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Synthesis of Gold Nanoparticles: An Ecofriendly Approach Using *Hansenula anomala*

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Supporting Information

ABSTRACT:

This work describes a bioassisted approach for the preparation of metal nanoparticles using yeast species *Hansenula anomala*. Gold nanoparticles were prepared using gold salt as the precursor, amine-terminated polyamidoamine dendrimer as the stabilizer, and the extracellular material from *H. anomala* as the bioreductant. It could also be demonstrated that, using our approach, small molecules such as cysteine can act as stabilizers as well. This synthetic approach offers a greener alternative route to the preparation of gold sols that are devoid of cellular and toxic chemical components. The ability of as-synthesized gold sol to function as biological ink for producing patterns for the analysis of fingerprints and to act as an antimicrobial reagent is evaluated. The generality of this toxin-free synthetic approach to other metals was assessed using palladium and silver.

KEYWORDS: antimicrobial agent, bioassisted approach, bioreductant, extracellular material, *Hansenula anomala*, metal nanoparticles, PAMAM dendrimers

1. INTRODUCTION

There have been fascinating developments in the area of nanotechnology in the recent past, with numerous methodologies formulated to synthesize nanoparticles (NPs) of predefined shape and size depending on specific requirements.[1−3] The general methodology includes either physical or chemical processes and involves the use of toxic, hazardous, and nonenvironmentally friendly chemicals. Hence, there is a growing need to develop an environmentally benign NP preparation that does not use toxic chemicals in the synthetic protocols.[4,5] Inspired by biological systems, researchers have turned their attention toward using biomacromolecules as a template for the synthesis of metal sols.[6] In such a biomimetic[7] approach, the use of microorganisms, in a deliberate and controlled manner, to synthesize NPs is a relatively new and exciting area of research with considerable potential for development.[8] The interactions between microorganisms (e.g., yeast, fungi, bacteria) and metals have been well documented.[9] The ability of such microorganisms to extract and/or accumulate metals is already employed in biotechnological processes such as bioleaching and bioremediation.[10−12] Although the area concerning the use of microorganisms in synthetic nanomaterials is limited, it is in great progress from the past few years.

Gold NPs (GNPs) are considered biocompatible, but chemical syntheses of such nanomaterials may still lead to the presence of toxic chemical species adsorbed on their surface.[13,14] These toxic chemicals may have adverse effects in medical and biological applications. Hence, the development of NP synthetic techniques that utilize ecofriendly biomacromolecules is well received in the research community.[15,16] The use of microorganisms or plants in NP synthesis can potentially eliminate the problem of toxicity by making the NPs biocompatible.[17] Earlier work involving microorganisms include the formation of iron oxides by bacteria and syntheses of metallic nanocrystals through the bioreduction of metal ions inside bacterial, viral, and fungal species.[18] Recently, enzymes were also employed as biocatalysts to synthesize or grow metal NPs. Some examples reported recently are the tyrosinase-stimulated generation of GNPs in the presence of tyrosine, the glucose-oxidase-mediated growth of GNPs, and the biocatalyzed synthesis of enlarged GNPs or gold nanorods.[19−21] Simple molecules like β-D-glucose and starch

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have also been used as reducing and stabilizing agents, respectively, for the preparation of GNPs through the green chemistry approach. The synthesis has been extended successfully to the direct use of starch-rich foods such as potato, carrot, and onion to synthesize GNPs.[22,23] The nonconventional yeast *Yarrowia lipolytica* has been shown to produce GNPs within the cells. However, researchers have not tried to prepare NPs free of cellular material.[24,25] Although these methods offer synthetic routes for a variety of metal and metal oxide NPs, they also leave traces of biomaterial. These biomaterials may significantly affect the properties and overall performance of as-prepared nanomaterials for a given application, including sensing applications. Hence, the identification of synthetic routes involving toxin- and biomass-free NP preparation is advantageous. The extraction of bioreductants from microorganisms or plants, followed by metal NP preparation, offers a solution to such problems.

From our earlier work with the yeast *Hansenula anomala*, we know that the yeast can donate electrons and act as a catalyst in a biofuel cell.[26] The reducing ability of this yeast is leveraged in the current work. Our studies on *H. anomala* have shown that the isolated membrane fraction of the species contains *Lactate dehydrogenase* (cytochrome *b*2), NADH *Ferricyanide reductase*, NADPH *F. reductase*, and cytochrome. In earlier reports, the presence of cytochrome *b*2 on the outer membrane has been advantageously used for the sensing of lactate.[27] The presence of electroactive enzymes in the membrane suggests that they can mediate the transfer of electrons generated during metabolism to any electron acceptor (metal ions or electrodes) directly without external mediators in the absence of oxygen. This concept has motivated us to use the reducing ability of this yeast to produce GNPs. If we make use of whole cells for the preparation of NPs, it will become a difficult task to harvest NPs free from cellular material. To prepare GNPs free from celluar material and to develop a preparation procedure that can be scaled up in the next stage, we thought of extracting the bioreductants from the yeast cells in order to use the same as a reagent for the preparation of GNPs. Hence, the yeast was allowed to undergo fermentation in the presence of lactate for 2 weeks to produce bioreductants.

Here we report the synthesis of GNPs by reducing an aqueous solution of a gold salt using bioreductants isolated from the yeast species *H. anomala*. The generality of this method and the applicability of isolated bioreductants to reduce other metal salt solutions to their respective metals is verified by extending the synthetic protocols to silver and palladium salts. The NPs thus prepared are stabilized by amine-terminated poly(amidoamine) (PAMAM) dendrimers. The possibility of using a small molecule such as cysteine as a stabilizer for GNPs is also demonstrated. The ability of prepared GNPs to function as biological ink, as an antimicrobial agent, and for analysis of fingerprints is explored using simple experiments.

2. MATERIALS AND METHODS

2.1. Materials. A generation 4 (G4) PAMAM dendrimer (ethylenediamine core) in methanol (10 wt %) and generation 5 (G5) in methanol (5 wt %) solution was purchased from Sigma-Aldrich. $H(AuCl_4)\cdot 3H_2O$ in 12.7% HCl was purchased from Merck. Cysteine and NADH were purchased from SRL India. The chemicals were used as received unless otherwise mentioned.

2.2. Isolation of the Bioreductants from *H. anomala*. *H. anomala* was cultured using standard culture media (see the Supporting Information) and grown for 48 h. The cells were harvested by centrifugation and were suspended in 25 mL of a phosphate buffer containing lactate (1 mM) solution. The solution was allowed to undergo anaerobic degradation for nearly 2 weeks under strictly deaerated conditions. Afterward, the cells were removed by centrifugation. The supernatant solution containing the bioreductants was isolated and used for NP preparation.

2.3. Synthesis of Metal NPs Using Isolated Bioreductants. Toward this goal, the gold salt (2 mg of gold) solution was initially mixed with G4 PAMAM dendrimers (0.025 g). Then the bioreductant solution (10 mL) prepared above was added. The reaction mixture turned from yellow to purple over a period of 3 h. The bioreductants were removed by dialysis, and the resulting solution was centrifuged at 10 000 rpm for 15 min at ambient temperature. Experiments were also performed by replacing G4 with G5 PAMAM dendrimers (0.025 g) and cysteine. The same procedure was followed for the preparation of Ag and Pd NPs.

2.4. Preparation of GNPs Using Whole Cells of *H. anomala*. In a typical experiment, *H. anomala* was cultured using standard culture media and grown for 48 h. The cells were then harvested by centrifugation, followed by suspension in 5 mL of phosphate buffer. To 1 mL of the cell suspension were added gold chloride (2 mg of gold), amine-terminated G4 PAMAM dendrimer (0.025 g), and 1 mM lactate. The sample was deaerated completely to maintain anaerobic conditions. The solution turned purple within a few hours, and the cells were separated from the supernatant by refrigerated centrifugation. Both the precipitate and supernatant were characterized by UV−vis spectroscopy and transmission electron microscopy (TEM).

2.5. Sodium Dodecylsulfate Poly(acrylamide) Gel Electrophoresis (SDS-PAGE) Experiments. SDS-PAGE experiments were run with electrophoresis unit of Amersham Bioscience U.K. Ltd. SDS-PAGE experiments were performed to identify and characterize the bioreductants and stabilizing groups present on the surface of the GNPs. SDS is the anionic surfactant that provides a negative charge to the proteins. We have used a procedure following discontinuous gel electrophoresis, i.e., two types of gels were used; $^1/_4$ of stacking gel (5%) and $^3/_4$ (12%) of separating gel. The SDS plates were cleaned with water and assembled in the gel stand. Regarding the separating gel, a 12% composition of the gel was prepared. In this, the acrylamide gels are formed by polymerizing 30% acrylamide with a cross-linker [bis(acrylamide)] in the presence of a catalyst (*N*,*N*,*N*′,*N*′-tetramethylethylenediamine) and an initiator (ammonium persulfate) with a suitable gel buffer. After the separating gel was poured in the plate, water was added to prevent inhibition of polymerization due to the presence of oxygen. Then, the stacking gel was formed above the separating gel. Before polymerization of the stacking gel, the comb was placed in order to get wells to load the sample. About 100 μL of the bioreductants was mixed with 25 μL of a sample buffer [containing the following: bromophenol blue, a pinch; 2-mercaptoethanol, 0.5 mL; SDS, 150 mg; glycerol, 1 mL; stacking gel buffer, 1.25 mL; distilled water, 7.25 mL) and kept in a water bath at 80 °C for about 4 min. This temperature will denature the proteins, which gives a perfect separation. Then the sample was cooled suddenly, and it was loaded in the lane. GNP solutions were also mixed with the sample buffer and loaded into different lanes. We have applied the current of 8 V/cm for the stacking gel and 15 V/cm for the separating gel during the experiments. After completion, the gel was stained for about 2 h using a staining solution consisting of coomassie brilliant blue (CBB; 200 mg), ethanol (50 mL), acetic acid (7 mL), and distilled water (43 mL). Then, it was destained by a destaining solution comprised of ethanol (30 mL), acetic acid (7 mL), and distilled water (63 mL) in order to obtain clear bands for the presence of proteins.

2.6. Biological Ink. A clean gold slide of dimensions 3 × 1.5 cm (1000 Å gold coating on silicon wafers with an intermediate adhesion layer of 100-Å-thick titanium, procured from Lance Goddard Associates, Foster City, CA) was immersed in a G5 PAMAM dendrimer solution (25 μL of a 5% solution in 2 mL of ethanol) for 48 h. Then the PAMAM dendrimer functionalized slide was washed in ethanol before immersion

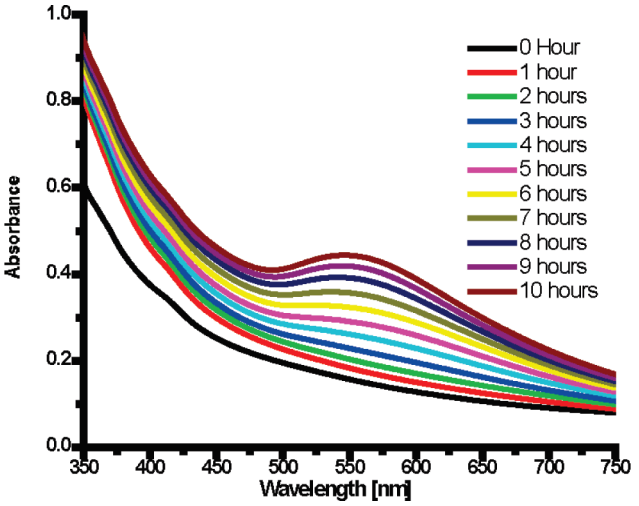

Figure 1. Absorbance spectra of GNPs, synthesized using isolated bioreductants and G4 PAMAM dendrimers, as a function of time.

in a gold chloride solution. After 15 min, the slide was removed, washed with water, and dried under dinitrogen gas. A rubber stamp containing the word "STUDENTS" was immersed in the bioreductant solution (which acts as ink), and then the stamp was pressed onto the dendrimer-functionalized gold slide gently to form an impression. In another experiment, the rubber stamp with the word "STUDENTS" was immersed in a GNP solution. The stamp with GNPs was pressed gently on a dry and clean gold slide to form an impression of the word "STUDENTS".

Another clean gold slide of dimensions 3 × 1.5 cm was immersed in a G5 PAMAM dendrimer solution (25 μL of a 5% solution in 2 mL of ethanol) for 48 h. Then the PAMAM dendrimer functionalized slide was washed in ethanol before immersion in a gold chloride solution. After 15 min, the slide was removed, washed with water, and dried under nitrogen gas. The copper grid used for TEM measurements was fixed at the end of a disposable pipet tip with an adhesive and was used as another stamp. The stamp containing the grid was immersed in the bioreductant solution (which acts as ink), and then the stamp was pressed on the dendrimer-functionalized gold slide gently to form an impression.

2.7. Antimicrobial Properties. Two Petri plates containing agar were inoculated initially with pure isolates of *Bacillus cereus* and *Pseudomonas putida* grown in nutrient broth for 24 h at 37 °C. A well was created in each plate. 100 μL of GNPs were introduced into the agar well and the inoculated plates were incubated for 24 h at 37 °C.

2.8. TEM. The morphology of the particles was estimated using a Technai G20 transmission electron microscope operating at an accelerating voltage of 200 kV. The samples for TEM were prepared by drop-casting the centrifuged product onto a carbon-coated copper grid (300 mesh, Electron Microscopy Science), and the solvent was allowed to dry in air at ambient temperature.

2.9. UV−Vis and Fourier Transform Infrared (FT-IR) Spectrophotometers. A Cary 5000 UV−Vis spectrophotometer from Varian was used for to record the absorption spectra of the samples. The samples were scanned through wavelength region 200−800 nm. FT-IR spectra of the samples were recorded using a Tensor FT-IR spectrophotometer from Bruker Optics. All of the spectra were recorded from 400 to 4000 cm^{-1}.

2.10. Particle Size Analysis. To measure the particle size(s) in solution, we used a Beckman Coulter Delsa Nano C particle size analyzer. The samples were initially filtered through a 0.4 μm syringe filter and then used for measurement of both the size and ζ potential.

2.11. Cyclic Voltammetry. Cyclic voltammograms were recorded using a PGSTAT (Autolab) potentistat/galvanostat. A calomel electrode was used as the reference electrode, platinum wire was used as the counter electrode, and a gold (diameter = 3 mm) disk electrode was used as the working electrode in a phosphate buffer at 50 mV/s.

3. RESULTS AND DISCUSSION

3.1. Preparation and Characterization of GNPs Using Isolated Bioreductants. The reduction of gold salt to GNPs was monitored by following the absorption spectra of the reaction mixture. Initially, the mixture of auric chloride, isolated bioreductants, and a G4 dendrimer solution did not show any absorbance at 550 nm (Figure 1). After 4 h, we observed a small hump formation at 550 nm, and the absorbance starts to intensify with time. From 8 h onward, the absorbance exhibited at 550 nm becomes essentially constant and the absorbance peak becomes well-defined, indicating complete conversion of the gold salt to GNPs. The absorbance at 550 nm corresponds to the surface plasmon resonance band of GNPs. A similar surface plasmon resonance absorbance is observed in the case of GNPs stabilized by a G5 dendrimer and cysteine (see the Supporting Information).

While the absorbance spectrum confirms the reduction of the gold salt to GNPs, the size and shape of the prepared GNPs were investigated using TEM. Figure 2A shows the TEM image of GNPs prepared using the isolated bioreductant solution with a G4 PAMAM dendrimer as the stabilizer. From the micrograph, it is evident that the particles are uniform and are nearly monodisperse in nature with an average size distribution of around 14 nm. Having confirmed the reduction of the gold salt to GNPs using bioreductants, we explored the effect of the stabilizer (dendrimer) on the particle size using a G5 PAMAM dendrimer. The use of a G5 dendrimer as the stabilizer led to a bigger particle size with an average size distribution of around 40 nm (Figure 2B). The concentrations of the G4 and G5 dendrimers (0.025 g) and the gold salt (0.002 g) were maintained constant during the experiments. The stabilizers form an envelope around the GNPs.

The size and ζ potential of the samples in solution were further analyzed using the particle size analyzer. The diameter and ζ potential of the G4-capped GNP were found to be 36.4 nm and −18.33 mV, respectively, while the G5-capped GNP showed values of 77.2 nm and −25.17 mV. In general, the magnitude of the ζ potential is directly related to the particle stability. The higher the ζ potential value, the greater would be the particle stability, resulting in the formation of smaller particles, as is evident from our G4- and G5-capped GNPs. Literature reports show that PAMAM dendrimers have been used as stabilizers or templates to synthesize two classes of GNPs, namely, dendrimer-stabilized GNPs (Au DSNPs) and dendrimer-encapsulated GNPs (Au DENPs). Au DSNPs are normally formed under mild reduction conditions and are composed of one GNP with a relatively large size (>5 nm) surrounded by multiple dendrimer molecules acting as stabilizers. In contrast, Au DENPs are synthesized by fast reduction and nucleation conditions and are made up of GNPs with sizes smaller than 5 nm entrapped within one single dendrimer molecule. The bioreductants are milder compared to the chemical reducing agents such as sodium borohydride. Hence, the bioreductants have led to the formation of Au DSNPS, and hence the sizes of the GNPs are larger. The reported sizes of G4 and G5 dendrimers alone are around 4.5 and 5.4 nm, respectively. If the particles are encapsulated by dendrimer molecules, they are expected to have small sizes similar to the diameter of the dendrimer. The formation of bigger particles suggests that the

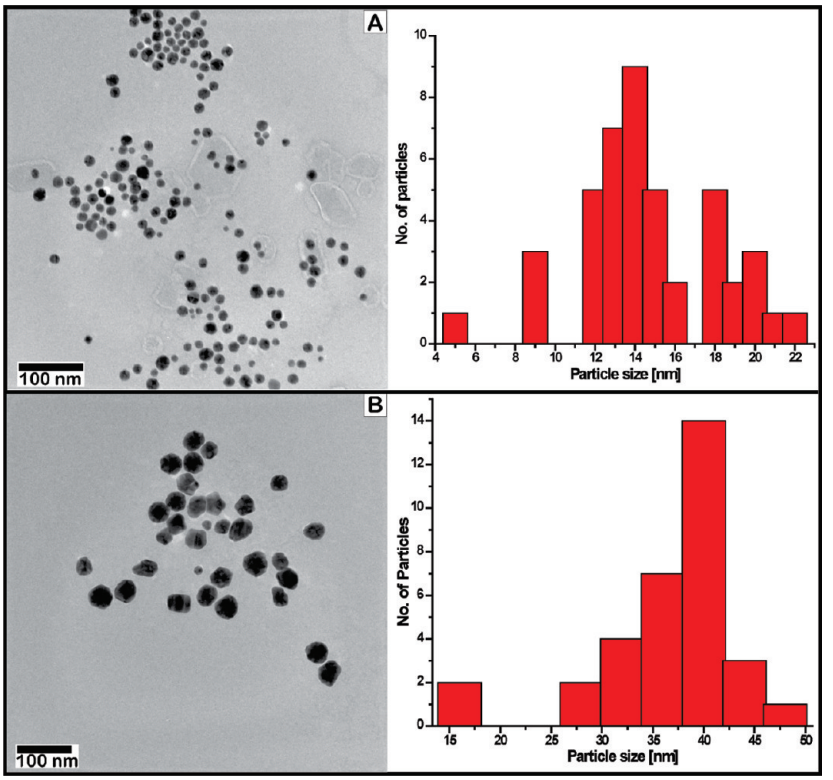

Figure 2. TEM image and particle size distribution graphs of GNPs prepared using PAMAM dendrimers (A) G4 and (B) G5.

particles are stabilized by dendrimer molecules and are not encapsulated inside the dendrimer molecules.[28−31]

The cytotoxicity of the stabilizers is a matter of concern when these GNPs are to be considered for in situ applications in biological systems. The study of the toxicity of dendrimers is controversial because a few reports suggest that they are nontoxic while others claim exactly the reverse.[32,33] In certain reports, it is claimed that the cytotoxic effect of dendrimers comes only above a certain threshold concentration. Functionalization of the amine groups has been shown to considerably reduce the toxicity of PAMAM dendrimers to a great extent in certain cases.[34,35] Hence, we also used our bioassisted approach to prepare GNPs in the presence of the simple, nontoxic molecule cysteine as the stabilizer. Figure 3 shows the TEM image of the cysteine-stabilized GNPs. This shows that our method can be tuned to the preparation of GNPs with different types of stabilizers.

3.2. Stabilization of GNPs by Dendrimers. SDS-PAGE experiments are run with bioreductant and GNP solutions and are shown in Figure 4. The bioreductant solution exhibits a band at 64 kDa, and the GNP samples exhibit a band at around 22 kDa. These results indicate that the proteins present in the bioreductant have no role in stabilizing as-formed GNPs. The presence of a dendrimer in the GNPs can be seen from the IR spectrum (see the Supporting Information). The interaction between the GNPs and dendrimers was investigated using FT-IR spectra. The FT-IR spectra of G4 and G5 dendrimers represent characteristic amide bands I and II at 1650 and 1550 cm^{-1} (see the Supporting Information), while the dendrimer-stabilized GNPs show a peak at 1640 cm^{-1}. The relatively small shift in the amide band from 1650 to 1640 cm^{-1} and the disappearance of the peak at 1550 cm^{-1} confirm encapsulation of GNPs by the dendrimer moiety.[36,37] However, the excess concentration of bioreductants

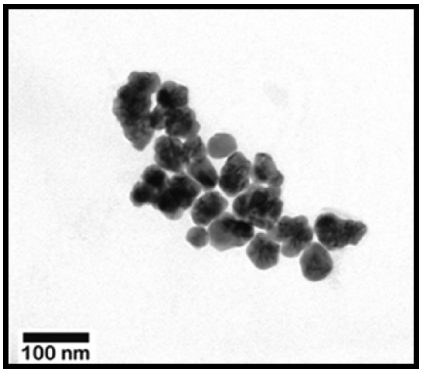

Figure 3. TEM image of GNPs prepared using cysteine as the stabilizer.

has led to the formation of GNPs stabilized by dendrimers and bioreductants. The presence of bioreductants is inferred from another set of SDS-PAGE experiments run with GNPs prepared with excess bioreductants (see the Supporting Information).

The stabilizing (NP stabilizer) ability of the dendrimer in the above syntheses was confirmed by a control experiment in which the gold salt and bioreductant were mixed without the addition of a dendrimer solution. Within 5 h of mixing of the solution, we noticed particle aggregation, which led to a large, insoluble particle formation. The solution appeared to be blue in color with a large gold aggregate (Figure 5A), while the presence of a dendrimer led to a clear purple solution, as shown in Figure 5B.

3.3. Synthesis of GNPs Using Whole Cells of *H. anomala*. The GNP synthesis carried out, as above, using whole cells (intracellular material) of *H. anomala*, without extraction of the bioreductant from *H. anomala*, resulted in a dark-purple solution.

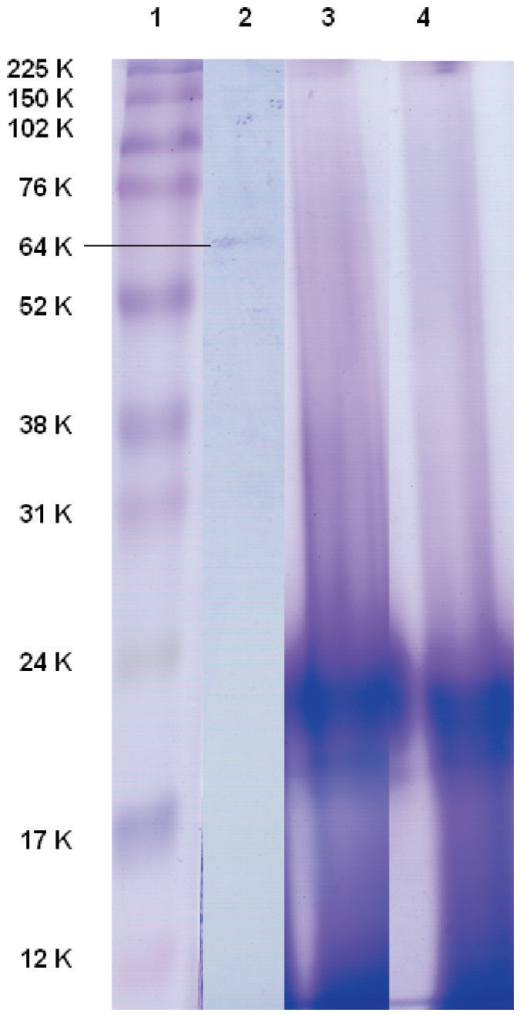

Figure 4. SDS-PAGE, stained with CBB. Lanes: 1, protein marker; 2, *H. anamola* bioreductant solution; 3, GNPs stabilized by a G4 PAMAM dendrimer; 4; GNPs stabilized by a G5 PAMAM dendrimer.

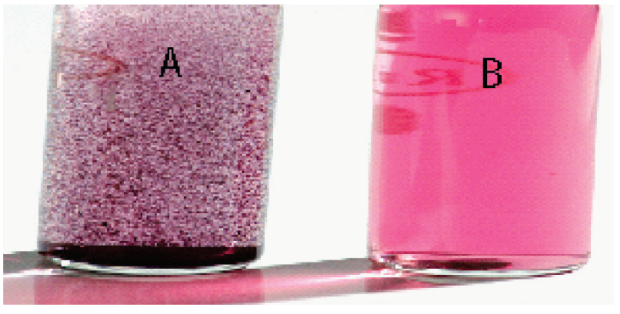

Figure 5. Digital photographs of the GNPs solution produced by the bioreductants in the (A) absence and (B) presence of a G4 PAMAM dendrimer.

The cells were precipitated by centrifugation, leaving a purple supernatant. The absorbance spectrum of the supernatant showed a broad peak at around 550 nm, confirming the reduction of the gold salt to GNPs, while the TEM analysis of the supernatant (Figure 6A) reveals the presence of polydisperse NP formation. The precipitated cells were redispersed in water and showed absorbance at around 550 nm, suggesting the reduction

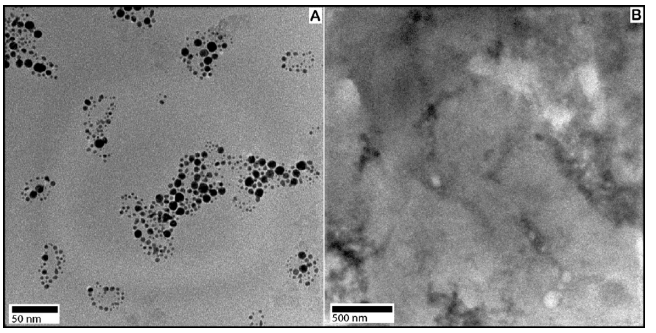

Figure 6. TEM images of GNPs synthesized using whole cell of *H. anomala*: (A) supernatant solution; (B) precipitated whole cell.

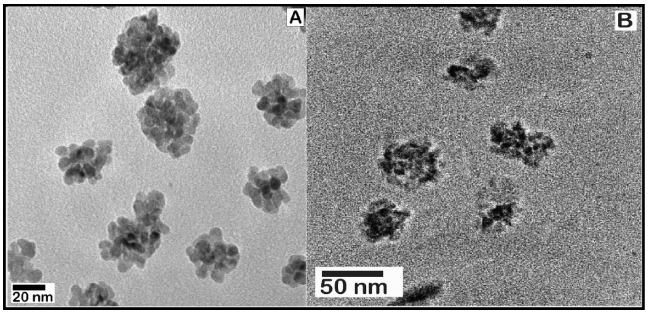

Figure 7. TEM images of (A) Pd NPs and (B) Ag NPs synthesized using bioreductants and G4 dendrimers.

of the gold salt inside the cell materials, as shown by the TEM analysis presented in Figure 6B. The presence of cell materials interferes with the particle size measurement, and efforts to clean cell materials, followed by the isolation of NPs, were not successful. These experiments suggest that though the reduction of the gold salt to gold nanomaterial is possible in the presence of whole cells and the presence of whole cells may complicate the isolation of biomass-free GNPs.

3.4. Applicability of the Synthetic Route To Prepare Other Metal NPs. The generality of the bioreductant-based reduction of the gold salt to GNPs with a dendrimer as a stabilizer is tested by extension of the synthetic protocols to other metal such as silver and palladium. The silver and palladium salts are mixed with isolated bioreductants, to which a G4 dendrimer solution was added as before. The appearance of a dark solution indicates respective metal (Ag and Pd) NP formation. TEM analysis reveals flowerlike structures for silver and palladium nanomaterials (Figure 7). The sizes of both nanomaterials are estimated to be around 35 nm. Hence, this synthetic approach can be used as a potential method for the preparation of a variety of biomass-free metal NPs. The appearance of the flowerlike structure is probably due to the aggregation of GNPs caused by the low concentration of dendrimers. In all of the preparations, the concentrations of the stabilizer and metal salt are maintained the same. The reduction of Au^{3+} ions requires three electrons. The reduction of Ag^{+} requires one electron, and the reduction of Pd^{2+} requires two electrons. Hence, for the same amount of bioreductants, the amount of metal ions reduced will be on the order of Ag > Pd >Au. Hence, more stabilizer molecules will be required in the case of Ag and Pd NPs. Because the concentrations of the PAMAM dendrimers are maintained the same, aggregation has developed in the cases of Pd and Ag NPs.

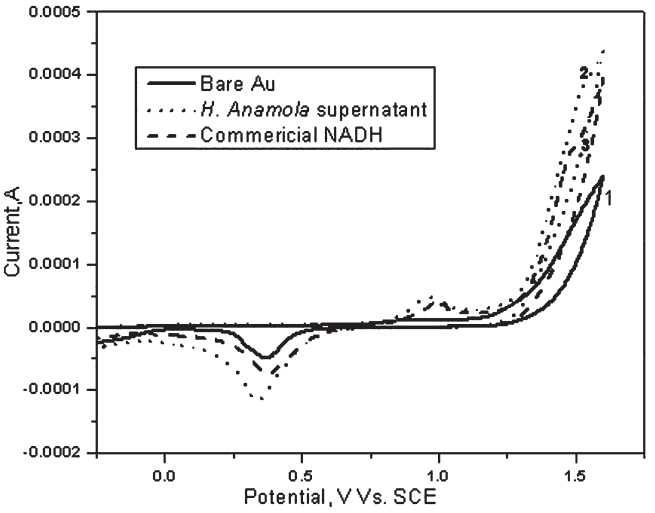

Figure 8. Cyclic voltammogram representing the electrochemical response for (1) a bare gold electrode, (2) a NADH commercial sample, and (3) a *H. anomala* bioreductant extract in phosphate buffer at 50 mV/s.

3.5. Identification of the Reductant. On the basis of the above experiments, it is clear that to have better control of the size and to prepare biomass-free NPs, one needs to extract the bioreductants from yeast *H. anomala*. Literature reports reveal that enzymes such as reductases and proteases are responsible for microbial NP synthesis.[25] The bioreductant extract was subjected to spectral, electrochemical, and qualitative analysis. The UV−vis spectrum of the bioreductant exhibited absorbance characteristics similar to those of NADH. Cyclic voltammograms of the bioreductant and the standard NADH solution both show the appearance of a redox peak at the same position, indicating that the bioreductant must be NADH (Figure 8). While the above observation agrees with the literature reports,[38] the role of NADH in the biosynthesis of metal NPs is well established also. NADH loses an electron to become NAD^+, while the released electron is utilized for the reduction Au^{3+} to Au^0.[39] The preparation of GNPs (as before) using a commercial NADH sample supports this hypothesis. The GNPs thus prepared using commercial NADH exhibited different shapes (see the Supporting Information). Although the cause of formation of differently shaped NPs is not clear, our experiment confirms that NADH isolated from the yeast *H. anomala* is responsible for conversion of the gold salt to GNPs. Our experience also shows that pure NADH is able to reduce the gold chloride solution, but it is not a good stabilizer because the color of the sol is lost after some time. However, the reduction of gold salts by NADH in the presence of a PAMAM dendrimer yields stable sols. The bioreductant solution was also subjected to qualitative analysis to test the presence of carbohydrates and reducing sugars by phenolsulfuric acid and Benedict's method, respectively. The phenolsulfuric acid test revealed the presence of carbohydrates in the bioreductant, and Benedict's method showed the absence of reducing sugars in the bioreductant. Hence, the reducing ability of bioreductants is due to NADH produced during the anaerobic fermentation of lactate by the yeast *H. anomala*.

3.6. Application of GNPs as Biological Ink and as an Antimicrobial Agent. Recently, some works have appeared that use enzymes as a "biocatalytically active" ingredient in the nanopatterning process.[40−42] Immobilized protease was used for

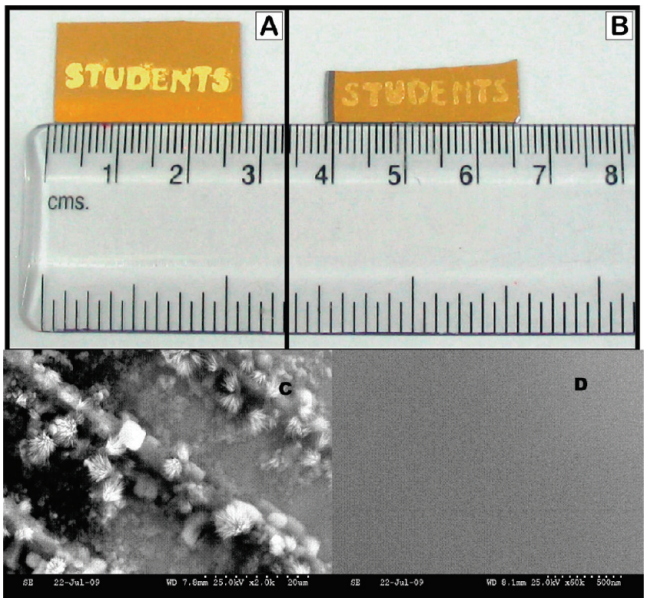

Figure 9. Digital photographs of the pattern created by the rubber stamp technique: (A) isolated bioreductant stamped on a dendrimer-functionalized gold salt containing substrate; (B) GNPs synthesized using an isolated bioreductant stamped on a substrate. SEM images of (C) A and (D) B.

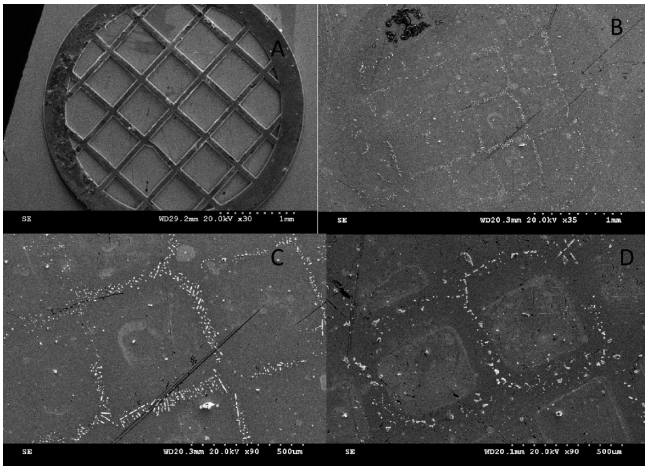

Figure 10. SEM images of (A) the copper grid and (B) the grid formed with the help of bioreductant ink. (C and D) Expanded views.

etching of the peptide layers, and DNase was implemented as a "biocatalytic ink" for the patterning of a DNA self-assembled monolayer. The use of enzymes and DNase as biological inks turns out to be very expensive because of the high costs associated with the enzymes and DNase. Isolation of the bioreductants from the anaerobic fermentation of yeast provides a viable and relatively inexpensive alternative protocol. Along this line, the suitability of using a GNP solution and the bioreductants as inks for rubber stamps has been verified as described in the Experimental Section. Figure 9A presents the pattern created by the rubber stamp with the bioreductants acting as the ink, Figure 9B presents a photograph of the gold slide containing pattern produced with GNPs as inks, while the corresponding scanning electron microscopy (SEM) images are shown in Figure 9C,D. The SEM image of

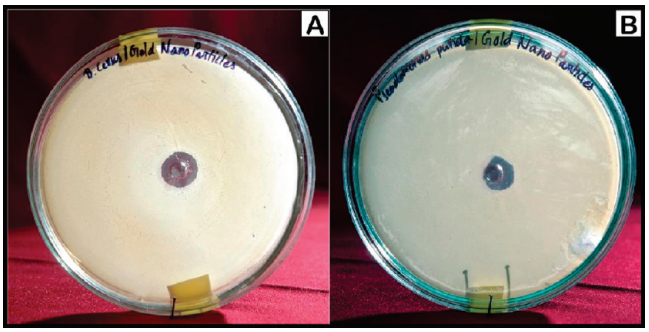

Figure 11. Digital photographs of agar Petri plates containing GNPs at the center that are inoculated with (A) *B. cereus* and (B) *P. putida*.

the slide shows that the pattern formed with the GNP ink consists of particles. The SEM image formed with the bioreductant ink shows linelike features. This indicates that the PAMAM dendrimers on the gold slide direct the preconcentrated gold ions in the form of a line. When the bioreductant ink encounters the gold ions, they are reduced along the line. Hence, the patterns show linelike features.

Figure 10A presents the SEM image of the copper grid used in TEM imaging, and parts B−D of Figure 10 show images of the copper grid stamp formed on the gold slide as described in the Experimental Section. Although a clear replica of the copper grid could not be formed, the features of the grid are translated onto the gold slide to a reasonable extent.

Parts A and B of Figure 11 indicate the antimicrobial properties of GNPs. The absence of microbial growth around the initially formed well indicates that the GNP suspension exhibits antimicrobial properties and arrests the growth of *B. cereus* and *P. putida*. The inhibition zone has a diameter of 8 mm in the case of *P. putida* and 12 mm in the case of *B. cereus* (see the Supporting Information). The antimicrobial properties of the NPs are likely to be due to the high surface area of the NPs, which can bind to bacteria and virus to a large extent and pose toxicity to the cells. Bacterial proteins present in the cell wall and cytoplasm play a vital role in the healthy functioning of the cell. When these proteins interact with NPs, the normal functioning of these proteins is disturbed, which ultimately causes the death of the cells. Gold has a greater tendency to react with sulfur- or phosphorus-containing soft bases. Therefore, the sulfur-containing proteins present in the membrane or inside the cells and phosphorus-containing DNA molecules are preferential sites for attack by GNPs. The gold ions also bind to thiol groups present in enzymes such as NADH dehydrogenases and disrupt the respiratory chain, facilitating the release of active oxygen species, leading to oxidative stress, and resulting in significant damage to the cell structures and ultimate cell death.[43]

Our results also show that the lyophilized GNP powders can be used for the analysis of fingerprinting. The scanned image of the fingerprint formed on a transparent sheet used for overhead projection (see the Supporting Information) shows the scope for using GNP powders for fingerprinting analysis.

4. CONCLUSIONS

In summary, we have demonstrated that anaerobic metabolism of lactate by *H. anomala* has resulted in the production of an electron-donor source. The isolated electron-donor source serves as a bioreductant for the preparation of silver, gold, and palladium metal NPs. Further, the monodispersity, size, and aggregation behavior of the metal NPs, especially GNPs, produced through the bioassisted route are controlled by the addition of PAMAM dendrimers of different generations. The bioassisted approach offers an environmentally friendly alternative route to metal NP synthesis, which otherwise will be prepared using toxic and nonenvironmentally friendly chemicals. The gold sol prepared through the bioassisted approach was evaluated for different applications. It has been demonstrated that the gold sol can be used as inks for rubber stamps, as antimicrobial reagents, and for the analysis of fingerprints.

■ ASSOCIATED CONTENT

Supporting Information. Subculturing of *H. anomala*, UV−vis spectra of GNPS showing the surface plasmon band, FT-IR spectra of GNPs, SDS-PAGE experimental results, TEM images of GNPs prepared with commercial NADH, the antimicrobial zone formed with the GNP solution with *B. cereus* and *P. putida*, and fingerprint analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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