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Green synthesis and photothermal application of superparamagnetic iron oxide nanoparticles for cancer hyperthermia

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Cancer remains a major global health challenge, with traditional treatments like chemotherapy and radiation often yielding limited long-term success. Hyperthermia, one of the oldest cancer therapies, is promising due to the disorganized blood supply in tumors, making them more sensitive to heat compared to healthy tissues. Nanomedicine, particularly nanoparticle-mediated hyperthermia, is emerging as an innovative treatment strategy. Iron oxide nanoparticles (IONPs) are of interest due to their ability to convert near-infrared (NIR) laser radiation into localized heat while functioning as contrast agents for imaging. In this study, we investigated the synthesis of IONPs using *Ganoderma lingzhi* (Reishi) extract (IONP@GL) by green synthesis method. The synthesized IONPs were characterized by their size, morphology, magnetic properties, and photothermal efficiency. IONP@GL exhibited good heat generation under 808 nm laser irradiation. These findings highlight the potential of green-synthesized IONPs for application in photothermal cancer therapy.

Keywords: green synthesis, photothermal therapy, hyperthermia, iron oxide nanoparticles, nanomedicine

INTRODUCTION

Cancer continues to be a significant global health challenge, with traditional therapies such as chemotherapy and radiation often resulting in limited long-term success [1]. As the incidence of cancer continues to rise, there is an increasing demand for more effective and innovative treatment strategies. Hyperthermia is one of the oldest cancer therapies. Due to their disorganized blood supply, tumors have a reduced ability to dissipate heat, making them more vulnerable to thermal stress compared to well-vascularized healthy tissue [2]. Additionally, hyperthermia has been shown to improve the efficacy of other therapies, including radiation and chemotherapy [3], which has renewed interest in its role in modern cancer treatment.

Nanotechnology presents a promising approach to achieving localized hyperthermia. Appropriately designed nanoparticles (NPs) can overcome biological barriers [4] and selectively accumulate in pathological sites. Accumulated in tumor tissue, the NPs could convert various external physical stimuli, such as magnetic fields or laser radiation, into localized heat [5]. For example, *Nanotherm*® iron oxide NPs have been approved for magnetic hyperthermia in the treatment of glioblastoma and prostate cancer [6], while *AuroShell* are experimental NPs used for laser-mediated (photo-thermal) ablation of solid tumors [7]. Additionally, *Thermodox*®, a thermally-activated drug carrier in human clinical trials, releases its payload at hyperthermic sites, allowing localized chemotherapy [8].

Among the various methods of inducing hyperthermia, nanoparticle-mediated laser hyperthermia has gained popularity due to its minimal invasiveness, spatiotemporal selectivity, and the availability of cost-effective laser equipment. Laser radiation can penetrate to depths of up to 1 cm under the skin, making photothermal therapy particularly suitable for treating superficial tumors or those located in body cavities. Near-infrared (NIR) lasers, which offer maximum tissue penetration, are typically used in these treatments. At wavelengths in the NIR range, the safety limit (maximum permissible exposure) is 0.33 W/cm^2 for 808 nm lasers and 1.0 W/cm^2 for 1064 nm lasers [9]. Iron oxide NPs (IONPs) are particularly attractive for laser hyperthermia because in addition to being good at converting NIR radiation into heat, they also function as contrast agents and can mediate multiple therapies [10]. Superparamagnetic IONPs which exhibit magnetic properties only in the presence of an external magnetic field, find applications as magnetic targeting agents in drug delivery [11] and as contrast agents in magnetic resonance imaging

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(MRI) [12].

Among the various methods for synthesis of IONPs, green synthesis offers an environmentally friendly and non-toxic approach [13]. This method uses plant extracts as reducing agents, making it a cost-effective alternative of other methods. An additional advantage of green synthesis is that biologically active molecules from the plant extracts can be adsorbed onto the surface of the nanoparticles, potentially enhancing their therapeutic properties.

The aim of the present study was to synthesize coated IONPs using *Ganoderma lingzhi* (Reishi) extract (IONP@GL), and demonstrate their potential in photothermal therapy. The green-synthesized NPs were compared with uncoated IONPs and characterized in terms of size, shape, magnetic properties, zeta potential, and photothermal efficiency. The results show that green synthesis can successfully produce superparamagnetic IONPs with efficient infrared-to-heat conversion properties, highlighting their potential for use in photothermal cancer therapy.

MATERIALS AND METHODS

Materials

Iron(III) chloride hexahydrate, iron(II) chloride tetrahydrate and NaOH were purchased from Merck KgaA (Darmstadt, Germany). *Ganoderma lingzhi* mushroom was purchased from a local pharmacy.

Preparation of Ganoderma lingzhi aqueous extract

For preparation of the aqueous extract of *Ganoderma lingzhi*, 1 g of mushroom powder was added to 100 mL of distilled water. The mixture was heated at 80°C for 60 min under continuous magnetic stirring. After cooling to room temperature, the extract was filtered using a vacuum filtration system (0.45 μm cellulose acetate filter) to remove any particulate matter. The final extract was used immediately for nanoparticles synthesis.

Synthesis of IONP@GL using Ganoderma lingzhi extract

IONPs were synthesized using an aqueous solution of $FeCl_3$ and $FeCl_2$ in a molar ratio of 2:1. The iron salts were dissolved in 200 mL of distilled water under constant stirring at 80°C for 30 min. Then 50 mL of the *Ganoderma lingzhi* extract was added to the solution. The pH of the reaction was raised to above 6 by dropwise addition of 0.1 M NaOH, and the mixture was stirred for another 30 min at 80°C. The resulting nanoparticles were separated using a magnet and washed three times with distilled water. The synthesis of the NPs was carried out under nitrogen atmosphere to prevent oxidation, and ultrasound was applied to reduce the formation of NP clusters.

Synthesis of uncoated IONPs

$FeCl_3$ and $FeCl_2$ in a molar ratio of 2:1 were dissolved in 200 mL of distilled water and stirred at 80°C for 30 min. After that, 0.1 M NaOH (25 mL) was added, and the mixture was stirred again for 30 min at 80°C under nitrogen atmosphere, and application of ultrasound. The resulting nanoparticles were washed three times with distilled water.

Characterization of IONPs

Transmission electron microscopy, TEM, (Talos F200X, Thermo Fisher Scientific, Waltham, MA, USA) was used to determine the morphology and size of the NPs. Elemental composition was determined using energy-dispersive X-ray spectroscopy, EDX, (Prisma E SEM, Thermo Scientific, Waltham, MA, USA). Magnetic characteristics and phase composition of the IONPs were determined by Mössbauer spectroscopy (WissEl-Wissenschaftliche Elektronik GmbH, Germany). Dynamic light scattering, DLS, (Microtrac, York, PA, USA) was used for particle size, size distribution and zeta potential measurements.

Photothermal performance evaluation

The photothermal performance of the synthesized IONPs was demonstrated by irradiating the samples with an 808 nm laser at a power density of 0.330 W/cm² for 15 min. NPs suspensions were prepared at concentrations of 0.025 mg/mL, and 0.05 mg/mL in distilled water. The temperature of the suspensions was monitored by infrared camera FLIR (FLIR Systems Inc., Boston, MA, USA).

RESULTS AND DISCUSSION

TEM showed that the synthesized IONP@GL had a predominantly spherical morphology with an average particle size of approximately 20 nm (Fig. 1 C). It is well known that IONPs with diameters of 20 nm or less are superparamagnetic [14]. The magnetic properties of the produced IONPs were evident when a static magnetic field was applied, allowing them to overcome gravitational forces, thus demonstrating their superparamagnetic behavior (Fig. 1 A and B).

EDX analysis showed that the uncoated IONPs exhibited peaks only for Fe and O (Fig. 1D), indicating their pure inorganic composition. In contrast, IONP@GL displayed an additional peak for C (Fig. 1E), confirming the adsorption of organic

molecules from the plant extract onto the surface of the NPs.

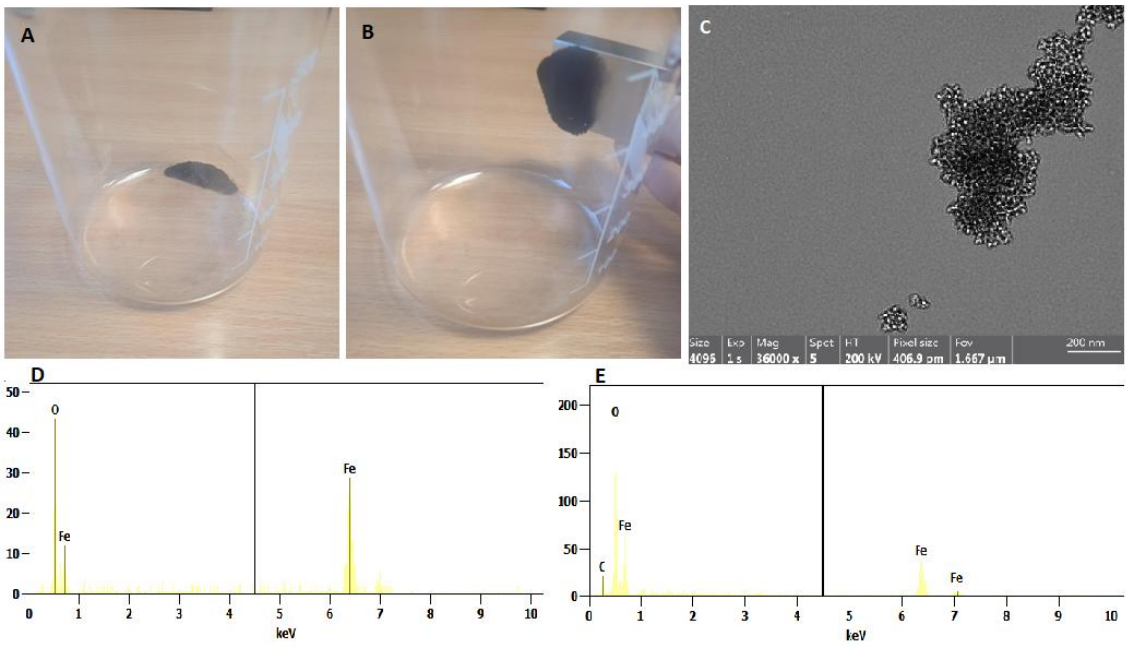

Fig. 1. Demonstration of superparamagnetic properties of IONPs (A and B); TEM micrographs of IONP@GL (C); EDX of uncoated IONPs (D) and IONP@GL (E).

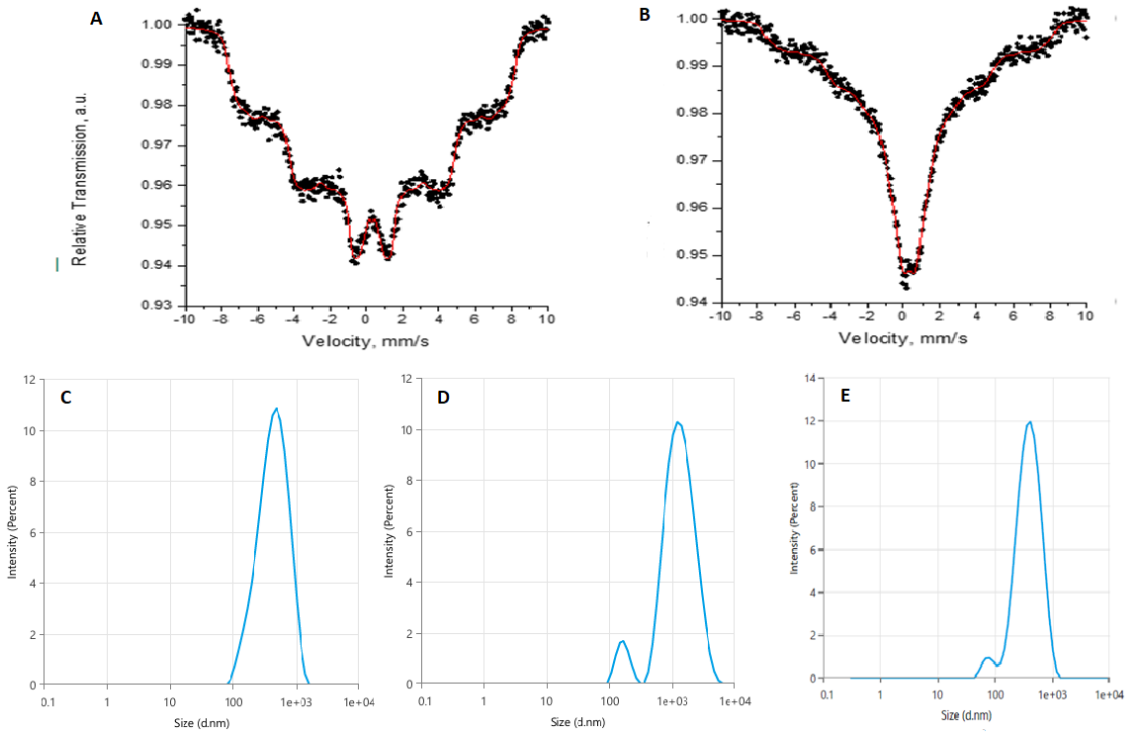

Fig. 2. Mössbauer spectra of uncoated IONPs (A) and IONP@GL (B); hydrodynamic size of uncoated IONPs (C); hydrodynamic size of IONP@GL before (D) and after additional ultrasound application (E).

The Mössbauer spectra of the samples (Fig. 2 A and B) resemble those of nanosized maghemite (γ-Fe_2O_3), with an average diameter larger than 10 nm [15]. The average magnetic field was determined to be 26.8 T for uncoated IONPs, and 19.4 T for IONP@GL.

DLS measurements show a significantly larger hydrodynamic size of NPs, around 160 nm for IONP@GL, suggesting the formation of clusters of NPs in suspension. To reduce aggregation, ultrasound was applied immediately after the synthesis for 3 h. This resulted in a reduction of the hydrodynamic size below 90 nm, and a noticeable decrease in the extent of cluster formation. The estimated polydispersity index was 0.216 for uncoated IONPs and 0.245 for IONP@GL. Zeta potential analysis showed a change from 41.3 mV for uncovered IONPs to -27.24 mV for IONP@GL. The change in polarity suggests adsorption of biomolecules from the plant extract onto the NPs surface.

During the irradiation of the samples, an initial temperature rise was observed at the point of penetration of the infrared laser light. Over time, this temperature rise deepened in the sample, indicating heat diffusion under prolonged laser exposure (Fig. 3A). Interestingly, IONP@GL exhibited a better photothermal effect at both concentrations. The IONP@GL samples demonstrated a 2°C increase in temperature compared to the uncoated IONPs under the same conditions (Fig. 3 B, C). A plausible explanation for the enhanced photothermal effect could be the presence of biomolecules from *Ganoderma lingzhi* on NPs, which reduce their agglomeration, in addition to application of ultrasound after NPs synthesis. This stabilization allows a larger number of NPs to remain dispersed, increasing the surface area available for interaction with infrared photons and for heat transfer to the surrounding medium [16]. The temperature increase in all samples was sufficient for mild hyperthermia.

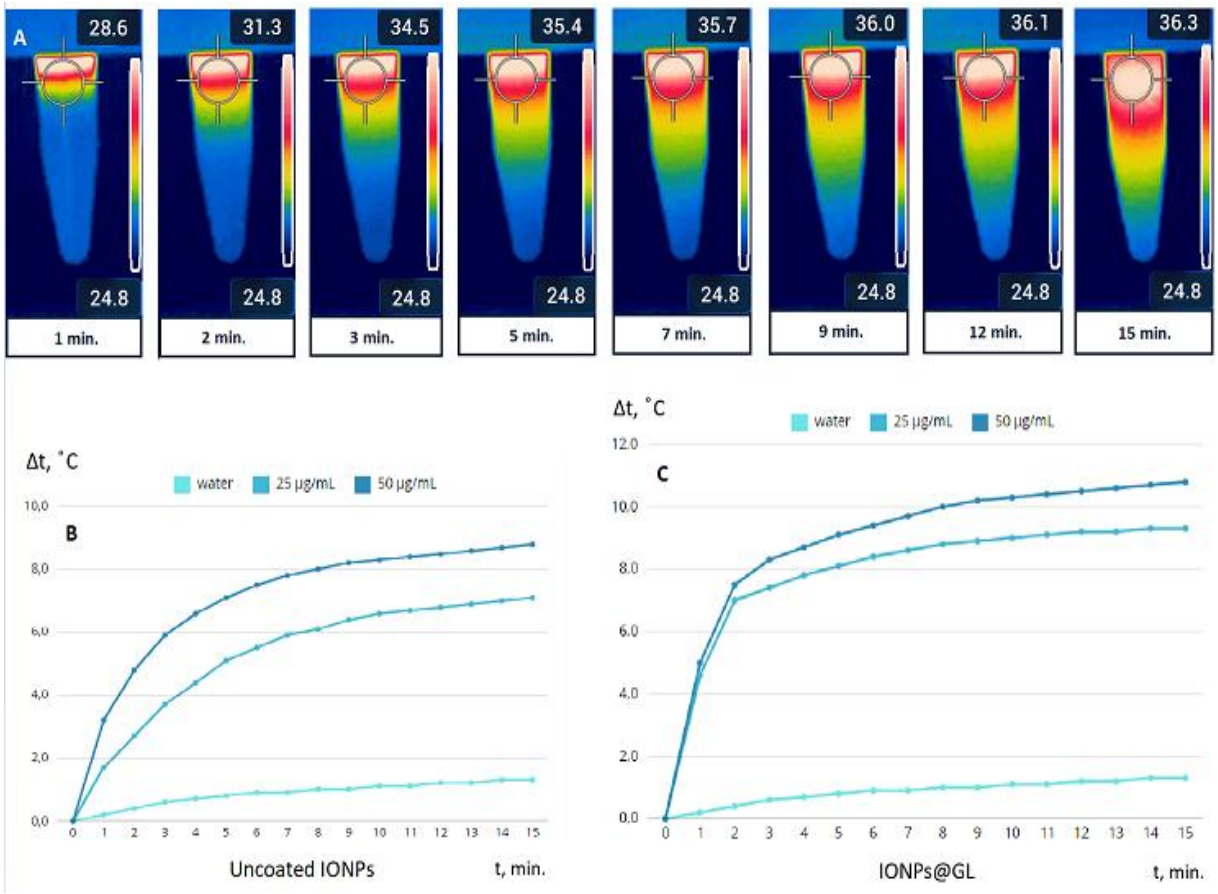

Fig. 3. Temperature changes with depth in a 50 µg/ml IONP@GL sample during 808 nm-laser irradiation (A); Temperature changes in uncoated IONPs at 25 µg/mL and 50 µg/mL concentrations during 808 nm-laser irradiation (B); Temperature changes in IONP@GL samples at 25 µg/mL and 50 µg/mL concentrations during 808 nm-laser irradiation (C).

CONCLUSION

This study successfully demonstrated the green synthesis of superparamagnetic iron oxide nanoparticles (IONP@GL) using *Ganoderma lingzhi* extract, and their promising photothermal performance. The IONP@GL samples exhibited a temperature increase that was by 2°C higher than that of uncoated IONPs under identical conditions. Both IONP@GL and uncoated IONPs at concentrations of 25 μg/mL and 50 μg/mL achieved sufficient temperature increase to induce mild hyperthermia, highlighting their potential for photothermal cancer therapy.

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