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Recyclable and stable silver deposited magnetic nanoparticles with poly (vinyl pyrrolidone)-catechol coated iron oxide for antimicrobial activity

Tamim Mosaiab [a], Chan Jin Jeong [b], Gyo Jic Shin [c], Kyung Ho Choi [c], Sang Kug Lee [c], Iksoo Lee [d], Insik In [e], Sung Young Park [a,b,*]

[a] Department of Green Bio Engineering, Korea National University of Transportation, Chungju-Si 380-702, Republic of Korea
[b] Department of Chemical & Biological Engineering, Korea National University of Transportation, Chungju-Si 380-702, Republic of Korea
[c] IT Convergence Material R & D Group, Korea Institute of Industrial Technology, Cheonan-Si, Republic of Korea
[d] R&D Center, NOROO Holdings Co., Ltd., Republic of Korea
[e] Department of Polymer Science and Engineering, Korea National University of Transportation, Chungju-Si 380-702, Republic of Korea

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ABSTRACT

This paper introduces a facile method to make highly stable and recyclable antimicrobial magnetic nanoparticles (NPs). Initially, magnetic iron oxide nanoparticles (IONPs) were coated with poly (vinyl pyrrolidone) conjugated catechol (PVP-CCDP). Afterward, silver nanoparticles (Ag^0) were deposited onto PVP-CCDP coated IONPs using remain catechol. The prepared nanoparticles showed long term (~4 weeks) colloidal stability and redispersibility, respectively, against external magnetic field and over a broad range of pH (4–12). The NPs were characterized by UV–vis, SEM, XPS, and XRD measurements. TEM and DLS analyses showed that the mean particle size of PVP-CCDP coated IONPs/Ag^0 were about 72 nm. The recyclable magnetic NPs possessed a high antibacterial effect against the model microbes *Staphylococcus aureus* and *Escherichia coli* and could be separated easily using magnet following antibacterial test for repeated uses and maintained 100% antibacterial efficiency during three cycles. In MTT assay, the magnetic nanoparticles possessed no measureable cytotoxicity to live cells.

1. Introduction

The antimicrobial properties of newly prepared compounds are intensively studied because of increasing bacterial resistance against frequently and excessively used conventional antibiotics. As a result, microbial threats on human health and safety have become a serious public concern. Therefore, antimicrobial materials that can effectively inhibit the growth of microorganisms have been attracted to the material researchers [1]. In recent times, quaternary ammonium salts [2–4], free halogen [5,6], metallic nanoparticles such as copper [7,8], gold [9], and silver [10–12], molecular engineered peptides [13] etc., have been reported in the development of antimicrobial materials. However, the main drawback of these reported materials is their short term longetivity and antimicrobial performance differs significantly. As a result, it is needed to apply the antimicrobial agents frequently.

Magnetic nanoparticles have shown multiple benefits in many applications [14]. Among them, iron oxide nanoparticles (IONPs) have been attracted due to their unique magnetic properties and ease of preparation with various biomedical applications such as magnetic resonance imaging (MRI) [15–17], for target drug delivery [18,19], cancer diagnosis and treatment [20], cell purification etc. [21]. Generally, IONPs were stabilized with formation of a polymeric layer on its surface by using dextran, starch, albumin, citrate, silicones, poly (ethylene glycol), etc. [22,23] though most of these dispersants lacked with a well-defined high affinity anchor group lead to broad particle size distribution [24]. It has recently been demonstrated that catechol derivatives of the amino acid L-3,4-dihydroxyphenylalanine (L-DOPA) which is abundantly present in the marine mussels *Mytilus edulis* exhibit strong affinity to metal oxide nanocrystals by using diol groups of catechol moiety [25,26]. Therefore, to make more stabilized functional IONPs, catechol derivatives can serve as an attractive ligand to encapsulate IONPs.

Recently, there are a few literature reports referring to the IONPs/Ag^0 [23,27,28], but most of the cases only surfactant was used to encapsulate IONPs and $NaBH_4$ as an extra reductant to form silver nanoparticles that results a lower stable magnetic nano-complex with less efficient antibacterial properties. For example, Wang et al. used Triton X-100 to coat IONPs and $NaBH_4$ to reduce silver ion resulting sedimentation of nanoparticles within two days in water [29]. Wong et al. has reported bacterial inactivation through silver coated magnetic nanoparticles and found that the effectiveness of inactivation efficiency in killing bacteria was above 5 hour exposure time [10].

Very recent, Amsted et al. used poly (ethylene glycol) conjugated DOPA as an anchor group for IONP stabilization using single DOPA

* Corresponding author at: Department of Chemical & Biological Engineering, Korea National University of Transportation, Chungju-Si 380-702, Republic of Korea. Tel.: +82 43 841 5225; fax: +82 43 841 5220.
E-mail address: parkchem@ut.ac.kr (S.Y. Park).

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moiety which results in good stability, however, this nanoparticles lack DOPA with further use reducing other metal ions under multi-layer modification [24]. IONPs also functionalized for MRI contrast agents by using oligo-PEG-DOPA by Na et al. but no manifestation shows that a small molecule can act as a versatile and robust anchor on a nanoparticle whose surface involves iron oxide [19,30]. In this work, we developed a recyclable and reusable antimicrobial agent to show long term bactericidal activity. To achieve our destiny, we conjugated a robust adhesive catechol derivative 2-chloro-3′, 4′-dihydroxyacetophenone (CCDP) to the backbone of poly (vinyl pyrrolidone) (PVP-CCDP). Since the catechol side chain exhibits extremely strong binding affinity, we anticipated that PVP-CCDP can easily be covalently anchored on the surface of IONPs via mussel-inspired adhesion (PVP-CCDP coated IONPs). The remaining catechol derivatives were further used to reduce $AgNO_3$ to form Ag^0 on the surface of magnetic PVP-CCDP coated IONPs (PVP-CCDP coated IONPs/Ag^0) which enables to show antimicrobial property. UV–vis, XPS, SEM, TEM, DLS, and XRD were used to characterize the NPs. Recycling antimicrobial activity was studied against both Gram-positive and Gram-negative bacteria by applying magnetic force. Finally, we tested in vitro cytotoxicity of the synthesized PVP-CCDP coated IONPs/Ag^0 using MTT assay.

2. Experimental procedure

2.1. Materials

Poly (vinyl pyrrolidone) (PVP, Mw ~40,000), 2-chloro-3′, 4′-dihydroxyacetophenone (CCDP), iron (II, III) oxide nanopowder less than 50 nm (Fe_3O_4), tetrahydrofuran (THF), ethanol, hexane, silver nitrate ($AgNO_3$, 99%,), trizma base (99%, Sigma), trizma HCl, (99%, Sigma), Deuterium oxide (D_2O), *Staphylococcus aureus*, *Escherichia coli*, MRS broth, Luria-Bertani (LB), and agar powder were purchased from Sigma Aldrich Reagent Company.

2.2. Synthesis of PVP-CCDP

4 g of poly (vinyl pyrrolidone) and 2.24 g of 2-chloro-3′, 4′-dihydroxyacetophenone (CCDP) were dissolved in 50 ml of ethanol in a 150 ml flask and purging the solution with nitrogen gas. The mixture was then stirred at 70–80 °C for 24 h. After the reaction time, the solvent was evaporated in rotary evaporator and precipitated by using cold diethyl ether. The product was then dried in vacuum dry oven. The yield of the product was 88.4%.

^{1}H NMR (400 MHz, D_2O, δ): 1.77–2.0 (m, 2H, $-CH_2-$ of PVP main chain), 2.12–2.23 (m, 2H, $-CH_2-$ of VP ring), 2.24–2.32 (s, 2H, $-CH_2-$ of VP ring), 3.15–3.28 (t, 2H, $-CH_2-$ of VP ring), 3.47–3.66 (m, −CH− of main chain), 6.83–7.39 (m, 1H, CH of catechol).

2.3. Preparation of PVP-CCDP coated IONPs

The synthesis procedure used for capping IONPs with PVP-CCDP was followed by Mattoussi and co-workers with a little modification [30]. Briefly, 50 mg of Fe_3O_4 nanoparticles were dispersed in 5 ml of THF and this solution was added dropwise into 250 mg of PVP-CCDP that was initially dissolved in 30 ml of ethanol. The resulted mixture was then stirred for 24 h at room temperature. After the reaction time, the solvent was evaporated in a rotary evaporator and precipitated by using hexane. The sample was then centrifuged to get a dark pellet of nanoparticles. After filtering the hexane, the product was readily dispersed in water and filtered via 0.45 μm of disposable syringe filter. Finally, the product was dried using a freeze drier for future experiment. The product yield was 56%.

2.4. Deposition of AgNPs onto the surface of PVP-CCDP coated IONPs

PVP-CCDP coated IONPs (200 mg) was placed in a reaction vessel and dispersed in 80 ml of Tris buffer (100 mM, pH 8.5) solution. A solution of $AgNO_3$ (0.12 mmol) in Tris buffer (20 ml) was slowly added to the reaction flask in the dark under vigorous stirring at room temperature overnight. The experiments were carried out in the dark due to photo responsiveness of $AgNO_3$. After the reaction, yellow–brown product of PVP-CCDP coated IONPs/Ag^0 was obtained and the sample was separated from the suspension by centrifugation and washed with water several times. Lastly, the product was collected through freeze drying. The product yield was 45%.

2.5. Material characterization

^{1}H NMR spectra were recorded on a Bruker Avance 400 spectrometer using deuterium oxide as the solvent. The UV–vis spectra were recorded using Optizen 2020UV, Mecasys Co. FE-SEM micrographs and EDX spectra were obtained with a (JSM-6700, GELO) where samples were prepared by powder. TEM pictures were investigated using FE-TEM, 200 kV, Technai F-20 (FEI, Netherlands). Particle size was measured by using dynamic laser light scattering (Zetasizer Nano, Malvern-Germany). XPS spectra were obtained by using an Omicrometer ESCALAB (Omicrometer, Taunusstein, Germany). X-ray diffraction (XRD) patterns were recorded by using a Bruker-D8-AXS diffractometer system equipped with CuKα radiation. The magnetic properties of the prepared samples were measured with an alternating gradient magnetometer (AGM, 2900-02 AGIM, PMC Co.). ICP-MS was used to analyze the composition, which was performed using a Bruker 820-MS/Varian ICP-MS.

2.6. Antibacterial activity PVP-CCDP coated IONPs/Ag^0

The antibacterial activity of the PVP-CCDP coated IONPs/Ag^0 was determined against the Gram-positive bacteria *S. aureus* and Gram-negative bacteria *E. coli* through a viable cell counting method [31]. A colony of *S. aureus* and *E. coli* grown on MRS and Luria-Bertani (LB) plates were used to inoculate 10 ml MRS and LB nutrient broth, respectively and incubated at 37 °C for overnight under shaking at 150 rpm (Lab Companion SI-600R Benchtop Shaker). Overnight cultures were measured by UV–vis spectroscopy at 600 nm with absorbance adjusted to 0.6 to confirm the turbidity standard according to McFarland scale, and at this stage the cultured contained ca. ~10^8 cells/ml. Then, the microorganism suspensions were diluted and 50 μl of both bacteria (5×10^5 CFU/ml) were placed in 5 ml nutrient broth with various concentration of PVP-CCDP coated IONPs/Ag^0 and incubated for 12 h at 37 °C. Then, 0.1 ml of suspension was collected, diluted, and spread on agar plates at different time intervals. Each dilution has three parallel groups. After being rubbed for 2 min, substances were incubated at 37 °C for 24 h and the bacterial colonies were inspected. Control experiment was also conducted in the absence of magnetic NPs.

2.7. Recycling of PVP-CCDP coated IONPs/Ag^0 for antibacterial application

PVP-CCDP coated IONPs/Ag^0 (20.0 mg) was incubated with 5 ml of cell suspension of both *S. aureus* and *E. coli* (10^5 to 10^6) in 15 ml conical tube at 37 °C for 120 min with 200 rpm. After 120 min of incubation, the tube was placed in a magnetic separation stand at room temperature for 10 min. The nanoparticles were attracted to the wall of the tube via magnetic force. After removing the supernatant from the tube, 0.1 ml of the supernatant was diluted appropriately and plated onto solid MRS and LB agar plates. Each viable bacterium formed into a bacterial colony that was counted after being incubated at 37 °C for 24 h. The recycled magnetite nanoparticles were incubated with a fresh sample of 5 ml of cell suspension in the same conical