

Advances in microbial biosynthesis of metal nanoparticles

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Abstract Metal nanoparticles are garnering considerable attention owing to their high potential for use in various applications in the material, electronics, and energy industries. Recent research efforts have focused on the biosynthesis of metal nanomaterials using microorganisms rather than traditional chemical synthesis methods. Microorganisms have evolved to possess molecular machineries for detoxifying heavy metals, mainly by employing metal-binding proteins and peptides. Biosynthesis of diverse metal nanoparticles has recently been demonstrated using such heavy metal detoxification systems in microorganisms, which provides several advantages over the traditional chemical synthesis methods. First, metal nanoparticles can be synthesized at mild temperatures, such as at room temperature, with less energy input. Second, no toxic chemicals or reagents are needed, and thus the process is environmentally friendly. Third, diverse metal nanoparticles, including those that have never been chemically synthesized, can be biosynthesized. Here, we review the strategies for the biosynthesis of metal nanoparticles using microorganisms, and provide future prospects.

Keywords Biosynthesis · Metal nanoparticles · Metal-binding proteins · Simple synthesis method

Introduction

Nanomaterials are at the center of academic and industrial attention owing to their numerous potential applications. Synthesis of nanomaterials and control of their characteristics and properties have been explored for diverse applications (Choi et al. 2010a, 2012a; Lee et al. 2010a; Kwon et al. 2012). Techniques and protocols for the synthesis of various inorganic metal nanomaterials have been developed for a wide range of applications, including biosensors and chemical sensors, bioimaging, catalysis, optics, electronics, drug delivery, and energy (Hergt and Dutz 2007; Xiang et al. 2007; Choi et al. 2010b, 2012b; Lee et al. 2010b; Yang et al. 2010, 2011). For example, various nanomaterials have been tested as specially controlled carriers in drug delivery systems for drug transport to the cellular target (Wilczewska et al. 2012) and used to convert solar energy directly into steam for sanitation and water purification (Neumann et al. 2013).

Although nanomaterials have great potential for further applications, production of nanoparticles, nanocomposites, and nanoscale materials, and the control of their characteristics and properties remain great challenges in the field of nanotechnology (Daryoush and Darvish 2013; Liu et al. 2013). Conventional method for the synthesis of inorganic metal nanomaterials often requires the use of organic solvents and/or high-energy input. Recently, there has been much interest in the synthesis of inorganic metal nanoparticles using environmentally friendly methods, rather than typical organic solvent-based synthetic approaches (Bhattacharya and Gupta 2005; Hutchison 2008; Daryoush and Darvish 2013). In the last few decades, microorganisms such as bacteria, yeast, and

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fungi have successfully been employed for the biosynthesis of inorganic metal nanomaterials (Dameron et al. 1989; Labrenz et al. 2000; Mukherjee et al. 2001; Sriprang et al. 2003; Bharde et al. 2006; Kang et al. 2008). Metal nanoparticles can simply be produced *in vivo* by cultivating specific microorganisms possessing metalloregulatory molecules, particularly proteins and peptides involved in metal detoxification process, under certain conditions by providing metal cations in the culture medium.

In this paper, we review recent trends and advances in the biosynthesis of inorganic metal nanoparticles using microorganisms. Although the mechanisms of *in vivo* reduction of metal ions are not clear, biosynthesis of increasing number of metal nanoparticles is being reported (Zhang et al. 2011). Thus, the metal detoxification mechanisms by metal ion regulators, transporters, ligands, and metal-dependent enzymes, and other binding proteins in microorganisms involved in such metal-reducing processes are first reviewed. Then, strategies for the biosynthesis of metal nanoparticles using various microorganisms and the controllability of morphology and size are reviewed. Finally, perspectives on the future use of biosynthetic techniques and their potential applications are discussed.

Reduction of heavy metal ions by microorganisms

Microbial cells need metal ions mainly as cofactors for the proper functions of various enzymes and proteins. However, heavy metal ions interfere with the normal protein functions of microorganisms and are extremely toxic. Thus, the cells have evolved the ability to manage proper metal-protein interactions (Totter et al. 2005). Indeed, several organisms, such as bacteria, algae, yeast, and fungi are capable of reducing metal ions through metalloregulatory mechanism upon exposure to metal ions (Dameron et al. 1989; Labrenz et al. 2000; Mukherjee et al. 2001b; Kang et al. 2008). Details on the cellular mechanisms for the uptake and storage of metal ions by specific transporters and their related enzymes have been described previously (Vignais et al. 2001; Clugston et al. 2004; Kuchar and Hausinger 2004; Rodionov et al. 2006). Based on such capabilities, microorganisms have long been employed in the bioremediation of toxic heavy metals (Stephen and Macnaughton 1999; Kowshik et al. 2003; Reith et al. 2009). For survival in harsh environments such as sludge and metal-enriched polluted environments, the reduction and reaction processes of metal ions in microbial cell metabolism serve a key role in the maintenance of cellular activities (Bazyliński et al. 1988; Labrenz et al. 2000; Cobbett and Goldsbrough 2002; Shankar et al. 2003; Konishi et al. 2007; Mitra and Rensing 2007). Formation of metal nanoparticles from heavy metal ions occurs through the reduction of the metal ions, resulting in the formation of insoluble complexes. This mechanism was recently employed for the biosynthesis of diverse metal nanoparticles using microorganisms

engineered to express heavy metal-binding proteins and/or peptides (Park et al. 2012).

Metal-binding polypeptides in microorganisms

Several microorganisms have been studied for their abilities to synthesize metal nanoparticles. However, the mechanisms of metal nanoparticle formation remain poorly understood. Furthermore, cellular structures and/or biomolecules that play important roles in the formation or biosynthesis of inorganic metal nanoparticles intracellularly or extracellularly are not well known. It has been proposed that cell walls could act as nucleation sites for the synthesis of metal nanoparticle seeds, and for further growth into metal nanoparticles. One of the well-established mechanisms is that certain peptides, such as phytochelatin (PC), or proteins, such as metallothionein (MT), are overexpressed in microorganisms upon exposure to heavy metal ions (Cobbett and Goldsbrough 2002). The roles of PC and MT have been investigated for an improved understanding of their roles in the biosynthesis of metal nanoparticles. In this section, we will briefly review the roles of PC and MT in the biosynthetic process.

PC is a well-known peptide that binds with heavy metal ions and has been employed for heavy metal detoxification processes. Previously, PC was isolated from cell suspension cultures after exposure to Cd ions (Hirata et al. 2005). PCs are generally composed of only three amino acids, L-cysteine, L-glutamate, and L-glycine and exhibit the primary structure of $(\gamma\text{-Glu-Cys})_n\text{-Gly}$, where n is in the range 2–5; PCs in different organisms have different chain lengths. PCs are generally overexpressed in cells upon exposure to heavy metal ions such as Cd, Cu, Hg, Pb, and Zn. PCs then form complexes with the metal ions through metal ion reduction and metal-binding affinity. PCs are generally found in higher plants (Grill et al. 1988; Gekeler et al. 1989), fungi (Grill et al. 1986), yeast (Gekeler et al. 1988), and microalgae (Grill et al. 1987).

MT is a low molecular weight, cysteine-rich, metal-binding protein, which was discovered during the identification of a Cd-binding protein present in horse kidneys (Cobbett and Goldsbrough 2002). Unlike PC, MT has been identified in animals and plants, as well as prokaryotes, like *Synechococcus* sp. Four different types of MTs have varying amino acid sequences and motifs based on their gene sequences (Cobbett and Goldsbrough 2002). MT can commonly bind with Cu, Zn, and Cd and has the highest binding affinity to Cu. The enriched cysteine in MT has a role in heavy metal-binding and absorption as a metal-chelating core (Cobbett and Goldsbrough 2002; Perales-Vela et al. 2006). Glutathione, which is composed of glutamate, glycine, and cysteine, is used for binding heavy metals and interacts with MT (Perales-Vela et al. 2006). The thiol group in cysteine and glutathione can act as a reducing agent for the formation of metal nanoparticles.

Biosynthesis of metal nanoparticles

There has been increasing interest in developing environmentally friendly methods of metal nanoparticle synthesis. Recently, many different types of biological templates, including peptides, nucleotides, proteins, and other biomolecules, have been employed in the synthesis of various types of inorganic metal materials (Hutchison 2008; Irvani 2011). Although these biological templates have limited control over the resulting crystal's structure and size (Kang et al. 2008), it has been proposed that rational use of constrained environments within microbial cells, such as the periplasmic space and cytoplasmic compartments, can modulate the size and shape of particles (Cobbett and Goldsbrough 2002; Grass et al. 2005; Reith et al. 2009).

Biosynthesis of metal nanoparticles has been proposed since the 1960s (Temple and Le Roux 1964; Bansal et al. 2012). Several examples of metal nanoparticle formation, mainly resulting from natural regulatory processes, have been well documented: zinc sulfide nanoparticles in bacteria; gold nanoparticles in Precambrian algal blooms, algal cells, and bacteria; CdS nanoparticles in bacteria and yeast; and magnetite nanoparticles in bacteria (Bansal et al. 2012; Sastry et al. 2004). Inorganic metal nanomaterials synthesized in various microorganisms are summarized in Table 1.

Most studies on biogenic nanoparticles have focused on the synthesis of noble metal nanoparticles, especially gold and silver, due to their high demand in ore leaching and metal recovery applications using microorganisms. Furthermore, the formation of metal nanoparticles using sulfide-reducing processes and mixing with metal ions to form metal-sulfide complex nanoparticles has been reported. Such combinatorial biosynthesis approaches have become important in improving production yields and the controllability of the morphology of noble metal nanoparticles.

All living microorganisms require metal cation transportation to maintain intracellular homeostasis and survival (Mitra and Rensing 2007; Hobman et al. 2007). Metal ions present around the cells can be transported across the membrane and into the cytoplasm. The cytoplasmic concentration of available metal ion is maintained by the flow equilibrium state of reverse uptake and efflux from the cytoplasm across the membrane (Andersen et al. 2001; Grass et al. 2005; Cervantes and Campos-Garcia 2007). Many membrane transporters can transport various transition metal cations, but detailed mechanisms are not well known. Cyanobacteria such as *Synechocystis* and *Synechococcus* sp. express metal-binding proteins and metal cations are assembled into the metal clusters such as Fe, Mn, and Cu in the cells for electron transfer in photosynthesis and respiration processes (Keren et al. 2002, 2004). Some microorganisms cope with heavy metal-induced damage by directly delivering metals, including Hg, Pb, and As, from periplasm to cytoplasmic metal-binding proteins

(Borremans et al. 2001). Furthermore, virulent microbial cells such as *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*, *Salmonella* sp., and *Vibrio cholerae* accumulate and export Ag particles by producing Ag-binding proteins for detoxification (Lobo and Vasconcelos 1950; Charley and Bull 1979; Kaur and Vadehra 1986; Starodub and Trevors 1990; Gupta et al. 1999; Dibrov et al. 2002; Shakibaie et al. 2003).

Biosynthesis of metal nanoparticles by wild-type microorganisms

Bacteria were the first microorganisms utilized in early studies on the synthesis of metal nanoparticles, due to the relative easiness of their cultivation and manipulation (Lee et al. 1996). Again, most early studies were focused on the synthesis of gold nanoparticles. *Bacillus subtilis*, *Cupriavidus metallidurans*, *Shewanella algae*, *Rhodopseudomonas capsulata*, *P. aeruginosa*, and *Shewanella oneidensis* have been employed for the biosynthesis of gold nanoparticles. In most of these studies, bacterial cells were incubated with gold chloride solution, resulting in the formation of nanoparticles of 5–200 nm in diameter (Beveridge and Murray 1980; Kashefi et al. 2001; Karthikeyan and Beveridge 2002; Lengke and Southam 2006; Konishi et al. 2007; He et al. 2007, 2008; Suresh et al. 2011). Depending on the experimental conditions, the gold ion precursors were converted into nanoparticles intracellularly or extracellularly. Through microbial reduction of gold ions, nanoparticles having octahedral, triangular, hexagonal, and spherical shapes were generally formed; these are similar to the typical structures of gold nanoparticles synthesized by employing conventional chemical synthetic methods (Beveridge and Murray 1980; Kashefi et al. 2001; Karthikeyan and Beveridge 2002; Lengke et al. 2006a, b, 2007; He et al. 2007, 2008; Park et al. 2010; Suresh et al. 2011). Furthermore, bacterial reduction of gold ions can occur by environmental bioremediation mechanisms in metallophilic bacteria, such as *C. metallidurans* (Reith et al. 2009), which is paramount to metal cycling and mineralization in metal-enriched environmental conditions.

Other types of metal nanoparticles can also be synthesized in bacteria. Interestingly, silver ions, which are known to be highly toxic to most microbial cells, can also be reduced and converted into silver nanoparticles using bacteria (Singh et al. 2008). Bacteria including *Lactobacillus* sp. and *Pseudomonas stutzeri*, isolated from silver mine, were used for the synthesis of silver nanoparticles having well-defined size and unique structure (Slawson et al. 1992; Joerger et al. 2000; Klaus-Joerger et al. 2001; Nair and Pradeep 2002; Zhang et al. 2005). Other inorganic metal nanoparticles synthesized using bacteria and photosynthetic cyanobacteria include Co, Cu, Hg, Li, Ni, Pb, Pd, Pt, Rh, Se, Te, CuO, CdS, PbS, ZnS, Fe₃S₄, Fe₃O₄, and Co₃O₄ (Aiking et al. 1982; Cunningham

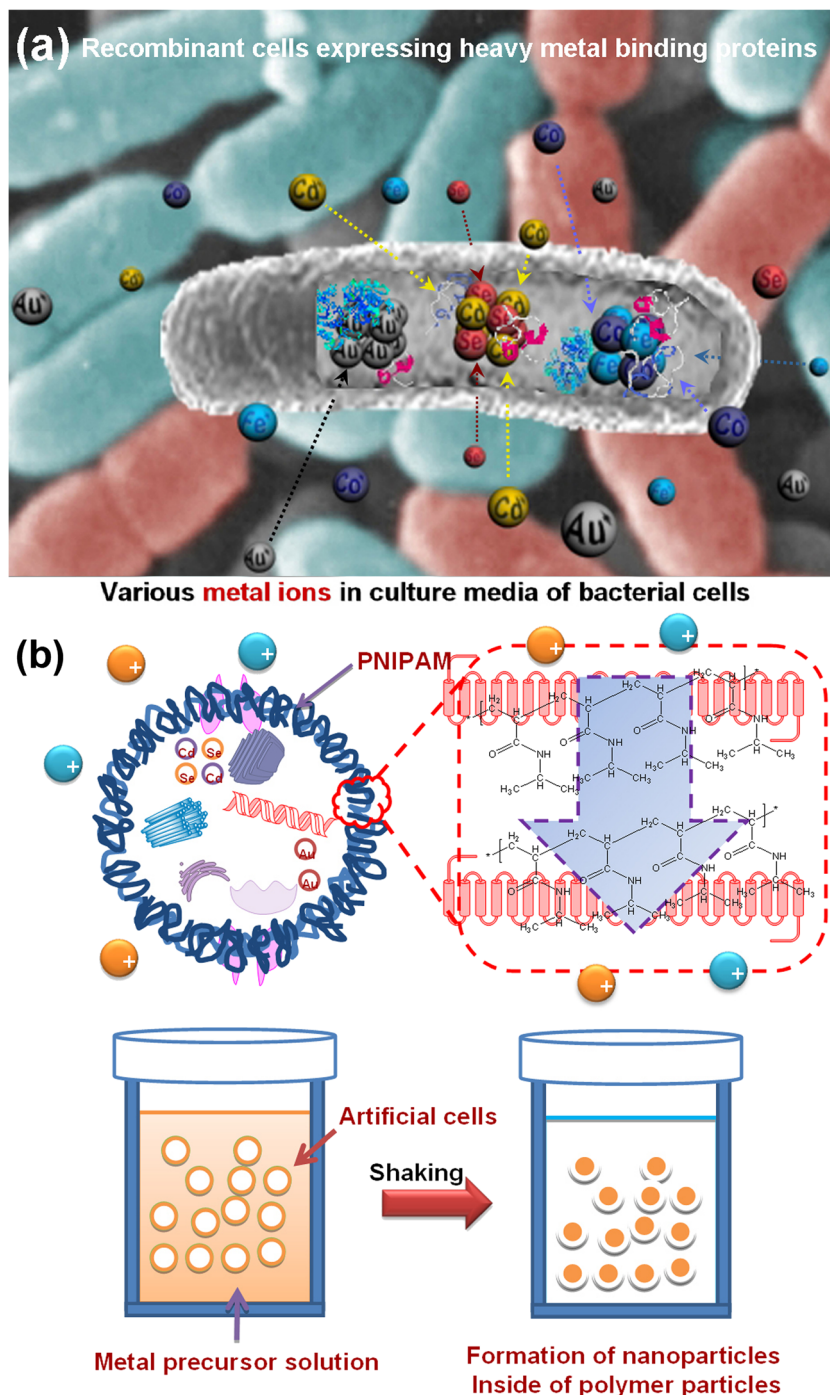
Table 1 List of the microorganisms employed for the synthesis of metal nanoparticles

Microorganisms	Metal	References
Bacteria		
<i>Aquaspirillum magnetotacticum</i>	Fe	Mann et al. (1984), Bazylinski et al. (1988)
<i>Bacillus subtilis</i>	Au	Beveridge and Murray (1980), Lengke and Southam (2006)
<i>Cupriavidus metallidurans</i>	Au	Reith et al. (2009)
<i>Shewanella algae</i>	Au	Kashefi et al. (2001), Konishi et al. (2007)
<i>Rhodopseudomonas capsulata</i>	Au	He et al. (2007, 2008)
<i>Pseudomonas aeruginosa</i>	Au	Karthikeyan and Beveridge (2002)
<i>Shewanella oneidensis</i>	Au	Suresh et al. (2011)
<i>C. metallidurans</i>	Hg, Pb	Borremans et al. (2001)
<i>S. oneidensis</i>	Pd	De Windt et al. (2005)
<i>Lactobacillus</i> sp.	Ag	Nair and Pradeep (2002)
<i>Corynebacterium</i> sp. SH09	Ag	Zhang et al. (2005)
<i>Mycobacterium tuberculosis</i>	Ag	Lobo and Vasconcelos (1950), Charley and Bull (1979)
<i>Salmonella typhimurium</i>	Ag	Gupta et al. (1999)
<i>Vibrio cholerae</i>	Ag	Dibrov et al. (2002)
<i>Desulfovibrio desulfuricans</i>	Pd	(Yong et al. 2002)
<i>Sulfurospirillum barnesii</i>	Se	Oremland et al. (2004)
<i>Sehnia anaerobacter shriffii</i>	Se	Oremland et al. (2004)
<i>Bacillus selenitireducens</i>	Te	Baesman et al. (2007)
<i>Serratia</i> sp.	Cu, CuO	Hasan et al. (2008)
<i>P. aeruginosa</i>	Ag, Co, Fe, Li, Ni, Pd, Pt, Rh, Ru	Srivastava and Constanti (2012)
<i>Pseudomonas stutzeri</i> AG259	Ag, Ag ₂ S	Slawson et al. (1992), Joerger et al. (2000), Klaus-Joerger et al. (2001)
<i>Pseudomonas syringae</i>	Cu	Zhang et al. (2006)
<i>Escherichia coli</i>	Ag, Cu, CuO, CdS	Starodub and Trevors (1990), Singh et al. (2010)
<i>Clostridium thermoaceticum</i>	CdS	Cunningham and Lundie (1993)
<i>Klebsiella</i> sp.	Ag, CdS	Kaur and Vadehra (1986), Aiking et al. (1982)
<i>Magnetospirillum magnetotacticum</i>	Fe ₃ O ₄	Philipse and Maas (2002)
<i>Thermoanaerobacter ethanolicus</i>	Fe ₃ O ₄	Roh et al. (2001)
Sulfate-reducing bacteria	Fe ₃ S ₄ , Fe ₃ O ₄	Watson et al. (1999), Reguera et al. (2005, 2007)
Cobalt-resistant bacteria	Co ₃ O ₄	Kumar et al. (2008a, b)
<i>Plectonema boryanum</i> UTEX 485	Au, Ag, Pt	Lengke et al. (2006a, b, 2007)
Recombinant <i>E. coli</i>	Au, Ag, Fe, Te, CdS, CdZn, CdSe, CdTe, ZnSe, CdCs, PrGd, SrGd, SrPr, FeAg, FeCo, FeMn, CdSeZn, FeCoNi, FeCoMn, CdSeZnTe and AuCdSeZn	Kang et al. (2008), Park et al. (2010), Jung et al. (2012), Lee et al. (2012), Seo et al. (2015)
Cyanobacteria	Fe, Mn	Keren et al. (2002, 2004)
Actinomycetes		
<i>Thermomonospora</i> sp.	Au	Ahmad et al. (2003c)
<i>Rhodococcus</i> sp.	Au	Ahmad et al. (2003b)
<i>Actinobacter</i> sp.	Au, Ag, Fe ₃ S ₄ , FeS ₂	Shakibaie et al. (2003), Bharde et al. (2007, 2008)
Yeast		
Yeast strain MKY3	Ag	Kowshik et al. (2003)
<i>Candida glabrata</i>	CdS	Dameron et al. (1989)
<i>Schizosaccharomyces pombe</i>	CdS	Dameron et al. (1989), Kowshik et al. (2002)
<i>Torulopsis</i> sp.	PbS	Kowshik et al. (2002)
Fungi		
<i>Verticillium</i> sp.	Au, Ag, Fe ₃ O ₄	Mukherjee et al. (2001a, b, 2002), Bharde et al. (2006)
<i>Collectotrichum</i> sp.	Au	Shankar et al. (2003)
<i>Fusarium oxysporum</i>	Ag, Au–Ag, CdS, CdSe, SiO ₂ , TiO ₂ , ZnO ₂ , BaTiO ₃ , Bi ₂ O ₃ , Fe ₃ O ₄	Mukherjee et al. (2002), Kumar et al. (2007), Ahmad et al. (2002, 2003a), Bansal et al. (2004, 2005, 2006), Senapati et al. (2005), Bharde et al. (2006), Uddin et al. (2008)
<i>Humicola</i> sp.	CuAlO ₂	Ahmad et al. (2007)

and Lundie 1993; Watson et al. 1999; Roh et al. 2001; Borremans et al. 2002; Kowshik et al. 2002a, b; Philipse and Mass 2002; Yong et al. 2002; Keren et al. 2002, 2004; Oremland et al. 2004; De Windt et al. 2005; Zhang et al. 2006; Lengke et al. 2006a, b, 2007; Konishi et al. 2007; Hasan et al. 2008; Kumar et al. 2008a, b; Park et al. 2010; Singh et al. 2010; Wang et al. 2010; Srivastava and Constanti 2012). Interestingly, magnetic nanowires have also been synthesized during the pili formation process of *Geobacter* sp. (Reguera et al. 2005, 2007).

Actinomycetes are another family of bacteria capable of synthesizing several kinds of metal nanomaterials. *Thermomonospora* sp. and *Rhodococcus* sp. were used to synthesize gold nanoparticles (Ahmad et al. 2003b, c). The shape and size of gold nanoparticles were different when synthesized using *Actinomycetes*, showing triangular, hexagonal, and spherical shapes up to 50 nm in diameter. Furthermore, some magnetotactic bacteria have been employed to synthesize magnetic nanomaterials such as Fe_3S_4 , FeS_2 , Fe_3O_4 , $\gamma\text{-Fe}_2\text{O}_3$, and FeS_2 (Mann et al. 1984; Bazylinski et al. 1998;

Fig. 1 Schematic diagrams for **a** in vivo and **b** in vitro synthesis of metal nanoparticles by recombinant bacterial cells expressing metal-binding proteins (Modified from Park et al. 2010; Lee et al. 2012)



Philipse and Mass 2002; Bharde et al. 2005, 2007, 2008; Reguera et al. 2005, 2007). Among these metal nanomaterials, Fe_3S_4 and FeS_2 were synthesized extracellularly under aerobic condition through the reaction of a ferric source with an exogenous sulfate source (Bharde et al. 2008).

Yeasts have also been found to synthesize inorganic metal nanoparticles (Dameron et al. 1989; Kowshik et al. 2002a, b). Silver ions were extracellularly reduced to silver nanoparticles by a silver-tolerant yeast strain, MKY3 (Kowshik et al. 2003). Using this yeast strain, large amounts of silver nanoparticles that are highly crystalline and 2–5 nm in diameter could be synthesized. In another study, two yeast species, *Candida glabrata* and *Schizosaccharomyces pombe*, were employed for the synthesis of CdS crystallites of 2 nm in diameter (Dameron et al. 1989). PCs were suggested to be able to control the nucleation and growth of the CdS nanoparticles.

Fungi can also be used to synthesize a variety of inorganic metal nanomaterials. For example, gold nanoparticles could be synthesized by exposing *Verticillium* sp. and *Fusarium oxysporum* to AuCl_4^- ions (Mukherjee et al. 2001b, 2002). In these studies, yellow-colored fungal cells were converted to purple when gold nanoparticles were synthesized near the cell walls, as examined by transmission electron microscopy. Similarly, silver nanoparticles of about 25 nm in diameter were synthesized by exposing *Verticillium* sp. cells to an aqueous AgNO_3 solution (Mukherjee et al. 2001a). Silver nanoparticles were primarily located at the external and internal cell boundaries, suggesting that cell walls might serve as nucleation sites. *F. oxysporum* has been reported to produce a wide variety of metal nanoparticles, including Ag, Au–Ag, CdS, CdSe, Co_3O_4 , SiO_2 , TiO_2 , ZrO_2 , BaTiO_3 , Bi_2O_3 , and Fe_3O_4 (Mukherjee et al. 2002; Ahmad et al. 2002, a; Bansal et al. 2004, 2005, 2006; Kumar et al. 2007, 2008a, b; Senapati et al. 2005; Bharde et al. 2006; Uddin et al. 2008; Jha et al. 2009). These fungi were simply exposed to the different types of metal or inorganic ion solutions for the synthesis of various types of metal nanoparticles.

Biosynthesis of metal nanoparticles by engineered microorganisms

Although wild-type microorganisms have been successfully employed for the synthesis of several types of metal nanoparticles as described earlier, biosynthetic efficiency is rather low, and the size and composition of nanoparticles need to be better controlled. Furthermore, there has been much interest in developing methods for the synthesis of more diverse metal nanoparticles having a wide range of properties and characteristics for various applications. Recently, biosynthesis of various metal nanoparticles using genetically engineered *Escherichia coli* expressing PC and/or MT has been reported (Kang et al. 2008; Park et al. 2010; Jung et al. 2012; Lee et al. 2012; Seo et al. 2015). A schematic for the in vivo synthesis of

metal nanoparticles by MT and PC in recombinant *E. coli* cells is shown in Fig. 1a. Here, recombinant *E. coli* is cultured to a suitable cell density, and then is incubated with the desired metal ion solution for the biosynthesis of the corresponding metal nanoparticles. It has been demonstrated that PCs form metal complexes with Cd, Cu, Ag, Pb, and Hg, while MTs do so with Cu, Cd, and Zn ions. To broaden the spectrum of metal nanoparticles, a recombinant *E. coli* strain was developed to express both PC synthase and MT genes. Co-expression of the PC synthase and MT can provide a unique cellular environment for the biosynthesis of metallic, bi-metallic, tri-metallic, semiconducting, noble, magnetic, and rare-earth metal nanoparticles, which cover more than one third of the elements in the periodic table. Indeed, metal nanoparticles composed of Au, Ag, Fe, Te, CdZn, CdSe, CdTe, ZnSe, CdCs, PrGd, SrGd, SrPr, FeAg, FeCo, FeMn, CdSeZn, FeCoNi, FeCoMn, CdSeZnTe, and AuCdSeZn could be successfully synthesized by a recombinant *E. coli* strain co-expressing PC synthase and MT (Park et al. 2010).

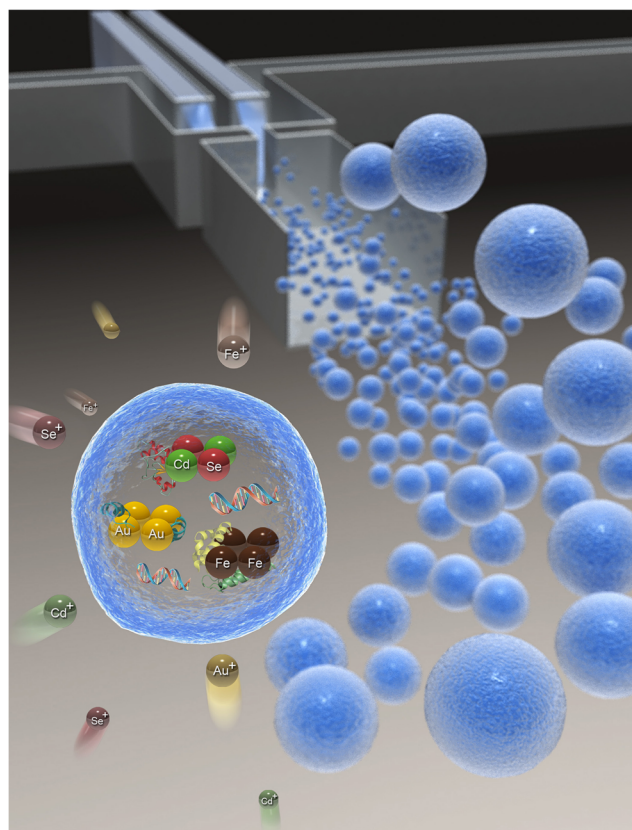


Fig. 2 Schematic diagram for the in vitro synthesis of metal nanoparticles by recombinant *Escherichia coli* cells expressing metal-binding proteins. This image illustrates the formation of an artificial bioreactor using a microfluidic device, hydrogel, and cellular extracts of recombinant *E. coli*. The fabricated microdroplets are polymerized and used as individual chemical bioreactors for the synthesis of different types of metal nanoparticles using encapsulated cellular components (Lee et al. 2012)

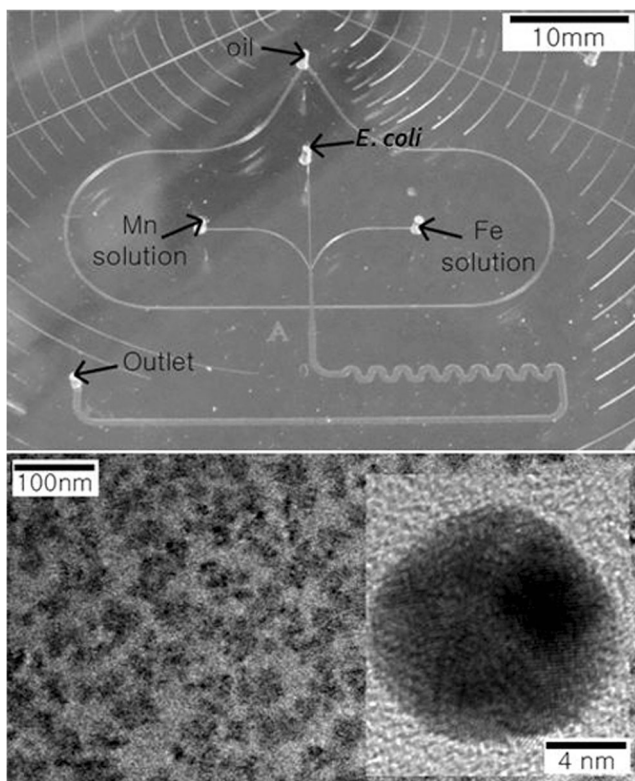


Fig. 3 Photograph of a polydimethylsiloxane (PDMS) microfluidic device for droplet generator (top) and a transmission electron microscopy (TEM) image (bottom) of produced biogenic nanoparticles (Modified from Jung et al. 2012)

Biosynthetic control of morphology and size of metal nanoparticles

For any type of metal nanoparticle, control over the morphology and particle size is important for industrial applications.

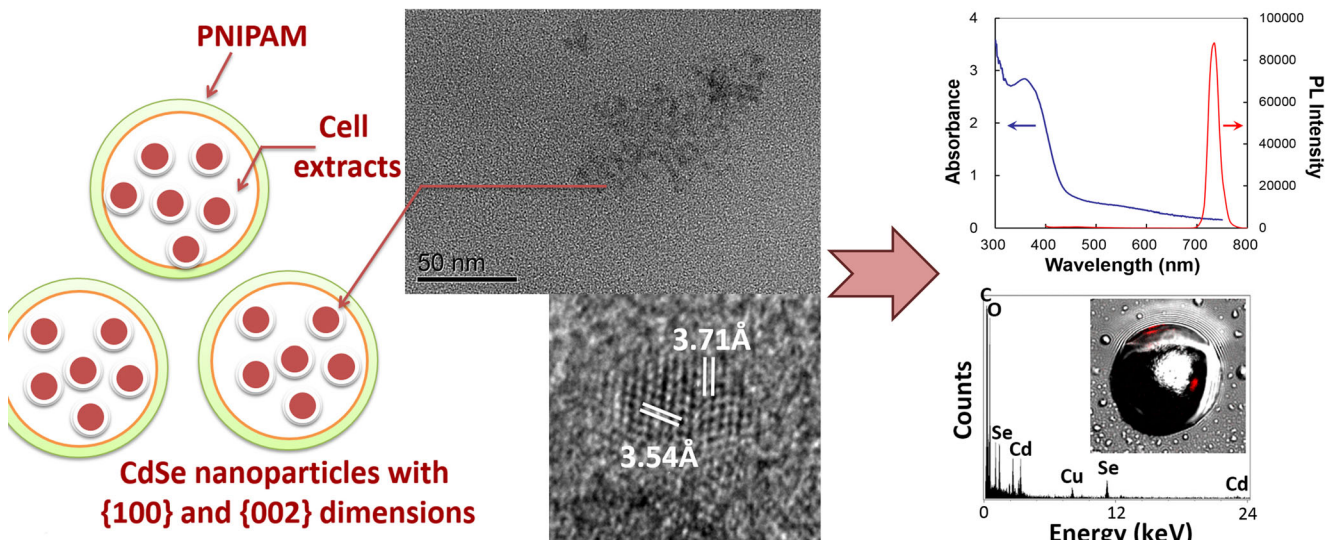


Fig. 4 In vitro synthesis of CdSe quantum dots (QDs) using the artificial cellular bioreactor. Fluorescent/optical microscopic image of the artificial cellular bioreactor and the TEM image of CdSe QDs in the artificial

The sizes of the nanoparticles including quantum dots (QDs) and magnetic nanoparticles can be controlled by varying the concentrations up to 5 mM of the treated metal ions (Park et al. 2010). The fluorescent QDs such as CdSe nanoparticles exhibited several unique emitted colors, depending upon their size in diameter. Thus, QDs of different sizes can be distinguished through the unique color. Moreover, they can be separated by using discontinuous sucrose density gradient prepared by layering decreasing sucrose concentrations and sequential centrifugation process (Pintoalphandary et al. 1995; Kumar et al. 2008a, b). However, microbial biosynthesis of metal nanoparticles is inherently inferior in this aspect due to the heterogeneity of cells, cell viability affected by metal toxicity, and varying metal ion concentrations around each cell (Jung et al. 2012). To overcome this problem, a microfluidic system generating microdroplets was developed. Recombinant *E. coli* cells and metal ions are encapsulated in microdroplets of nanoliter volumes. Individually encapsulated recombinant *E. coli* cells were able to synthesize homogeneous nanoparticles through the control of the droplet size and the concentrations of the treated metal precursors of above 5 mM, and other reaction conditions (Jung et al. 2012; Lee et al. 2012). Furthermore, the synthesized nanoparticles such as CdSe QDs in the droplets can be easily distinguished through the unique colors.

In another system for the synthesis of metal nanoparticles, the cell extracts of recombinant *E. coli* expressing metal-binding proteins were also encapsulated in microdroplets to serve as an artificial bioreactor for in vitro biosynthesis of diverse metal nanoparticles, as shown schematically in Fig. 1b. By using this in vitro biosynthetic system, different kinds of metal nanoparticles could be synthesized (Lee et al. 2012). Recombinant *E. coli* cells were allowed to fully express

cellular bioreactor are shown together with the UV/vis and fluorescent emission characteristics (Modified from Lee et al. 2012)

Table 2 Applications for the biosynthesis of metal nanoparticles

Nanoparticles	Applications	References
Au	Biosensors	Mann et al. (1984)
	Drug delivery	Mann et al. (1984), Bazylnski et al. (1988), Seo et al. (2015)
	Bioremediation	Beveridge and Murray (1980), Lengke and Southam (2006)
Ag	Biosensors	Mann et al. (1984)
	Drug delivery	Reith et al. (2009)
	Bioremediation	Kashefi et al. (2001), Konishi et al. (2007)
	Antibacterial agents	Duran et al. (2005, 2007), Singh et al. (2008), Fayaz et al. (2010)
Au–Ag	Microscopy, catalysts	Senapati et al. (2005)
Ag ₂ S	Biofilm	Sastry et al. (2004)
BaTiO ₃	Imaging	Bansal et al. (2006), Li et al. (2011)
	Microelectromechanical devices	Bansal et al. (2006), Jha and Prasad (2010)
Cu, CuO	Biosensors	Padil and Cernik (2013)
	Antibacterial agent	Iravani (2011), Padil and Cernik (2013)
	Cancer therapy	Iravani (2011)
	Bioremediation	Guo et al. (2010)
CdS	Biosensors	Kowshik et al. (2002a, b)
	Drug delivery	Mohanpuria et al. (2008)
	Electronic devices	Kowshik et al. (2002a, b), Mandal et al. (2006)
	Imaging	Zhang et al. (1998), Watson et al. (1999), Lee et al. (2004)
	Bioremediation	Cunningham and Lundie (1993), Nies (1999), Korbekandi et al. (2009)
	Biomarker, cell labeling agent	Chan et al. (2002) Yang et al. (2005), Li et al. (2011)
CdSe	Biosensor	Kumar et al. (2007)
	Imaging	Park et al. (2010), Taniyuchi et al. (2011), Li et al. (2011)
	Bioremediation	Cunningham and Lundie (1993), Nies (1999), Korbekandi et al. (2009)
CdSeZn	Imaging	Park et al. (2010)
CdZn	Imaging	Park et al. (2010)
	Bioremediation	Cunningham and Lundie (1993), Nies (1999), Korbekandi et al. (2009)
CdTe	Imaging	Bao et al. (2010a, b), Park et al. (2010), Taniyuchi et al. (2011)
	Labeling agents	Bao et al. (2010a, b)
	Bioremediation	Cunningham and Lundie (1993), Nies (1999), Korbekandi et al. (2009)
Co	Bioremediation	Nies (1999), Korbekandi et al. (2009)
	Surface modification	Srivastava and Constanti (2012)
Co ₃ O ₄	Magnetorheological devices	Kumar et al. (2008a, b)
Fe	Cleaning of contaminated land and water	Srivastava and Constanti (2012)
Fe ₃ O ₄	Biosensor	Shan et al. (2005)
	Drug delivery	Xiang et al. (2007), Felfoul et al. (2007), Chertok et al. (2008), Fan et al. (2009), Meng et al. (2010)
	Detection	Tanaka et al. (2004)
	Solar energy, electrical battery	Mohanpuria et al. (2008)
	Electronic devices	Mandal et al. (2006)
	MRI	Felfoul et al. (2007)
Fe ₂ O ₃	Cancer treatment	Hergt and Dutz (2007), Meng et al. (2010)
	Drug delivery	Xiang et al. (2007), Felfoul et al. (2007), Chertok et al. (2008), Fan et al. (2009), Meng et al. (2010)
	Detection	Tanaka et al. (2004)
	DNA analysis	Fan et al. (2009), Meng et al. (2010)
	Gene therapy	Hergt et al. (2005), Sun et al. (2007), Fan et al. (2009), Meng et al. (2010)
	MRI	Chertok et al. (2008), Fan et al. (2009)
FeS ₂	MRI, biomedical applications	Bharde et al. (2008)
Fe ₃ S ₄	Bioremediation	Mandal et al. (2006)

Table 2 (continued)

Nanoparticles	Applications	References
	Solar energy, electrical battery	Mohanpuria et al. (2008)
	Biomarkers, cell labeling agents	Yang et al. (2005), Li et al. (2011)
FeCo	Drug delivery	Park et al. (2010)
FeMn	Imaging	Park et al. (2010)
FeCoNi		
FeCoMn		
Li	Catalysts	Srivastava and Constanti (2012)
Ni	Bioremediation	Nies (1999)
	Catalysts	Srivastava and Constanti (2012)
Pd	Bioremediation	Yong et al. (2002), Windt (2005), Irvani (2011)
	Catalysts	Sobjerg et al. (2009), Irvani (2011)
PbS	Biomarkers, cell labeling agents	Chan et al. (2002), Yang et al. (2005), Li et al. (2011)
	Imaging	Zhang et al. (1998), Watson et al. (1999), Kowshik et al. (2002), Lee et al. (2004)
	Bioremediation	Guo et al. (2010)
Pt	Biosensor	Bhattacharya and Mukherjee (2008), Li et al. (2011)
	Cancer treatment	Bhattacharya and Mukherjee (2008)
	Bioremediation	Konishi et al. (2007), Govender et al. (2009), Li et al. (2011)
Ru	Catalysts	Srivastava and Constanti (2012)
	Energy production	Srivastava and Constanti (2012)
Se	Biosensors	Wang et al. (2010), Li et al. (2011)
	Bioremediation	Fesharaki et al. (2010)
	Biomedical therapy	Fesharaki et al. (2010), Ramamurthy et al. (2013)
SiO ₂	Biosensors	Jha et al. (2009), Li et al. (2011)
Te	Antibacterial agents	Zare et al. (2012)
	Bioremediation	Zare et al. (2012)
TiO ₂	Biosensors	Jha et al. (2009), Li et al. (2011)
ZrO ₂	Catalysts	Bansal et al. (2004), Li et al. (2011)
Zn	Bioremediation	Nies et al. (1999), Korbekandi et al. (2009), Guo et al. (2010), Irvani (2011)
ZnS	Biomarkers, cell labeling agents	Chan et al. (2002), Yang et al. (2005), Li et al. (2011)
	Drug delivery	Mohanpuria et al. (2008)
	Imaging	Zhang et al. (1998), Watson et al. (1999), Lee et al. (2004)
	Bioremediation	Mandal et al. (2006) Korbekandi et al. (2009), Guo et al. (2010)
ZnSe	Imaging	Park et al. (2010), Taniyuchi et al. (2011)
	Bioremediation	Korbekandi et al. (2009), Guo et al. (2010)

heavy metal-binding proteins and were lysed. All soluble components containing the nucleation sites such as cell membranes were mixed with monomers of temperature-responsive hydrogel to generate polymeric microparticles using microfluidic devices, as depicted in Fig. 2. In contrast to the previous method of employing the whole cell, these microparticles act as individual chemical reactors containing metal-binding proteins for the synthesis of different metal nanoparticles by simple dispersion of the produced microdroplets in the targeted metal precursor solution. Most of the studies of biogenic nanoparticles in microorganisms were focused on whether microbial particles were formed or not. Whereas metal nanoparticles can be synthesized by wild-type and

recombinant microorganisms in vivo with relatively non-uniform size and low yield, in vitro biosynthesis using artificial microreactors allow synthesis of metal nanoparticles of homogeneous size to a higher yield (Lee et al. 2012). This allows the microdroplet method useful for the production of metal nanoparticles for high-throughput screening, molecular monitoring, and other biomedical applications.

Also, recombinant *E. coli* cells expressing heavy metal-binding proteins were grown to high cell density in a microfluidic device (Jung et al. 2012), and then mixed with metal precursors in the microfluidic channel (Fig. 3a). Monodisperse FeMn paramagnetic nanoparticles were pseudo-continuously produced outside its outlet port under uniform

reaction condition (Fig. 3b). This system can be considered a portable and environmentally friendly bioreactor that can be used for the continuous synthesis of metal nanoparticles using microfluidic techniques. It has a great potential for quantitative analysis in biomedical assays and high-throughput drug screening (Hergt et al. 2005). This system was also successfully used for the controlled synthesis of iron(III) oxide (Fe_3O_4), quantum alloy (CdSe), and gold nanoparticles. A representative concept of this system for metal nanoparticle synthesis, and CdSe QDs synthesized by this system are shown in Fig. 4.

Future perspectives

Metal nanoparticles have widely and commonly been used in biomedical, biological, and biosensing applications, as listed in Table 2. There has recently been much interest in developing more environmentally friendly methods for the synthesis of metal nanoparticles. Wild-type and recombinant microorganisms have been employed for the *in vivo* and *in vitro* synthesis of diverse metal nanoparticles. Such strategies have a good potential to replace the conventional chemical synthetic approaches. It is thus important for the microbial synthetic system to achieve cost-effective, large-scale synthesis of diverse metal nanoparticles of controlled size and shape. As described above, several different approaches for the synthesis of metal nanoparticles based on different systems, such as wild-type versus recombinant microorganism strains, whole live cells versus cell extracts, and bulk phase versus microdroplet can be examined. For example, the use of recombinant cell extracts, rather than live cells, to produce large amounts of metal-binding proteins will be useful for the biosynthesis of nanoparticles composed of much toxic metal elements, which cannot be efficiently assembled into nanoparticles *in vivo*. Although the use of microdroplet-based approaches and/or the use of *in vitro* cell extract-based approaches can control the size, polydispersity, chemical composition, and shape of nanoparticles for diverse industrial applications to some extent, there is still much room for further improvement. Fundamental to this is a more thorough understanding of the mechanisms of metal nanoparticle assembly by metal-binding proteins and/or peptides, and in particular metal reduction mechanisms.

Microbial metal nanoparticle synthesis systems reviewed in this paper have been demonstrated to synthesize diverse metal nanoparticles (Park et al. 2010). Scanning through the elements in the periodic table, however, immediately suggests that there are more metal nanoparticles that have not yet been synthesized by chemical methods. Such novel metal nanoparticles that have not yet been synthesized can potentially serve as new nanomaterials for exciting industrial applications. Of course, it should not be forgotten that microbial metal nanoparticle synthesis is based on metal detoxification process.

The metal nanoparticle synthesis systems described in this paper can be employed for the removal or recovery of metal ions: adsorption and removal of metal ions in water purification and treatment processes and recovery of valuable metals from locations where these metals are present at low concentrations. For these important application opportunities, it is expected that *in vivo* and/or *in vitro* microbial system will become increasingly important for the environmentally benign and cost effective synthesis of metal nanoparticles and synthesis of novel nanoparticles.

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Conflicts of interest The authors declare that they have no competing interests.

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