allowed for about 15 min. The gloves were allowed to dry for 24 h under room temperature conditions after being coated with AuNPs.

**Disk diffusion assay**

Bacteria cell cultures were adjusted to McFarland standard No. 0.5 before being disseminated on MHA and SDA for bacteria and fungi, respectively. The plates were covered with AuNPs-gloves and incubated overnight at 37 °C. To assess the diffusion from AuNPs-gloves, the inhibition zones were examined.
Antimicrobial efficacy evaluation using Au NPs coated gloves

The surface of glove (2.5 cm × 2.5 cm) was exposed to 1.0 × 10^6 CFU ml⁻¹ of several Gram-negative and Gram-positive microorganisms, as well as yeast. A microscope cover slip was kept on top of the bacterial inoculums to examine a uniformly thin layer of inoculums. Submersion in 10 ml of D/E Neutralizing Broth terminated all antibacterial activity after exposure. The resulting culture was collected and diluted serially for quantitative culturing. Comparatively, uncoated gloves were examined thrice after exposure for about one hour to the challenge inoculum, and the found live organisms were measured. A log 10 reduction was estimated by comparing the number of microorganisms retrieved from uncoated gloves to the number of organisms retrieved from NPs coated gloves.

Characterization

Optical nature of Au NPs was measured at 1nm resolution and wavelength ranging from 350 to 800 nm in the Ultraviolet–Visible (UV–vis) spectrophotometer (Thermo Scientific Evolution 201 model). Maximum level of absorbance was shown by the NPs solution at a wavelength of 532 nm. UV–vis spectrophotometer was also used to conduct the Energy Dispersive x-ray (EDX) spectroscopic analysis of the sample confirms its elemental composition. Crystallinity, size and morphology of the Au NPs was identified using High Resolution Transmission Electron Microscopy (HR-TEM). A drop of Au NPs solution was placed on the copper grid (carbon coated) and vacuum dried for preparing the samples and examined under TEM. JEOL-JEM controlled at a voltage acceleration of 200 kV was used for image capture analysis of TEM. By using XRD-Brunker D8 Advance with Cu Kα radiation, thin layer of XRD was measured. Glass plate coated with Au NPs and vacuum dried was used to prepare the sample.

Results and discussion

We reported the production of Au NPs using G.glauca extract in order to verify the G.glauca value in green nanotechnology. Although, in mild conditions the formation of Au NPs was not difficult, the reaction could not be completed just before 24 hr of the incubation. To overcome this issue, we slightly heated 1mm of HAuCl₄ with G.glauca extract at 60 °C in 10 µM NaBH₄ in trace amounts. The indicator for the Au NPs formation is the change in the color of the solution to violet in not less than 5 min. A characteristic plasmon resonance band seen on the surface of Au NPs, centered at a wavelength of 532 nm was recorded from the absorption spectra of resulting solutions in the UV–vis spectrophotometer (figure 1). Au NPs presence in a poly dispersed form was indicated by the SPR peaks with broad nature.

Fourier Transform Infrared Spectroscopy (FTIR analysis) was used to detect the functional groups of biogenic Au NPs and G.glauca leaf extract. The FTIR spectra of both biosynthesized Au NPs and plant leaf extract were compared to evaluate the difference before and after bio-reduction (figure 2). IR spectra of G.glauca leaf extract displays sharp peak at 3415 cm⁻¹ because of H band stretching vibrations (OH) of phenols, alcohol groups and may also include proteins NH group [22]. One more strong band was noticed at 1585 cm⁻¹ which determines the C–C band stretching vibrations of aromatic compounds [23]. Band at 1411 cm⁻¹ is assigned to bending of –OH because of R–COOH group [24]. Peak at 780 cm⁻¹ is because of N–H stretching for 1° and 2°
amines [25] and band at 1067 cm$^{-1}$ is due to polyphenols C–N stretching vibrations [26]. In protein biomolecules, the amino acid residues are held together with the help of amide linkages [27]. The FTIR spectra of Au NPs displays sharp peaks at 3469 and 1640 cm$^{-1}$, attributed to the stretching vibrations of O–H band [28] which indicates the presence of phenol groups and stretching vibrations of N–H band of protein amide I resulted because of the stretch in carbonyl group [26] respectively. Decreased peaks at 706 and 1392 cm$^{-1}$ are due to deformation of C–H group in aromatic hydrocarbons [22] and stretching vibrations of C–N band of aromatic amines [27] respectively. The above results suggest that biomolecules like polyphenols and proteins are probably involved in capping of Au NPs and in Au$^{3+}$ ions reduction.

X-ray powder diffraction (XRD analysis) was used to determine the crystalline nature of bio-fabricated Au NPs. The findings presented sharp peaks related to 311, 220, 200 and 111. Bragg’s reflection depends on face centered cubic (fcc) structure with 2$\theta$ estimations of 77.86°, 64.73°, 44.33° and 38.17° (figure 3). Various shapes and sizes of synthesized Au NPs (with the help of G. glauca leaf extract) were explained by TEM analysis. Most of the synthesized Au NPs are sphere shaped, some of them were decahedral, rod, hexagonal and triangular in shape and with size ranging from 30–50 nm (figure 4(A)). Energy dispersive x-ray analysis (EDAX spectrum) was used to confirm the purity and chemical composition of fabricated biogenic Au NPs. The EDAX pattern of Au NPs displayed strong signals representing Au atoms and weak signals representing oxygen, nitrogen and carbon (figure 4(B)). The phytochemicals which were bounded to the fabricated biogenic Au NPs could be the reason behind the formation of weak signals.

**Antimicrobial properties of AuNPs-gloves**

Antibacterial property of Au NPs-gloves was investigated towards multidrug-resistant clinical strains of fungi, Gram-positive bacteria, and Gram-negative bacteria that cause clinical infections. Both clinical isolates and reference strains were found to be inhibited by the AuNPs-gloves. Fungi, Gram-positive bacteria and Gram-
negative bacteria had inhibition zones of 7.3–7.6 mm, 10.25–11.80 and 5.9–9.9 mm, correspondingly [table 1]. However, the mechanism of Au NPs’ action on bacterial cells are still being debated. The significant antimicrobial property of Au NPs and the potential mechanism of antimicrobial activity may be because of the enhanced intracellular ROS generation resulting the oxidative stress to the microbial cells [29]. Furthermore, increased zeta potential has frequently been described as a diagnostic for membrane injury [30–32]. On the other hand, researchers have found stronger effects against Gram-positive microorganisms due to electrostatic repulsion among the NPs and outer lipid membrane of Gram-negative microorganisms, which has a higher negative charge [33, 34]. Greater performance in Gram-positive microorganisms may have emerged from the synergistic action of gold NPs and ZnO existed in natural rubber. The combination of Au NPs and ZnO have shown to increase the generation of ROS and cause plasmid DNA damage [35].

Within one hour, gloves coated with Au NPs eradicated a broad range of Gram-negative and Gram-positive microbes, and yeasts (figures 5(A), (B)). When tested against standard strains and many clinical isolates, the gloves exhibited a decreased activity of an average of 5 logs (p 0.01). K. pneumoniae NPRCoE 160602 and E. coli NPRCoE 161001 showed a drop of two to three logs.

Table 1. Agar disk diffusion approach showing the antibacterial property of Au NPs-gloves.

| Zone of inhibition (nm) | Microorganisms | Au NPs- gloves | Uncoated gloves |
|------------------------|----------------|----------------|-----------------|
| Clinical isolates      |                |                |                |
| E. coli NPRCoE 161001  | 8.6 ± 1.31     | 6.30 ± 0.14    |
| C. albicans NPRCoE 160120 | 7.3 ± 0.11 | —*           |
| K. pneumoniae NPRCoE 160602 | 6.95 ± 0.12 | 6.40 ± 0.28 |
| A. baumannii NPRCoE 160510 | 7.32 ± 0.00 | —*           |
| P. aeruginosa NPRCoE 160901 | 5.95 ± 0.11 | —*           |
| E. coli NPRCoE 161001  | 8.60 ± 1.31     | 6.30 ± 0.14    |
| Methicillin-resistant  |                |                |                |
| S. aureus NPRCoE 160801 | 11.70 ± 2.35 | 6.40 ± 0.42    |
| Reference Strains      |                |                |                |
| P. aeruginosa ATCC 27853 | 7.35 ± 0.38 | —*           |
| A. baumannii ATCC 19606 | 9.87 ± 0.45 | 6.35 ± 0.95    |
| S. aureus ATCC 25923   | 10.25 ± 0.44   | 6.35 ± 0.31    |
| E. coli ATCC 25922     | 5.75 ± 1.23    | 6.15 ± 0.06    |
| C. albicans ATCC 90028 | 7.53 ± 0.11    | —*           |
| E. faecalis ATCC 29212 | 10.50 ± 0.99   | —*           |
| K. pneumoniae ATCC 700603 | 6.65 ± 0.64 | 6.30 ± 0.17    |

Figure 4. HR-TEM image (A) and EDS spectrum (B) of Au NPs.
Conclusions

Biogenic Au NPs (by using G.glauca leaf extract) were obtained using one-pot synthesis method. The fcc phase of the fabricated Au NPs was confirmed from XRD analysis. Most of the synthesized Au NPs were spherical in shape, some of them were decahedral, rod, hexagonal and triangular in shape. The size of synthesized nanoparticles ranges from 30–50 nm, which was confirmed with the help of TEM analysis. The purity of synthesized Au NPs was confirmed with the help of EDAX analysis. Further, prepared Au NPs were dip-coated onto the surface of gloves and their antimicrobial results showed that the Au NPs-coated gloves demonstrated good antibacterial effect, notably against multidrug-resistant bacteria. Hence, the highly effective Au NPs-coated gloves may be ideal candidates for preventing or minimising indirect microbial transmission and cross-contamination in nursing care.

Data availability statement

No new data were created or analysed in this study.
Conflicts of interest

Authors declare that there are no conflicts of interest associated with this work.

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