Synthesis, Characterization and Antibacterial Activity of Glycol Chitosan Modified Superparamagnetic Nanoparticles

B.H. Chen[*,**] and B. Stephen Inbaraj[*]

[*]Department of Food Science, Fu Jen University, Taipei 242, Taiwan
[**]Graduate Institute of Medicine, Fu Jen University, Taipei 242, Taiwan, 002622@mail.fju.edu.tw

ABSTRACT

Superparamagnetic iron oxide nanoparticles (IONPs) are particularly attractive as antibacterial agents owing to their ease of precise bacterial biofilm- and cell-targeting *in vivo* by magnetic driving. The IONPs coated with water-soluble glycol chitosan (GC-IONPs) was synthesized by coprecipitation method, followed by characterization and evaluation of antibacterial activity. Characterization of IONPs by FTIR, XRD, TEM and VSM revealed both bare and GC-coated IONPs to be superparamagnetic belonging to magnetite, with a saturation magnetization value of 70.3 and 59.8 emu/g and average diameter 9 and 8 nm respectively. For antibacterial activity, both bare and GC-coated IONPs were more effective against *E. coli* ATCC 8739 and *S. enteritidis* SE 01 than the commercial antibiotics linezolid and cefaclor. But, for *E. coli* O157:H7 TWC 01 and *S. aureus* ATCC 10832, GC-IONPs showed higher potency than bare IONPs. The GC-coated IONPs could be a potential nanomaterial for *in vivo* applications in biomedical fields.

Keywords: iron oxide nanoparticles, superparamagnetic, glycol chitosan, coating material, antibacterial activity

1 INTRODUCTION

Recent advancements in the field of nanotechnology provide attractive solutions for synthesizing alternative antimicrobial agents and reducing biofilm formation [1]. For instance, silver nanoparticles have been proved successful in exhibiting antimicrobial activity to reduce infections on implanted devices, preserve food and develop antimicrobial finished textile fabrics [2]. However, recent reports have raised concerns on potential environmental and health risks associated with the release of ultrafine silver particles from a range of consumer products containing silver nanoparticles as antimicrobial agents [3]. Therefore, there is a need to develop alternative nanoparticles modified with non-toxic and biocompatible materials/ polymers. Superparamagnetic iron oxide nanoparticles (IONPs) with tailored surface chemistry has been widely used for various *in vivo* applications including magnetic resonance imaging, tissue repair, immunoassay, detoxification, hyperthermia, drug delivery and cell separation [4]. The surface modification not only helps to overcome the agglomeration problem resulting from large surface to volume ratio, but also ease the demands of some specific applications [5]. Among various coating materials, chitosan has been widely employed because of its inherent antimicrobial property [6]. But, its application is limited by poor water solubility, as its highly deacetylated form is soluble only at low pH in organic acids [6]. Unfortunately, most approaches to improve its suitability for physiological conditions have resulted in a reduction of free amine-containing residues [7]. To remedy this problem, we chose glycol chitosan (GC), a hydrophilic chitosan derivative soluble in water at all pH, as a coating material to modify the surface of iron nanoparticles. GC contains free amine groups along the polymer chain and has a tendency to behave as a typical polycation at lower pH due to protonation of amine groups [7]. The objectives of this study were to synthesize glycol chitosan modified superparamagnetic IOPs (GC-coated IOPs) by the classical co-precipitation method (see Fig. 1), characterize its structural and magnetic properties, and evaluate its antibacterial activity against four pathogens by comparing with two commercial antibiotics.

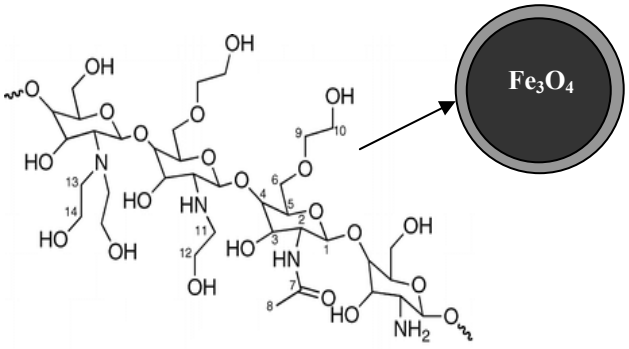

Figure 1: Schematic diagram showing glycol chitosan coated on iron oxide nanoparticles

2 EXPERIMENTAL

Synthesis and Characterization of GC-coated IONPs

An aqueous mixture containing 6.1 g of ferric chloride and 4.2 g of ferrous sulfate was prepared followed by adding a few drops of Conc. HCl for complete dissolution of iron salts. The mixture was then vigorously stirred at 2000 rpm with a concomitant bubbling of nitrogen gas to prevent oxidation of ferrous ions and raising the temperature to 85°C. When the mixture attained 85°C, 20 mL of ammonium hydroxide was rapidly added to precipitate iron

oxide nanoparticles and the solution mixture turned from a light orange color into black. Next, 2.0 g of GC dissolved in 50 mL of DD water was added to the reaction mixture at 85°C and the stirring was continued for 1 h. At the end of the reaction, the precipitated IOPs coated with GC were washed several times and vacuum dried at 0 mm Hg and 40°C for 24 h for characterization by FTIR, XRD,TGA, TEM and VSM. The bare IOPs were prepared by adopting the same procedure but skipping the addition of GC.

Antibacterial Activity Assay

Four test pathogens namely *Escherichia coli*–ATCC 8739, *E. coli* O157:H7–TWC 01, *Salmonella enteritidis*–SE 01 and *Staphylococcus aureus*–ATCC 10832 were cultured aerobically in tryptic soy broth (10 mL) overnight and antibacterial activity was evaluated by using spot-on-lawn on Mueller-Hinton agar. Ten different doses (0.5, 1, 2, 4, 8, 16, 32, 64, 128 and 256 μg/mL) of NaPGA- and CaPGA-coated MNPs as well as antibiotics linezolid and cefaclor were prepared separately, followed by spotting 100 μL of each dose on the soft agar and incubating at 37°C for 24 h. Based on the zone of inhibition, the minimum inhibitory concentration (MIC) of nanoparticles/ antibiotics required to inhibit the growth of pathogens was determined.

3. RESULTS AND DISCUSSION

Characterization of GC-coated IONPs

Comparision of FTIR spectra of bare and GC-coated IONPs with that of pure GC revealed the presence of characteristic peaks of both GC and IONPs (582 cm^{-1} for Fe-O) in the spectra for GC-coated IONPs, implying the coating of GC on the surface of IONPs. The typical peaks of GC identified were at 1627 cm^{-1} corresponding to amide bond of undeacetylated part in GC, 1546 cm^{-1} to N-H bending vibration of free amine group of deacetylated portion, 1110 cm^{-1} to glycosidic linkage (ether bond) and 1064 cm^{-1} to C-N vibrations. Based on the X-ray diffractograms, the estimated lattice parameters (8.3945 for bare-IONPs and 8.3922 Å for GC-IONPs) and six diffraction peaks at 30° (2 2 0), 35° (3 1 1), 43° (4 0 0), 53° (4 2 2), 57° (5 1 1) and 62° (4 4 0) correlated well with the standard value suggesting the synthesized IONPs to be mainly magnetite. From the weight loss in TGA curve recorded at a temperature range from 25 to 700°C, the amount of GC coating on IONPs was estimated to be 8.5%. The TEM images showed the morphology of synthesized IONPs was roughly spherical in shape and the particle-size distribution analysis showed the mean diameter of bare and GC-coated MNPs to be in the range of 8–9 nm. Vibration sample magnetometry (VSM) data revealed no noticeable coercivity or remanence indicating the superparamagnetic behavior and the saturation magnetization value was determined to be 70.3 and 59.8 emu/g, implying a large portion of magnetic property is retained even after coating of IONPs with GC (see Fig. 2).

Antibacterial Activity of GC-coated IONPs

The antibacterial effect of both bare and GC-coated IONPs as well as antibiotics linezolid and cefaclor was evaluated based on the inhibition zone by agar dilution assay and the MIC values obtained as a mean of 9 replicates are plotted in Figure 3. Of the 3 gram-negative bacteria tested, both bare and GC-coated IOPs were more efficient against the growth of *E. coli* and *S. enteritidis* than linezolid and cefaclor, as their MIC values were much lower than that for the antibiotics. For gram-negative *E. coli* O157:H7 strain, both bare (256 μg/mL) and GC-coated IOPs (128 μg/mL) exhibited a much higher MIC value than the antibiotics (32 and 16 μg/mL), implying a high dose of IOPs is necessary to attain the same antibacterial activity as the antibiotics. Nevertheless, it is worth pointing out that the GC-coated IOPs were 2-fold more efficient than the bare IOPs in inhibiting *E. coli* O157:H7 growth. Similarly, a lower antibacterial activity was observed for both bare and GC-coated IOPs toward the gram-positive *S. aureus* than the antibiotics. Yet, the GC-coated IOPs did show a 4-fold higher potency in anti-*S. aureus* activity than the bare ones. Compared to linezolid, the antibiotic cefaclor was twice more potent in inhibiting all the tested pathogens, probably due to the difference in bactericidal mechanism. Since the pH of bacterial medium (7.3) used was lower than the pK_a of GC (6.0) [8], the NH_2 groups in GC may fail to be protonated, thereby preventing the antibacterial mechanism through surface charge interaction using amide groups. It has been well documented that when both bacterial and adherent surfaces are hydrophobic, the bacterial adhesion can be greatly enhanced, but if both are hydrophilic, the adhesion would proceed with difficulty [9]. Thus, the pronounced antibacterial activity of GC-coated IONPs toward *E. coli* and *S. enteritidis* may be accounted for by the hydrophilic nature of both the gram-negative strains and nanoparticles. Likewise, the hydrophilic nature of *E. coli* O157:H7 may be responsible for the 2-fold higher in antibacterial activity for GC-coated IONPs than for the bare ones. However, the difference in antibacterial activity between the two tested *E. coli* strains (ATCC 8739 and O157:H7) as affected by IONPs and antibiotics should be caused by the varying degree of hydrophilicity. Moreover, the extracellular polymeric substance produced by *E. coli* O157:H7 may mask the hydrophilic property by enhancing the adhesion ability of cells [10]. Generally, the gram-negative *S. aureus* strains are considered to be hydrophobic in nature. But, according to the hydrophobicity–hydrophilicity evaluation of 15 *Staphylococcus* strains by Reifsteck et al. [11], the Wood 46 strain of *S. aureus* employed in our experiment was classified to be hydrophilic, accounting for a 4-fold rise in antibacterial activity for GC-coated IOPs compared to the bare ones.

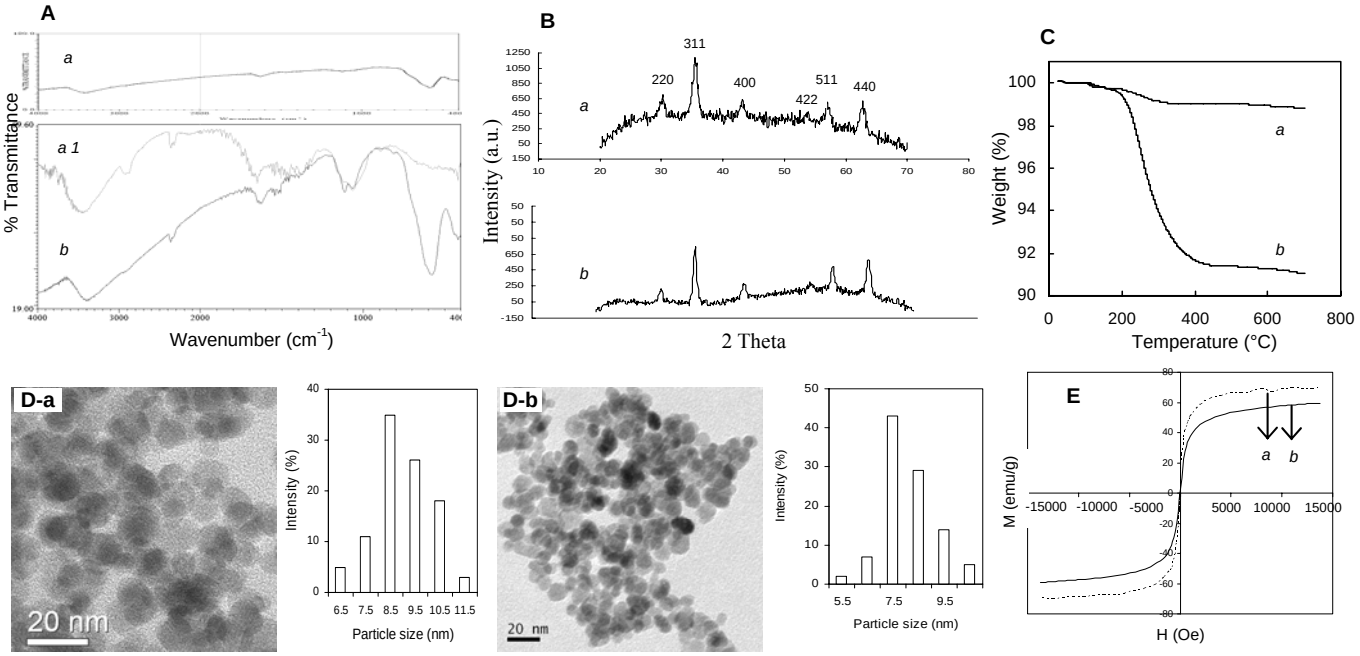

Figure 2: Characterization of GC-coated nanoparticles. (A), FTIR spectra of bare-IONPs (a), pure GC (a1) and GC-IONPs (b); (B), XRD data; (C), TGA data; (D), TEM images; (E), VSM data

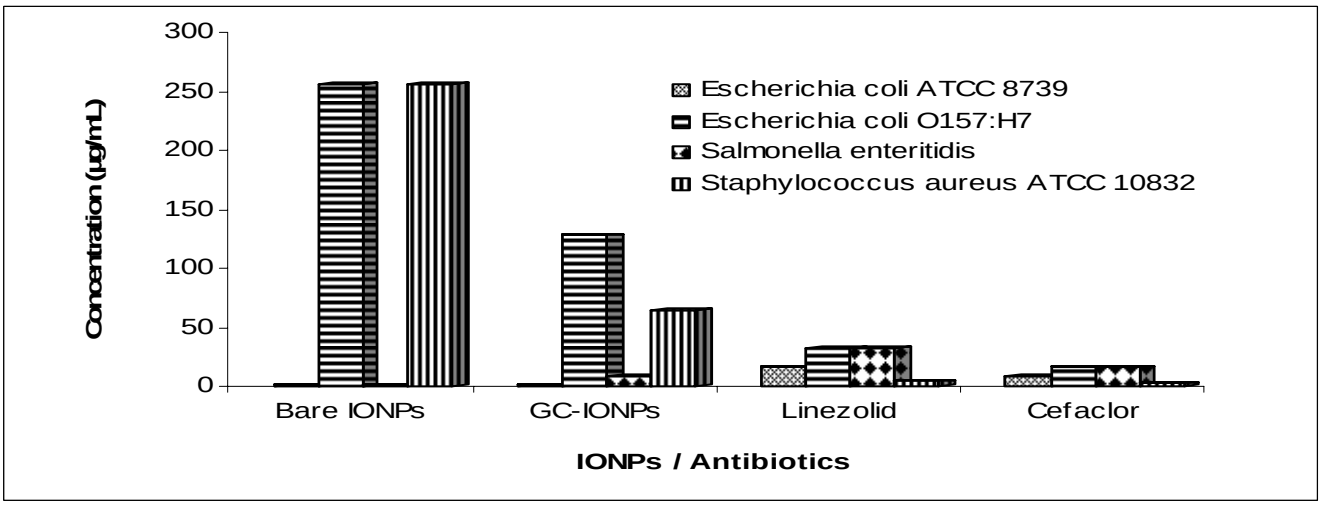

Figure 3: Minimum inhibitory concentration of bare IONPs and GC-IONPs against tested pathogens as determined by agar dilution assay

4 CONCLUSION

The outcome of this study demonstrated the antibacterial activity of glycol chitosan-coated-iron oxide nanoparticles and thus may be a promising nanomaterial for further in vivo applications in biomedical fields.

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