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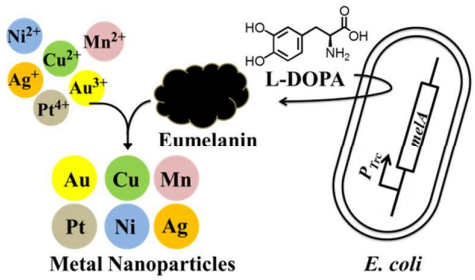

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ARTICLE TYPE

Biosynthesis and display of diverse metal nanoparticles by recombinant *Escherichia coli*

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This study used the biomolecule, eumelanin, as an agent for the reduction of metal ions. Our results demonstrate the effectiveness of synthesizing diverse metal nanoparticles through the use of recombinant *E. coli* expressing *Rhizobium etli* tyrosinase, MelA. Gold nanoparticles were recovered using cells with gold binding peptides on the surface. This study illustrates the possibility of using *E. coli* to produce and display diverse metal nanoparticles in a green chemistry synthetic route.

Nanomaterials are excellent platforms for applications involving diagnostic imaging, sensing and labeling.[1, 2] Functionalized metal nanomaterials have been studied extensively due to their unique photophysical, electronic, and magnetic properties with a wide range of potential applications in biomedicine and other industries.[3-5] The preparation of metal nanomaterials generally involves the chemical reduction of metal salts using strong reducing agents.[6, 7] In nature, many microorganisms respond to metal stress through the precipitation of toxic metals from solutions. For example, *Cupriavidus metallidurans* is able to detoxify toxic gold ions through a reduction of Au(I/III) to metallic gold.[8] Numerous researchers have been attracted by the robustness of biosynthesizing nanoscale materials *via* the green chemistry synthetic route at ambient temperature in aqueous solutions.[9-12] Mugdha *et al.* recently explored the synthesis of cell-associated gold in yeast *Yarrowia lipolytica* by inducing the production of melanin.[13]

Eumelanin (EuMel), a type of melanin pigment, has been identified in prokaryotic as well as eukaryotic cells.[14] The function of melanin in microorganisms remains unclear; however, metals have been shown to bind the structure of melanin.[15] Tyrosinase is a key enzyme in EuMel production, catalyzing the oxidation of 3-(3,4-Dihydroxyphenyl)-L-alanine (L-DOPA) and further to dopaquinone and melanin. Studies of EuMel indicated that this pigment contains carboxyl, amine, hydroxyl groups, quinone and semiquinone groups.[16] The conversion of the phenolic groups to the quinone groups could serve as reducing agents for the transformation of metal ions into elemental forms. Recombinant *E. coli* cells have been shown to produce dark melanin pigment when expressing *melA* gene from *Rhizobium etli* CFN42.[17, 18] Herein, this study reports a new approach to the tunable bioreduction of diverse metal ions through the genetic incorporation of *Rhizobium* tyrosinase gene (*melA*) in recombinant *E. coli* cells with exogenous metal ions.

We began by testing the biosynthesis of gold nanoparticles *in vitro*. *R. etli* cells were incubated with 1mM of L-DOPA for 12 h, resulting in a dark brown color. The UV- visible spectra of the solution was shown in Fig. S1A, ESI†. The typical absorbance spectra of EuMel was observed.[19] Next, the supernatant was collected through centrifugation at 12,225 g for 15 min. In the presence of L-DOPA and gold ions, a reddish color change was observed in the solution (Fig. 1A, S1B). The absence of either L-DOPA or gold ions failed to result in any change in color, indicating that the formation of gold nanoparticles depends on the presence of EuMel as well as gold ions. Similar result was observed from chemically produced EuMel (Fig. S1B). We then conducted transmission electron microscopy (TEM) analysis of gold nanoparticles (Fig. 1B). Figure 1B presents a TEM image of the gold nanoparticles produced through the incubation of EuMel with 2mM Au^{3+} salt.

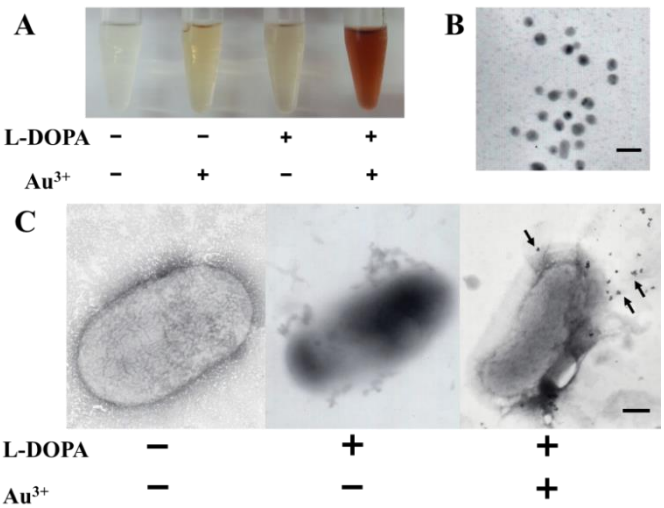

Fig. 1 EuMel from *R. etli* mediates the synthesis of gold nanoparticles. (A) Photograph of solutions in the presence or absence of 1 mM L-DOPA and Au^{3+}, respectively. (B) TEM image of gold nanoparticles synthesized by EuMel. Scale bar: 20 nm. (C) TEM images of *R. etli* cells. Cells were incubated with or without 1 mM L-DOPA for 12 h. Subsequently, 2mM of Au^{3+} or water was added. Scale bar: 200 nm.

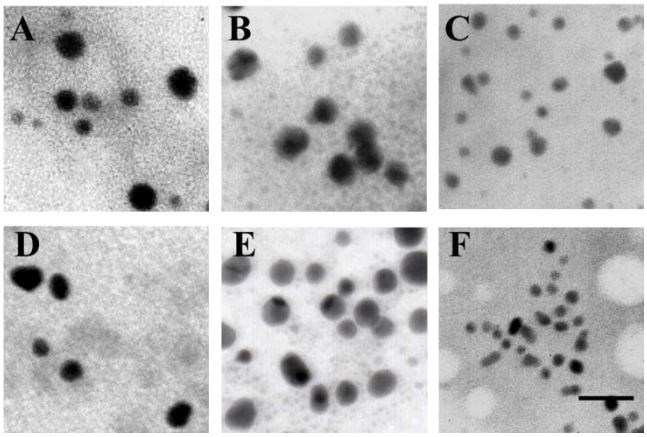

Fig. 2 TEM images of diverse metal nanoparticles synthesized by recombinant *E. coli*. 100nM IPTG and 1 mM L-DOPA were used to induce the expression of EuMel for 12 h. EuMel solution was incubated with the corresponding 2mM of metal ions. (A) Au, (B) Ag, (C) Cu, (D) Mn, (E) Pt, and (F) Ni. Scale bar : 50 nm.

The TEM analysis showed that the particle size was distributed with an average particle diameter of 11.7±5.8 nm (n = 50). To further validate the formation of gold nanoparticles in *R. etli* cells, we performed TEM analysis for *R. etli* cells. Notably, extracellular gold nanoparticles appeared when incubating cells with L-DOPA and gold ions for 12 h (Fig. 1C, arrows). The formation of gold nanoparticles was not observed in the absence of either L-DOPA or gold ions.

We then tested whether it was possible to synthesize gold nanoparticles with EuMel based on the fact that recombinant *E. coli* cells express the plasmid carrying the *R. etli* tyrosinase (*melA*) gene under the control of promoter P_{Trc} on a pBbE1k plasmid.[20] EuMel was observed by inducing the exponential-phase of *E. coli* cells through the addition of 100 nM IPTG and 1mM L-DOPA. The absorbance spectrum was similar to chemically produced melanin (Fig. S1C). The concentration of EuMel was calculated from standard curves (Fig. S2). After 12 h, the supernatant containing EuMel was collected and incubated with 2mM of Au^{3+} solution at room temperature for 3 min. We again observed a color change similar to that previously described. The spectrum of the solution revealed a maximum absorption peak at 535 nm, which is attributed to the surface plasmon band of gold nanoparticles (Fig. S3). We next prepared a variety of metal nanoparticles such as Ag, Cu, Mn, Pt, and Ni by mixing EuMel solution with 2mM metal salts. TEM and energy-dispersive X-ray spectroscopy (EDS) analyses were conducted to provide further confirmation of the metal particles. Representative TEM images of nanoparticles are shown in Fig. 2 A-F. The spherical particles presented a size distribution of 12 to 20 nm as calculated from TEM images (Table S4). Histograms of particle size distribution are presented in Fig. S4A-F, inset. The elemental composition of the representative field was according to EDS spectra (Fig. S4A-F). We then examined the possibility of tuning the size of the nanoparticles by altering the concentration of the reactants. Gold nanoparticles were used as an example with which to compare the diameter of nanoparticles formed from various metal ions and EuMel concentrations. As shown in Table S5A, the diameter of the gold nanoparticles increased with the concentrations of Au^{3+}

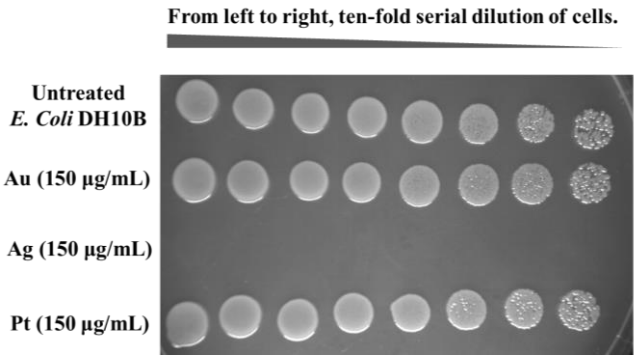

Fig. 3 Growth and comparison of various metal nanoparticles treated cells. Quantitative survival measurements of *E. coli* DH10B in the presence or absence of 150µg/mL of Au, Ag, or Pt nanoparticles.

up to 2 mM. No further increase in size was observed at higher concentrations (Fig. S5A). In addition, we also evaluated the effect of EuMel concentration on particle size. The average diameter of Au nanoparticles increased slightly under higher concentrations of EuMel (Table S5B and Fig. S5B). These results suggest that the size of the gold nanoparticles is tunable between approximately 7 and 13 nm, by altering the concentration of the reactants.

We then tested the application of the EuMel-mediated metal nanoparticles as effective antibacterial agents. We compared the toxicity of Au, Ag, and Pt nanoparticles against *E. coli* DH10B by evaluating the number of colony forming unit (CFU) following the treatment of cells supplemented with 150 µg/mL of nanoparticles for 8 h. Bacterial growth was examined by spotting of cells onto agar plate. Figure 3 shows that Ag nanoparticles exhibited better antibacterial property than do Au/Pt nanoparticles. Furthermore, we compared the antibacterial activity of Ag nanoparticles prepared by EuMel and $NaBH_4$ (Fig. S6). Interestingly, EuMel-mediated Ag nanoparticles showed better antibacterial property.

We then sought to synthesize and display the metal nanoparticles on cell surface simultaneously. The metal particles were displayed by fusing metal binding peptides with FhuA, an outer membrane iron transporter protein.[3] We selected a gold binding peptide[21] to examine the display of gold nanoparticles by fusing it with FhuA (FhuA-GBP). A strain containing pTrc-melA

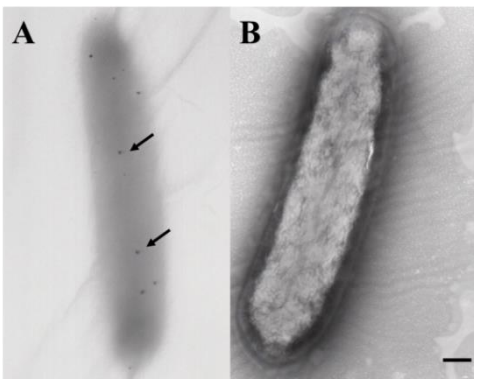

Fig. 4 Displaying gold nanoparticles on cell surface *via* FhuA-GBP (A) Following incubation with 1 mM L-DOPA, 100nM IPTG, 0.2% arabinose, and 2mM of Au^{3+}, the cells were washed and analysed. (B) Similar procedure was performed as described in (A), but arabinose was omitted in the medium.

and pBAD-fhuA-gbp plasmids was grown in the presence of inducers, 1mM L-DOPA, and 2mM Au^{3+} followed by TEM analysis of the gold deposition on the cell surface (Fig. 4). As shown in Fig. 4A (arrows), the binding of gold nanoparticles occurred in cells expressing FhuA-GBP. Binding of gold nanoparticles to cells without arabinose showed the minimal background, suggesting that the observed binding was not due to nonspecific gold nanoparticle aggregation (Fig. 4B). These findings demonstrate the effectiveness of the proposed method in the synthesis and display of metal nanoparticles.

In summary, this paper presents a simple, room-temperature, and the green chemistry synthetic method for the biosynthesis of nanoparticles. We illustrated how the EuMel biosynthesis pathway in *R. etli* can be exploited for the synthesis of metal nanoparticles through the use of recombinant *E. coli* with L-DOPA. While we demonstrate that the facile recovery of gold nanoparticles by gold binding peptide-tagged cells *via* surface display, the efficiency of gold nanoparticle recovery is low. A comprehensive screening of surface display carrier or metal binding peptide should allow us to enhance the applicability of the method in the conjugation of bacterial surfaces with diverse metal nanoparticles synthesized from EuMel.

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Notes and references

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† Electronic Supplementary Information (ESI) available: [Supporting Tables S1-5 and Supporting Fig.S 1-6.]. See DOI: 10.1039/c000000x/

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