Microbe-Mediated Extracellular and Intracellular Mineralization: Environmental, Industrial, and Biotechnological Applications

Wen Qin, Chen-yu Wang, Yu-xuan Ma, Min-juan Shen, Jing Li, Kai Jiao, Franklin R. Tay,* and Li-na Niu*

Microbe-mediated mineralization is ubiquitous in nature, involving bacteria, fungi, viruses, and algae. These mineralization processes comprise calcification, silification, and iron mineralization. The mechanisms for mineral formation include extracellular and intracellular biomineralization. The mineral precipitating capability of microbes is often harnessed for green synthesis of metal nanoparticles, which are relatively less toxic compared with those synthesized through physical or chemical methods. Microbe-mediated mineralization has important applications ranging from pollutant removal and nonreactive carriers, to other industrial and biomedical applications. Herein, the different types of microbe-mediated biomineralization that occur in nature, their mechanisms, as well as their applications are elucidated to create a backdrop for future research.

1. Introduction

Biomineralization is a process utilized by diverse biological systems to produce a broad variety of the nanostructured mineralized structures found in nature and within certain tissues of viruses and unicellular organisms which include bacteria, fungi, and algae. It is a prevalent phenomenon with all six taxonomic kingdoms containing members that are capable of forming minerals. To date, more than 60 different minerals have been identified from organisms.[1] For example, sulfide-oxidizing bacteria are involved in the formation of marine sedimentary phosphorite deposits and contribute to nature’s phosphorus cycle.[2]

Different kinds of mineralization occur in nature, including calcification, silification, and iron mineralization.[3–5] Based on taxonomic distribution, the most commonly found biominerals are the carbonate and phosphate salts of calcium that are used in conjunction with organic polymers such as chitin or collagen to provide mechanical support in shells and bones. These biocomposites possess structures that are stringently controlled from the nanometer to the macroscopic level, producing complex architectures that provide multifunctional properties. Such a range of control over mineral growth is beyond what may be achieved in contemporary mechanical engineering and is highly desirable for materials design as well as biotechnological applications.

Much attention has been paid to the study of microbe-mediated mineralization and its potential applications in medicine, pollutant removal, and equipment improvement. For example, some diseases involving biomineralization may be cured through the study of pathogenic microorganisms. Minerals produced by microbes that have antimicrobial activities may be applied as coatings for medical devices. Bacterial mineralization is also important in geological processes and biotechnology. The process of microbial mineral precipitation in different situations can be mimicked and utilized in biotechnology, such as metal remediation, carbon sequestration, enhanced oil recovery, and construction restoration.[6]

In addition, biominerals produced by microbes in the laboratory may be used as drug carriers because of their excellent biocompatibility. By modifying some of the proteins in these microorganisms, the morphology and size of the synthesized biominerals may be controlled according to actual needs. Herein, we elucidate the different types of microbe-mediated biomineralization that occur in nature, their mechanisms, as well as their applications, to create a backdrop for future research.

2. Microbe-Mediated Mineralization in Nature

2.1. Bacterium- and Archaeon-Mediated Mineralization

2.1.1. Calcification

Bacteria play an important role in the calcification process of natural minerals and within organisms (Table 1). It is...
important to discern between mineralized bacteria and bacteria that really produce calcified minerals. With respect to natural calcium-based minerals, aerobic bacteria such as *Salinivibrio* and *Virgibacillus* species (sp.) contribute to the formation of MgCa(CO₃)₂, which is considered the precursor of ordered dolomite.[7] Biotic precipitation of CaCO₃ accounts for nearly 80% of the total carbon removal from the earth’s surface.[8] Calcifying bacteria such as *Bacillus sphaericus* have been used for biodeposition of CaCO₃ crystals on the surface of limestone for conservation purposes.[9] Other natural minerals produced by carbonatogenic bacteria, such as francolite (carbonate-rich fluorapatite)[10] and calcite deposition in Colombian mines,[11] have also been reported.

Within organisms, bacteria-mediated calcifications can be identified in crustaceans, marine organisms, plants, and in human organs. Terrestrial crustaceans form calcium deposits to store calcium. Symbiotic bacteria have been found to fill the most of the calcium bodies (organs containing large amounts of calcium) in *Titanethes albus*, an isopod crustacean. This large number of bacteria may produce an extensive acid polysaccharide matrix that provides nucleation sites for mineral deposition.[13] Bacteria colonies found in shrimp fossils may also contribute to biominalization and diagenesis.[14]

Sponges exhibit the highest diversity of bacteria among marine invertebrates. Endosymbiotic bacteria located in sponge cells produce calcitic spherules that cover the surface of the host sponges. The archocyte-like cells in sponges are capable of detecting potential symbiotic bacteria due to the presence of adhesion-related proteins. After being phagocytized, the symbiotic bacteria are kept alive and divide within the vacuole. *Hemimycale* (a calcifying bacterium), which lacks cell walls, has been reported to live in symbiosis with sponges and maintains of collagen scaffolds antimicrobial sol–gel chemistry, mesoporous silica, and endodontic materials.

Table 1. Summary of the contribution of bacteria to calcification of natural minerals and formation of calcified deposits within organisms.

| Location            | Bacteria                                      | Biomineral          | Ref.  |
|---------------------|-----------------------------------------------|---------------------|-------|
| Natural minerals    | Proteobacteria and firmicutes *Lysinibacillus*, *Bacillus*, *Stenotrophomonas*, *Brevibacillus*, *Methylobacterium*, *Aeromicrobium*, and *Acinetobacter* sp. | Aragonite           | [11]  |
|                     |                                               | Calcite             | [11]  |
|                     |                                               | Dolomite            | [11]  |
|                     |                                               | Vaterite            | [11]  |
|                     | Proteobacteria and actinobacteria              | Calcite (predominant), aragonite, vaterite identified in calcitic cave speleothems | [12]  |
| Organisms           |                                               |                     |       |
| Crustaceans         | *Titanethes albus*                            | Calcified matrix-associated bacteria | [13]  |
|                     | Fossil of shrimps                             | Calcifying bacteria |       |
|                     | Marine organisms                              |                     |       |
|                     | Sponges                                       | *Hemimycale*        | [15]  |
|                     | Plants                                        | *Calcitic spherules*|       |
|                     | Iroko trees                                    | *Carbonate*         | [16]  |
|                     | Humans                                        |                     |       |
|                     | Oral cavity—supragingival calculus             | *Neisseria flava*, *Aggregatibacter segnis*, *Streptococcus tigirinus*, and *Morococcus cerebrosus* | [17]  |
|                     | Kidney tubules                                | *Neisseria flava*, *Aggregatibacter segnis*, *Streptococcus tigirinus*, and *Morococcus cerebrosus* | [18]  |
|                     | Placental calcification                        | Nanobacteria        | [19]  |
|                     | Kidney                                        | *Pseudomonas*, *Gardnerella*, *Lactobacillus*, *Enterobacteriaceae* sp. |       |

Franklin R. Tay received his Ph.D. from the University of Hong Kong in China and his endodontic training from the Medical College of Georgia, USA. He is currently Professor, and Chair of the Department of Endodontics, College of Dental Medicine, Augusta University, Georgia, USA. His research interests include biominalization of collagen scaffolds antimicrobial sol–gel chemistry, mesoporous silica, and endodontic materials.

Li-na Niu received her Ph.D. from the School of Stomatology, the Fourth Military Medical University in China and had her postdoctoral training at Augusta University, Georgia, USA. She is the Changjiang Scholars Distinguished Young Professor of the Ministry of Education in China and is currently working at the Fourth Military Medical University. Her major scientific interest is on the application of nanotechnology in biomaterials.
an intergrowth relationship with its host. Existing in calcibacteriocyte vacuoles, the bacteria enhance sponge survival via Ca^{2+} detoxification and complementary skeletal formation against threat from enemies,[13]

Plants such as Iroko trees in the Ivory Coast and Cameroon are unique in having highly mineralized roots and trunks, where bacteria thrive and play an important role in the mineralization process. These plants produce a large amount of oxalate, which is consumed by oxalotrophic bacteria as carbon and energy sources. Oxidation of the oxalate salts produces carbonate and hydrogen carbonate ions, which are precipitated as CaCO_{3} within the trees in the presence of calcium.[16]

Calcibacteria can also induce calcification in human body organs such as the oral cavity, the kidneys, and placental tissues.[18–20] The exoskeleton of crustaceans is composed of chitin and calcium salts that form an inorganic–organic hybrid composite material.[21] The function of the exoskeleton is the protection against predators, excretion, sensing, supporting, feeding, and acting as a barrier. Mineralization of chitin by different forms of CaCO_{3} adds hardness to the exoskeleton. Calcium-sequestering bacteria can deposit calcium salts in tissues, especially in persistent inflammatory lesions. Several species of bacteria are capable of producing calcium phosphate deposits and are partly responsible for the formation of calculus around bacteria are capable of producing calcium phosphate deposits, especially in persistent inflammatory lesions. Several species of calcium-sequestering bacteria can deposit calcium salts in tissues, especially in persistent inflammatory lesions. Several species of bacteria are capable of providing a matrix for the deposition of calcium phosphate.[18] Calculifying nanoparticles, previously referred to as nanobacteria,[23,24] have been isolated from placental calcification specimens. These protein–mineral entities represent remnants and by-products of physiological mechanisms used for calcium homeostasis.[19] A potential correlation between bacteria and urinary stones has also been reported. Bacteria identified from urinary stones include *Pseudomonas*, *Gardnerella*, and *Lactobacillus* sp., as well as *Enterobactericeae*, a large bacteria family that includes *Escherichia coli*. Three potential mechanisms by which bacteria contribute to urinary stone disease have been proposed. The first is that the bacteria can adhere to calcium oxalate crystals. The second involves production of citrate lyase to decrease the urine citrate levels. The third mechanism involves the formation of bacteria-crystal aggregates that combine with renal tubular epithelium and induce the expression of stone matrix proteins.[20] Such findings suggest that stone diseases induced by calcifying bacteria may be alleviated or cured by antibacterial therapy.

Extreme biomineralization occurs in environments with extremely harsh conditions such as psychrophilic, thermophilic, anaerobic, alkaliphilic, acidophilic, and halophilic conditions, as well as environments with high or toxic metal-ion concentrations.[25] At a temperature of −15 °C, *Planococcus halocryophilus* Or1 increases the expression of carbonic anhydrase, which is responsible for the mineralization of calcium carbonate. Consequently, more calcium carbonate is deposited in the cell envelope. This phenomenon broadens our understanding of psychrophilic adaption and provides the opportunity for biomimetic mineralization at extreme low temperature.[26]

Because such interactions are direct and indirect indicators of life, they are highly relevant to the study of the archeological earth environment as well as the search for evidence of life on other planetary surfaces.[17,28] Bacteria employ three behavioral strategies to deal with mineralizing conditions: 1) evasion of mineralization by the formation of biofilms, 2) random embedding within the mineral, and 3) control over the mineral shape during its formation. The latter has been coined "pseudomineralization" and enables fine control over mineral morphology with changes in external calcifying conditions. The diversity of bacterial responses toward mineralization contributes to their survival under different physicochemical conditions and selective pressures.[29] Changes in environmental chemistry spur the evolution of bacteria-mediated biomimeralization.[30]

### 2.1.2. Silification

Bacteria have been reported to participate in natural silicific precipitations. The silica amorphization process in the Imawari Yeuta cave is the first reported to be connected with microbes.[31] Silica precipitation and speleothem formation are induced by the activity of heterotrophic or autotrophic filamentous bacteria such as cyanobacteria. The presence of tubular casts and filamentous structures is attributed to the silification of microbe cells and metabolic products. Bacterial communities that settled on the cave first cause the dissolution of quartz; the solubilized silica subsequently reprecipitates on the bacterial cell membranes.

Bacteria-induced silification has also been detected in some organisms. Silification is the predominant process of biomineralization in sponges. Sponges produce various minerals including calcite, aragonite (i.e., calcareous sponges),[32] and/or amorphous silica (siliceous sponges) in the form of spicules around a collagenous or chitinous skeleton to provide strength and flexibility.[33,34]

Cyanobacteria have been reported to induce silification in microfossils and modern extreme environments such as hot springs and sediments where amorphous silica or silicic acid is supersaturated and the pH is often extreme.[35,36] Recent study has also provided evidence that silicate is precipitated by *Synechococcus* sp., a kind of cyanobacteria, on the extracellular polymeric substance (EPS) associated with decomposing picophytoplankton. The EPS functions in providing an organic template that selectively incorporates supersaturated silica. *Synechococcus*-based silica accounts for 50% of the biogenic silicate inventory in the oceans, a feature that is indicative of the importance of bacterial silification.[37]

### 2.1.3. Iron Mineralization

Bacteria-induced iron mineralization produces iron oxide (Fe_{3}O_{4}; magnetite) and ferrimagnetic iron sulfide, including predominantly greigite (Fe_{3}S_{4}) and to a lesser extent pyrrhotite (Fe_{1−x}S), and mackinawite (Fe_{0}S). The predominant bacteria involved include α-Proteobacteria, δ-Proteobacteria, γ-Proteobacteria, and *Nitrospira* sp.[39–42] These bacteria have been observed in a wide range of ecosystems, from marine sediments, lagoons,
Magnetotactic bacteria are important both in nature and in humans. They play an important role in the euxinic marine system iron cycle. Because greigite (Fe$_3$S$_4$)-producing MTB require sulfur for magnetosome biosynthesis, their occurrence is correlated with the many episodic euxinia experienced by the earth's ocean in the geological past. As such, the presence of greigite magnetofossils in geological records has been suggested as an indicator of euxinia (conditions where water is free of oxygen and is sulfidic because of increased level of free H$_2$S).

Biogenic magnetic nanoparticles have been reported in various human organs, including the heart, liver, spleen, adrenal glands, ethmoid bone, and brain. Magnetic remanence (residual magnetism) has been identified recently in the human body, particularly in the brain. Magnetite is preferentially partitioned in the human brain, specifically in the cerebellum and brain stem. Biogenic remanent magnetite deposition in the human brain is similar to magnetite crystalline grown by MTB and is believed to be biologic precipitations as part of the body’s iron metabolism. More recent work opined that the occurrence of magnetite nanoparticles in the brain is caused by the extraneous release of anthropogenic materials and air pollution.

One of the questions that arises from the discovery of externally derived magnetite in brain tissues is whether or not these nanoparticles adversely affect human health. A recent study reported that excessive accumulation of magnetic iron oxide nanoparticles in the cells of the central nervous system (particularly astrocytes) may result in the disruption of normal iron metabolism/homeostasis, which is a hallmark characteristic of several neurodegenerative disorders. This is an issue the warrants further research.

Extreme biomineralization is also seen in iron mineralization, which may be explained from an evolutionary perspective. Authigenic pyrite, which is formed during the early diagenesis stage in the bottom sediments of oceans, has a biochemical origin associated with the activity of sulfate-reducing bacteria. The diagenetic process produces frambooidal pyrite (i.e., sub-spherical aggregates of crystals of sub-micrometer sizes) under extremely low temperatures (in the Arctic and Antarctic), in H$_2$S-rich zones (in the Caspian Sea and the Black Sea), and in space with releasing methane gas (in the Laptve Sea). The hyperthermophilic acidophile Sulfolobus solfataricus is a DNA-binding protein-synthesizing archeon with the ability to oxidize Fe(II) to Fe(III). The species grows best in temperatures around 80 °C, pH levels between 2 and 4, and enough sulfur for it to metabolize to obtain energy. Mineral formation mitigates oxidative damage to these archaea. Such a phenomenon enables the primordial life-forms to accommodate the toxicity generated by reactive oxygen species during the evolution of an oxygenic atmosphere.

2.2. Fungus-Mediated Mineralization

Although most attention has been focused on bacterial-induced biomineralization, fungi also participate in this process. Fungi produce organic acids such as oxalic acid and contribute to the genesis of various metal complexes such as metal–oxalate. They play a significant role in organic matter recycling. Fungi are crucial in the transformation of various mineral and metallic...
compounds such as uranium phosphate and metallic lead. This results in their wide implications in bioremediation. Specifically, fungi show great potential in the coprecipitation of different ions and the transformation of diverse precipitations. For example, strontium (Sr) is able to replace calcium (Ca) during the formation of carbonate minerals because Ca and Sr exhibit similar chemical properties. This probably accounts for the precipitation of hybrid Ca/Sr carbonate.

Fungi-mediated mineralization can be found in plants. The symbiosis between plants and the fungus Arbuscular mycorrhiza has a history of several hundred million years. Fungal mycelia are common in natural environments and are significant in helping plants acquire phosphorus from soil in zones that are inaccessible to the roots of those plants. Organic phosphate mineralization in soil is mainly dependent on the phosphatases derived from microbes or plant excretion. As the principle storage form of phosphorus in many plants, phytin acid can release phosphate radicals at certain pH values. The A. mycorrhzala fungal mycelium is capable of absorbing NH4+ ions and cause hyphosphere acidification. Such a process improves phosphatase activity and consequently promotes the mineralization of phytin and increases the absorption of phosphorus from phytin. It also explains the correlation among acidification, mineralization, and transformation of phosphorus into organic phosphate moieties.

The urease-positive fungus Pestalotiopsis sp. derived from calcareous soil can deposit Ca2+ and Sr2+ ions in the form of CaCO3, SrCO3 and (Ca,Sr)CO3 when cultured in urea-amended media and identical CaCl2 and SrCl2 concentrations. Urease-positive fungi may be used for bioremediation or biorecovery of Sr or other metals and even radionuclides that form insoluble carbonates. Other fungi that are present in montmorillonite and goethite clays have been reported to participate in biomineralization. Oxalate is a key metabolite produced by fungi and plays a significant role in many metal and mineral transformations. Whewellite (calcium oxalate) produced by fungi accounts for biodeterioration of rocks and mineral-based building materials. Fungi-mediated oxalate biosynthesis may have potential applications in environmental biotechnology such as metal and radionuclide leaching, biorecovery, detoxification and bioremediation, and in the production or deposition of biominerals or metallic elements with catalytic or other properties.

In the presence of high concentrations of calcium and nitrate, the fungal metabolism can induce the precipitation of calcium carbonate by denitrification. Laboratory biomineralization of calcite (a form of CaCO3) or otavite (CdCO3, in the presence of CdCl2 supplement) has been achieved with the use of the urease-positive fungus Neurospora crassa. These urease-positive fungi play a potential role in the synthesis of novel biominerals and in metal bioremediation/biorecovery. Two other urease-positive isolates (Pestalotiopsis sp. and Myrothecium gramineum) are capable of precipitating vaterite (another form of CaCO3) as [CaCO3, (Ca,Sr)CO3] and olekminskite [Sr(Sr,Ca)(CO3)2] by co-precipitation of Sr into vaterite.

Yeasts are eukaryotic single-cell fungi that exist in environments that are rich in sugar, such as the skins of fruits and berries. They can also be isolated from marine environments. Natural mineralization induced by yeast is rarely reported. However, yeast-mediated mineralization has been achieved in the laboratory setting. The most common precipitations induced by yeasts are metal chalcogenides nanoparticles, such as the synthesis of intracellular CdS and PbS quantum dots (QDs; radii lower than the Bohn excitation, i.e., <10 nm) by Schizosaccharomyces pombe, Torulopsis sp., and Rhodospirillum diobovatum, via uptake of these heavy-metal ions from soil and water. The protein-capping in cadmium telluride (CdTe) QDs render these nanoparticles highly soluble in water. Being highly biocompatible, CdTe QDs have bioimaging and biodiagnostic applications. Yeasts are tolerant to extreme environmental conditions, including metal toxicity. Another advantage of yeast is its ability to differentiate between different metals such as selenium, antimony, and mercury based on their toxicity. Because of its cost effectiveness, yeast has been applied for biosorption of toxic metal ions including uranium, chromium, cadmium, and lead.

Yeasts are also used as biotemplates for synthesis of materials via coprecipitation around the biotemplate core. For example, tricalcium phosphate microspheres incorporating strontium can be synthesized in this manner. The microspheres exhibit excellent biocompatibility and can be used as a potential drug release system for bone regeneration. As a biotemplate, yeast plays a significant role in the fabrication of Mg-doped CaCO3 microcapsules. Preadsorption of magnesium by yeast cells from a Mg2+-containing solution is followed by the induction of CaCO3 precipitation within the cultured yeast cells. These microcapsules possess high specific surface area and may be used as therapeutic drug carriers or for encapsulation of biomacromolecules.

Biosorption of metal ions is the basis for biomineralization. The first step in biosorption involves attachment of metal ions to the cell surface through complexation with cell-surface functional groups. Another explanation for precipitation on cell surfaces is the presence of acid phosphatases, which meditate decomposition of organic phosphates and subsequent accumulation of lead on the cell surface. The second step in biosorption involves metal binding. Attaining equilibrium is relatively slow as the weak acid groups dissociate and different cations compete for attachment. Metal binding to the yeast cell wall is subsequently followed by the nucleation and formation of mineral precipitations around the living or dead biomass. Because of their ability to attract a wide range of metal ions, yeasts enhance mineralization via 6 different methods, which have been extensively investigated in recent studies:

1) Immobilization in solution: Saccharomyces cerevisiae (brewer’s yeast) exhibits better affinity for metal ions in the presence of 3% alginate extract, producing biomass/polymer matrix beads. Biosorption is affected by parameters such as the pH of the solution, initial concentration, biosorption consumption, and contact time. This method makes it possible for the yeast cells to remove metal ions more effectively. The method is cost-efficient because of its potential for reuse and recovery.

2) Installation of anionic functional groups: Installing phosphate functional groups on yeast cells provides them with negative charges that enhance the effectiveness of biosorption. With more negative charges, phosphorylated yeast cells
can absorb heavy-metal ions such as Gd²⁺, Cu²⁺, Pb²⁺, and Zn²⁺ more effectively. They also exhibit high efficiency in the absorption of rare-earth ions such as Ce³⁺, Dy³⁺, Gd³⁺, La³⁺, Nd³⁺, Pr³⁺, and Yb³⁺. Thus, phosphorylated yeast cells have great potential for use as novel biosorbents.[91]

3) Utilization of an organic phosphate source: Yeast cells can transform soluble metal species into insoluble minerals through phosphatase-mediated bioprecipitation. In the presence of an organic phosphorus source as the sole source of phosphorus, yeast exhibits higher phosphorus activity for mediating uranium via the formation of uranium phosphate biominal. This has potential applications for environmental uranium removal.[83]

4) Irradiation: With the ability to survive irradiation, pre-exposure of S. cerevisiae to irradiation increases its biosorption capability, which has practical applications in bioremediation.[92]

5) Genetic level enhancement: New strains of S. cerevisiae have been produced by integration of recombinant human MT2 and GFP-hMT2 genes into yeast cells. These new strains exhibit improvement in copper ion bioremediation because of the expression of proteins involved in biosorption that are derived from the exogenous genes.[93]

6) Bio-electrospaying: This is a technique for immobilizing S. cerevisiae onto the surface of poly(e-caprolactone)/chitosan/rectorite ternary composite-based nanofibrous mats, which renders the application of yeasts less costly and more environmentally friendly. The advantages of this technique include the low cost of S. cerevisiae and the simplicity in recollection and reuse of the biosorbents.[94]

2.3. Virus-Mediated Mineralization

Because of their small sizes and lack of unique chemical or isotopic signature, viruses have been used as templates for organizing materials on the nanoscale. Data on virus-mediated mineralization in nature is scanty compared with bacterium- or fungus-mediated biominalization. Viruses can self-mineralize in nature under metal ion-abundant conditions, such as those identified from hot springs and hydrothermal vents; the mineralized state represents a transient state that augments the survivability of the viruses in the absence of host cells.[95]

For example, biosilicification increases the heat resistance capability of viruses to thermal denaturation or desiccation. Mineralization also endows the viruses with increased infectivity by enabling them to bypass tissue and cellular barriers more easily, and to utilize the mineralized shell as a camouflage to evade detection by the components of the host's immunological system; the latter include molecular pattern-recogizing innate defense cells and antibodies produced by B lymphocytes.[95]

The major attention on virus-induced mineralization is the use of viruses as templates for laboratory synthesis of nanoparticles via biomimetic mineralization strategies.[95] Bacteriophages and other viruses have been reported to interact with minerals, especially iron oxyhydroxides.[96] Phages represent important iron-binding ligands in the marine environment. The primary site for phage-mineral contact is the viral capsid, which is composed of proteins containing reactive carboxyl and amine groups. Bacteriophages in marine environment possess the ability to concentrate dissolved iron and transform the ions into iron oxides.[97]

With respect to virus-mediated mineralization in the laboratory setting, tobacco mosaic virus (TMV) has been used as a template for the synthesis of metal–organic frameworks[98] owing to its anisotropic structure and chemically addressable amino acid residues on both the external and internal surfaces.[99] Different protein shows different affinity to metal ions. For example, CdS, PbS, and ferric oxide mineralization are mainly dependent on specific metal-ion binding to the glutamate and aspartate surface groups, while the silica mineralization is dependent on binding to the surface arginine and lysine groups. As the virus exhibits tolerance to high temperature and are stable in different pH environments, TMV may be used for the synthesis of exotic materials such as high-aspect-ratio composites and protein-confined inorganic nanowires.[100]

Cowpea mosaic virus (CPMV) has been used as a template for controlled mineralization of silica nanoparticles[101] after chemical modification with mineral/metal specific peptides or increasing negative charges on the virus surface.[102] Hollow mesoporous silica nanocapsules have been synthesized using CPMV-mediated synthesis for drug delivery.[103] In the presence of CPMV as templates, zinc phosphate [Zn₃(PO₄)₂] nanoparticles are self-assembled into nanosheets with a high degree of isotropy. These nanosheets are subsequently self-organized into a 3D structure that has potential use in tissue regeneration.[104]

2.4. Other Microbe-Mediated Mineralization in Sea and Soil

The ocean is said to be the origin of the lives, which is the habitat of many kinds of algae and microbes. There are two major forms of biominalization in marine phytoplanktons: silicification induced by diatoms, chrysophytes, synurophytes, dictyochophytes, and choanoflagellates and calcification induced by coccolithophores.[105] Biomineralization by the marine phytoplankton plays an important role in carbon and nutrient cycling in the oceans.

Diatoms are frequently investigated in biosilicification because of their unicellular characteristics and silicified cell walls. Biomolecules such as peptides, polynucleins, and saccharides are associated with biosilica formation. Silafin, a complex post-translationally modified peptide,[106] and the membrane protein silicanin-1, which is highly conserved throughout all diatom species,[107] play important roles in biosilica deposition. Mannose-6-phosphate, a phosphorylated monosaccharide that is strongly expressed in the cell wall of Stephanopyxis turris, significantly influences the biosilica precipitation behavior of this diatom.[108] Apart from amino acids such as serine, threonine, and hydroxyproline, proteinaceous materials such as frustulins, pleuralins, silafins, silacidins, and circlins, as well as polynucleins such as chitin are also involved in diatom biomineralization. These molecules function as a scaffold as well as a template for the mineralization process. The complex organic network plays an important role in improving the biominaler properties in diatoms.[109]

Biocalcification is very common among photosynthetic organisms in the ocean. The calcium and bicarbonate concentrations...
in the ocean have slowly become supersaturated throughout the geological timeframe, which result in extracellular or intracellular calcification of these organisms. [106] Coccolith biomineralization induced by coccolithophores produce extracellular layers of CaCO₃ platelets. [110] The most common species include Emiliania huxleyi and Gephyrocapsa oceanica. Other species such as Coccolithus braarudii and Calcidiscus leptoporus are relatively less heavily calcified. [111] Calcite coccoliths are produced intracellularly by nucleation of calcite crystals in Golgi-derived coccolith vesicles. As the coccoliths mature, the endomembranes associated with the vesicles becomes more complex and play an important role in transportation of ions and development of morphology. The mature coccolith is subsequently released to the cell surface via exocytosis. [112] Most haptophytes are predominately calcified although some species may be covered with silica scales that resemble a “silicified coccolithopore,” as in the case of Prymnesium neolepis. [113] Formation of siliceous coccoliths is due to the presence of silicon transporters on their membranes. [114] The role of Si in coccoliths production has yet to be determined, but may be involved in stabilization of the intermediate form of CaCO₃ in P. neolepis. [115] Multiphase mineralization may also be seen in the diatom Didymosphenia geminata which produces a cytoskeleton that consist of both biogenic silica and calcite nanofibers. [116]

Other single-celled marine protists (i.e., eukaryotes that cannot be classified as plants, fungi, or animals) such as choanoflagellates [117] and radiolarians [118] have also been identified to induce mineralization. Their silicification processes are less well studied compared with algae.

Microbial mineralization of soil organic matter plays an important role in cycling of carbon, nitrogen, and phosphorus. Microbial mineralization is influenced by factors such as soil temperature, soil structure, and character of soil microorganisms. [119] Microbial mineralization of nitrogen and phosphorus is largely determined by C:N:P ratios in soils, depending on the decomposition state of organic matter. [120] Bio-geochemical cycles (including cycling of C, N, and P) are biologically coupled through microbial immobilization and mineralization. [121] It appears that carbon mineralization is the original driving force for the mineralization of other elements. Study has shown that microbially mediated mineralization is not driven by the microbial need for phosphorus, but by microbial carbon acquisition. [122] Below certain threshold soil C:N ratios (28–40) or N:P ratios (42–60), net nitrogen mineralization occurs and increases with decreasing ratios. Net phosphorus mineralization occurs below the threshold soil C:P (1000–1400) or N:P ratios (40–44) and also increases with decreasing C:P and N:P ratios. [120]

The effect of climatic temperature on soil mineralization is also significant and has received much attention in light of global warming threats. Study shows that the rate of soil organic carbon (SOC) mineralization significantly increases with increasing temperature, [119] due to improvement in the ability of microbes to decompose the SOC in soils. Difference in temperature pattern (constant or fluctuating) may also influence SOC mineralization. [123] With the advent of global warming, the global mean temperature has been predicted to increase by another 1.5–2 °C by the end of this century. [124] The impact of extreme temperature on soil mineralization has also been investigated in vitro. These environment disturbances affect soil mineralization as a result of changes in microbial community activity. Extreme temperature events may result in faster cycling of nitrogen and carbon but slower cycling of phosphorus, consequently resulting in the decoupling of phosphorus cycling from those of carbon and nitrogen. This may result from different stress responses of the microbial community to these processes. Extreme temperature influences the microbial community composition, subsequently resulting in changes in extracellular enzyme activities. Enzymes synthesized by microorganisms are involved in protein and glucan depolymerization in soil mineralization to produce NH₄⁺, PO₄³⁻, and NO₃⁻. [121]

3. Mechanisms of Microbe-Mediated Mineralization

Biogenic mineralization may be biologically induced or biologically controlled in nature. Biologically induced mineralization is the result of cell metabolism; however, the cells do not directly control where or how the precipitates are produced. Biologically controlled mineralization occurs in isolated compartments within a living organism, producing highly ordered precipitates in which the size, texture, and orientation are controlled by the organism. For the sake of convenience, these mineralization processes are collectively referred to as microbe-mediated mineralization herein.

Minerals produced by microbes may be broadly classified into extracellular and intracellular mineralization. In extracellular mineralization, the cell produces an organic matrix outside or around the cell, in an area that eventually forms the site of mineralization. The cell may actively pump cations through the cell membrane into the destined mineralization site. Alternatively, the cations may be concentrated within vesicles inside the cell, which are subsequently exported through the membrane and disintegrated within the matrix.

Intracellular mineralization occurs within specialized intracellular vacuoles or vesicles that direct the nucleation of biominerals within the cell via highly concerted metabolic activities. These compartmentalized crystallization environments exert control over the biomineral composition and morphology. [125] Examples of microbial metabolic activities that result in intracellular mineralization include: i) oxygenic photosynthesis (e.g., cyanobacteria); ii) ureolyis (e.g., Bacillus sp. that utilize urea as a nitrogen source); iii) ammonia oxidation (e.g., nitrifying bacteria); iv) nitrate reduction (e.g., denitrifying bacteria); v) ammonification of amino acids (e.g., soil Myxobacteria); vi) iron oxidation (e.g., iron-oxidizing bacteria); vii) sulfur oxidation (e.g., sulfur-oxidizing bacteria); viii) sulfur reduction (e.g., sulfate-reducing bacteria); and ix) methane oxidation to bicarbonate under anoxic conditions (e.g., methanotrophic bacteria or archaea). [9, 126, 127] Once formed, the biominerals may be exported outside the cell or remain within the cell.

3.1. Extracellular Biomineralization

There are several requirements for extracellular mineral deposition: 1) sufficient raw materials, such as the concentration of...
dissolved inorganic carbon, 2) pH (often alkaline condition), mediated by microbe metabolic activities, including sulfate reduction, organic acid degradation, and urea hydrolysis,[9,128] and 3) the availability of nucleation sites.[6,9]

3.1.1. Production of Raw Material

For extracellular mineralization to occur, it is first necessary for the microbes to attain sufficient soluble materials. For example, saprophytic bacteria and fungi are capable of phosphorus solubilization. The organic (carboxylic acid) and inorganic acids (e.g., hydrochloric acid) secreted by the phosphorus solubilizing bacteria can lower the pH to dissolve inorganic phosphate (e.g., \( \text{Ca}_3(\text{PO}_4)_2 \)).[129] Increasing the activity or amount of microbe biomass can accelerate the release of C and N from soil organic matter.[130] Another mechanism to increase the concentration of soluble ions is catalysis by enzymes such as alkaline phosphatase. Such a mechanism can be seen in E. coli. These microbes release orthophosphate ions following the hydrolysis of organic phosphates; precipitation of calcium phosphate occurs on supersaturation of the orthophosphate ions.[131]

3.1.2. Initial Biomineralization

Classical nucleation theory (CNT) and nonclassical nucleation (NCN) theory have been proposed to elucidate the initial stage of mineralization. The former theory is successful in explaining the nucleation phenomenon when it is involved in purely qualitative considerations.[132] In CNT, crystal growth is mediated by atom, molecule, or ion deposition. In contrast, NCN is mediated by particle aggregation or self-assembly.[133]

In CNT, nucleation refers to the commencement of a phase transition, which is the irreversible formation of the first nucleus as a nascent supersaturated phase.[133] Nucleation involves overcoming a barrier or activation energy.[134] For microbial mineralization, the nucleation site may be the EPS produced by microbes or proteins present on the surface of those cells. The EPS consist of high-molecular-weight polysaccharides and proteins, as well as macromolecules such as DNA, lipids, and humic substance secreted by the microbes as natural polymers. The EPS have a strong correlation with nucleation[135] because of the presence of polysaccharides and proteins that contain carboxyl, phosphate, amine, and hydroxyl groups.[136] They play an important role in microbial mineralization by sequestering metal cations via binding to those charged groups. Upon degradation of the EPSs, the supersaturated localized cations are precipitated as minerals (Figure 1A).[6] For example, yeast secretes proteins, which can combine with \( \text{Cd}^{2+} \) or \( \text{Te}^{2+} \) and induce nucleation.[137] Nucleation on the surface of microbes has been reported as well. For example, platinum and palladium are first absorbed on the periplasm of Desulfovibrio vulgaris, a species of Gram-negative sulfate-reducing bacteria in the Desulfovibrionaceae family. These ions then erupt through the outer membrane of the bacteria and accumulate on the cell surface.[138] Fungal hyphae contain a thick calcareous coating, which is the nucleation site for the precipitation of calcite. Formation of calcium oxalate proceeds via accumulation of \( \text{Ca}^{2+} \) on the fungal surface. The calcium ions react with oxalic acid secreted by the fungi as an oxidation product of carbohydrates.[140] Molecules present on the surface of microbes play an important role in nucleation. For example, iron reacts directly with the carboxyl and hydroxyl groups of amino acids present in cell surface proteins, resulting in the formation of metal–protein complexes through metal oxidation reactions.[141] The molecules in the EPS or on the surface of the microbes contain negatively charged functional groups with which metallic ions can combine to induce extracellular mineralization.[142] Surface enzymes such as nitrate reductases that participate in ion reduction are important for nanoparticle formation.[143] Tryptophan, which possesses an indole group, is known to reduce gold ions to synthesize Ag nanoparticles.[144] Likewise, polylysine can also reduce metal ions.[145] Apart from surface enzymes, proteins present on the cell membrane or cell wall of microbes also have the ability to reduce ions,[146] such as reduction of Ag(I) to Ag(0) to form Ag nanoparticles.[147] For example, surface proteins of the fungus Fusarium oxysporum bind to silver in their thiol regions to form SH-Ag bonds that participate in the conversion of Ag(I) to Ag(0).[148]

In NCN, prenucleation clusters (PNCs), which are considered as solutes, have no relationship with phase separation, at least in the initial stage. Prenucleation clusters are around 1–3 nm in size on average and have no phase boundary with the surrounding solution.[133] These highly dynamic, stable, hydrated-ion-association complexes undergo liquid–liquid phase separation to produce nanodroplets with distinct interfaces; the latter subsequently aggregate to produce amorphous mineral precursor intermediates that eventually crystallize by loss of hydration water and solid-state transformation.[149] The PNCs for calcium carbonate are held together by the ionic bond between a calcium cation and carbonate anion instead of covalent linkage.[150] The PNCs in microbe-mediated calcium carbonate precipitation produce amorphous calcium carbonate (ACC)[151] and in microbe-mediated calcium phosphate precipitation produce amorphous calcium phosphate (ACP).[152] Extracellular organic material in the EPS matrix can serve as nucleation sites to facilitate precipitation of ACC.[153] Compartmentalization plays an important role in biomineralization,[154] with the phospholipid bilayer membrane acting as a selective diffusion barrier.[155] Compared with macromolecules present in an organic matrix, the surface chemistry of the phospholipid bilayer and its selective permeability for certain materials contribute to the stability of amorphous precursors. Biological confinement also influences the kinetics of phase transformation.[156] However, the exact mechanism of how PNCs grow to reach a critical size is still unclear. It has been hypothesized that PNCs grow to a critical size based on the ion-by-ion growth or individual aggregation, and that biomineralization occurs within a hydrogel environment formed by specific proteins. Internal pores within the hydrogel environment serve as “limited volume reaction vessels” that enable nucleation to occur.[157]

Intracellular biomineralization is influenced by molecular crowding, which alters the thermodynamic activities of small
species and induces diffusion limitations that can enhance or suppress the formation of mineral precursors and their self-association. Ion-association and nucleation of the solid mineral phase are inhibited in a crowded environment by macromolecules such as poly(ethylene glycol). The same processes are promoted by other macromolecules such as bovine serum albumin. Under the conditions of macromolecular crowding, the ultimate result represents a trade-off between multiple factors, including enhancing thermodynamic activities of certain reactants and susceptibility to both diffusion and altered hydration dynamics. This is a significant phenomenon in biomimetic mineralization.

Stabilization of mineral precursors in vesicle-based confined volumes is a widespread strategy for transporting amorphous mineral precursors and regulating crystalline mineral precipitation. Recent progress in the understanding of nucleation and crystallization phenomena shows that metastable fluidic mineral precursors can be transiently stabilized by additives. Investigations of the effect of intrinsically disordered protein domains with simple and repetitive amino acid compositions on the stability of ion clusters, liquid-condensed phases, and amorphous mineral precursors indicate that the natively unfolded, disordered regions of such protein molecules are responsible for confining hydrated mineral precursors, transiently suppressing mineral precipitation and crystallization. Prior to mineral nucleation, ions and ion clusters lower the solubility of intrinsically disordered proteins, conditioning those domains with low sequence complexity toward self-association and conformational transitions that favor reorganization into amyloid-like β structural conformations. These organic–inorganic interactions modulate ion–ion association, stabilize PNCs and amorphous mineral phases, and transiently prevent mineral crystallization (Figure 2). The binding and displacement of organic molecules from ions, ion clusters, liquid–liquid phase-separation droplets, and particles representative of the mineral phase are affected by i) the molar mass of the additives and their affinity to a particular mineral phase, ii) the changes in energy landscapes of conformational ensembles in response to ionic conditions and interfacial adsorption, and iii) the physicochemistry of the mineral–solvent interface.
3.1.3 Subsequent Growth

This is an important process that ultimately determines the morphology and size of the final crystal. Similar to nucleation, crystal growth may be accounted for by the classical crystallization process and nonclassical crystallization (NCC) process.

According to the classical crystallization concept, nucleation is predominant in highly supersaturated solutions while crystal growth is predominant in low supersaturated solutions. The Ostwald rule of stages is the major principle that governs classical crystallization, which is the ordered arrangement of material into a more stable crystalline structure. This process may proceed layer by layer by adding single atoms or molecules to the nucleation site, which is driven by solvent supersaturation. In this process, different parameters may result in different growth rate of every single crystal facets, which, in turn, determines crystal morphology.

Not all extracellular mineralization is clear-cut. Post-nucleation mineralization of the hyperthermophilic archaeon, *Sulfolobus acidocaldarius*, for example, begins with extracellular mineralization outside the proteinaceous surface layer (S-layer) and subsequently beneath the S-layer within the cytosol (Figure 1b). After nucleation (step #1), mineral portions in form of domes are synthesized over the initial portions of the S-layer/mineral assemblies (step #2), which is followed by the fusion of the mineral assemblies (step #3). In this manner, the entire cell surface is eventually covered by a continuous mineral layer, leaving behind a nonmineralized layer related to the original S-layer (step #4). Fossilization of the S-layer subsequently occurs through the extracellular mineralized layer and intracellular mineralization occurs on the cell membrane beneath the S-layer (step #5).

According to the NCC concept, oriented attachment and mesocrystal formation are important in generating crystal architectures which cannot be produced by the classical pathway. Mesocrystals are considered the intermediate products in this process, which are composed of primary units without coherent, crystalline material connection. The primary units achieve crystallographic alignment despite spatial segregation from one another. Identical crystallographic orientation leads to the connection of PNCs after the formation of precursors. The mesocrystals ultimately fuse into oriented aggregates as a new single crystal. Proteins have been identified to play an important role in the guided assembly and organization of mesocrystals into higher-ordered structures.

Regulation of crystallographic orientation results in the generation of biominerals that exhibit superstructural organization at multiple length scales. Coccoliths produced by coccolithophores, for example, is intermediate in structural complexity compared with single crystals produced by prokaryotes and the hierarchical composites in multicellular organisms.

3.2 Intracellular Biomineralization

Elements for intracellular mineralization may enter the cell directly at the mineralization site or are transported to the exact site where mineralization occurs after cell entry. In the latter process, ions are concentrated in intracellular membrane-bound vesicles in the form of a highly disordered solid phase, which is then transported to the final mineralization site. Dedicated organelles, such as ion pumps and channels used for the cellular uptake, are often involved in such a process. Chemical and structural modifications sometimes occur inside
the cell to temporarily contain the imported elements. Magnetite biomineralization represents an excellent example of microbe-mediated intracellular mineralization. Other biomineralization processes induced by algae have also been reported.

The mechanism of magnetite biomineralization may be explained by a multistep process that occurs inside membrane-bound organelles—the magnetosomes (Figure 3A). The first step involves invagination of the cytoplasmic membrane. Ion transporters including FeoB1 and FeoB2 are involved in transporting iron into the cytoplasm and subsequently into magnetosomes, or directly into the magnetosomes. The imported Fe$^{2+}$ ions are encapsulated by a membrane, which is the precursor of bacterial magnetite particles (BacMP). Reduction of Fe$^{2+}$ to Fe$^{3+}$ occurs on contact of the Fe$^{2+}$-ion-containing vesicles with the cytoskeletal filaments, with the released electron transported into the cellular electron pool through the magnetosome transport chain. Finally, proteins attached to the BacMP initiate nucleation and also regulate the morphology...
of the magnetite crystals. Related proteins such as mms6, mamGFDC, msmS, mamIT, mamR, mmsF, and mamP promote and regulate the growth of magnetite crystals to their correct shape and size.\textsuperscript{[168,170]} Subsequent crystal growth may be elucidated as follows: cubo-octahedral particles are formed with initial isotropic growth. This is followed by anisotropic growth and elongation along the destined direction. The diverse twisted shapes of magnetite crystals are due to the offset between the initial and final elongation directions.\textsuperscript{[171]} External physical conditions may also have a minor influence on the biosynthesis of magnetite nanoparticles.\textsuperscript{[172]}

Coccoliths are individual plates of CaCO\textsubscript{3} formed by coccolithophores (single-celled algae such as \textit{Emiliania huxleyi}), which are arranged around the cell into a coccosphere. Coccoliths are produced by the vesicles and Golgi apparatus within the marine algae cells. Intracellular calcification commences by budding of membrane-bound bodies containing acidic polysaccharides, a disordered calcium phase, and carbonate that originate from the Golgi apparatus. These bodies are subsequently deposited in an intracellular membrane system known as “coccolith vesicle-reticular body”.\textsuperscript{[173]} During this stage, polysaccharides inhibit precipitation of CaCO\textsubscript{3}. The vesicle expands as more and more segregated bodies are supplied by the Golgi apparatus. The hollow space with less polysaccharide inside the coccolith vesicle provides the necessary environment for the precipitation of CaCO\textsubscript{3}. Contact with the polysaccharide on the vesicle membrane terminates this process. Finally, the reticular body disappears and the coccolith vesicle is extruded to join the shell of coccoliths, the coccosphere exoskeleton (Figure 3B).\textsuperscript{[112]}

A similar process also exists in the intracellular silica precipitation by diatoms. The organisms build complex, hard, but porous cell wall frustules that are composed primarily of silica. Silica deposition vesicles (SDVs) originate from the endoplasmic reticulum and Golgi apparatus.\textsuperscript{[174]} The SDVs contain silica-forming proteins known as silaffins and long-chain polyamines.\textsuperscript{[169]} These molecules function as the catalysts for the hydrolysis of Si(OH)\textsubscript{4} hydroxyl groups to produce solid amorphous silica.\textsuperscript{[175]} The nanopatterned biosiliceous organic–inorganic hybrid composite is precipitated within the SDVs and ultimately become the frustules (Figure 3C).\textsuperscript{[176]}

The efflux of heavy-metal ions into cells can cause a series of reactions to reduce the negative effect to the cells. The antioxidant defensive system of the microbe will be induced by these heavy ions to reduce the content of heavy metals within the cells by facilitating their efflux.\textsuperscript{[177]} Many ion carriers, such as desferrioxamine E, desferrioxamine B, and coelichelin, have been demonstrated to reduce heavy metals such as Cd\textsuperscript{2+}. These ion carriers may be responsible for maintaining metal homeostasis.\textsuperscript{[178]} A possible explanation of heavy-metal precipitation may be a self-protective, survival mechanism of the microbe.

4. Microbes as Tiny Cell Factories for Biosynthesis of Inorganic Nanoparticles

Nanoparticles have many applications because of their nanoscale, which will be elucidated in subsequent subsections. Present chemical and physical synthesis methods have many drawbacks, such as high cost, low efficiency, and toxicity to the environment. Consequently, much attention has been devoted to the use of microbes as cost-effective and ecofriendly nanoscale factories for synthesizing metallic nanoparticles\textsuperscript{[179,180]} (Table 2).

4.1. Inorganic Nanoparticles Synthesized by Bacteria

Gold nanoparticles (AuNPs) have unique characteristics such as tunable optical–electronic properties, excellent chemical reactivity, flexibility in functionalization, and nontoxic behavior, resulting in their widespread applications.\textsuperscript{[181]} Bacteria have been used as a cost-effective means to synthesize AuNPs. Biosynthesis of AuNPs has been reported in different prokaryotic organisms such as \textit{E. coli}, \textit{Bacillus subtilis}, \textit{Pseudomonas aeruginosa}, and \textit{Rhodopseudomonas capsulate}. More recently, \textit{Deinococcus radiodurans}, a polyextremophilic bacterium that can survive cold, dehydration, radiation, vacuum, and acid, has been used to provide a reducing microenvironment for transformation from Au\textsuperscript{3+} into AuNPs or detoxification of heavy metals under in situ oxidative stresses.\textsuperscript{[182]} The marine bacterium, \textit{Marinobacter pelagius}, has also been used to synthesize AuNPs in a cost-effective manner.\textsuperscript{[187]}

Bacteria-mediated synthesis of AuNPs involves interaction of Au(III) with the hydroxyl, amine, phosphoric, and carbonyl groups of bacterial proteins, which causes the reduction of Au(III) to Au(0) via the intermediate Au(I) state.\textsuperscript{[182]} Certain proteins embedded in the bacterial membrane catalyze the formation and stabilization of AuNPs.\textsuperscript{[183]} Intracellular synthesis of AuNPs by thermophilic \textit{Geobacillus} sp. strain ID17 is mediated by a nicotinamide adenine dinucleotide phosphate (NADPH)-dependent reductase that reduce Au\textsuperscript{3+} to elemental gold.\textsuperscript{[184]}

Biotechnological applications of AuNPs are affected by the shape and size of the synthesized nanoparticles. In immunological response, for example, 40 nm spherical nanoparticles express the highest level of specific antibodies against West Nile virus, while rod nanoparticles result in only 50% antibody production against the virus.\textsuperscript{[185]} Consequently, controlling the shape and size of nanoparticles is essential in biosynthesis. The morphology and dimensions of AuNPs are strongly dependent on biosynthesis conditions, such as temperature, concentration of metallic salt, and pH. The bacterium \textit{Rhodopseudomonas capsulate} produces AuNPs of different sizes and shapes when incubated with HAuCl\textsubscript{4} salts at different pH values. It can form spherical nanoparticles at pH 7.0 and nanoplatelets at pH 4.0, indicating that pH may be the most significant element in controlling this process.\textsuperscript{[185]}

The application of silver nanoparticles (AgNPs) is extensive, ranging from industrial uses such as electronics, biosensing, clothing manufacture, food storage, paints, sunscreens, and cosmetics,\textsuperscript{[190]} to medical applications which include treatment for wounds, burns, and bacterial infections caused by drug-resistant microorganisms.\textsuperscript{[199]} Environmentally friendly biosynthesis of AgNPs by microorganisms has received much attention in recent years because of the unique properties of AgNPs.

An important characteristic of AgNPs is their antibacterial ability. Studies have identified that AgNPs potently inhibit the growth of diverse bacteria such as \textit{E. coli}, \textit{Vibrio cholera},
that after exposure to Ag\(^+\) amino groups of proteins secreted by bacteria and the aldehyde protein-capped AgNPs is dependent on the interaction between the low concentration of AgNPs synthesized by *P. aeruginosa* proliferation of cervical cancer cells is completely inhibited by *Bacillus* sp., *sp.*, and *sp.* In addition, the *Proteus* *Staphylococcus* *Corynebacterium* *Aeromonas* *Salmonella* *sp.*,

It has been suggested that after exposure to Ag\(^+\) ions, the CusCBA actively pumps Ag\(^+\) ions to nano-

Silver is a heavy metal with a relatively high toxicity in prokaryotes. However, some bacteria have evolved different mechanisms that regulate the intracellular concentrations of Ag\(^+\). An example of how one of these mechanisms is utilized for intracellular synthesis of AgNPs by a silver-resistant *E. coli* strain 116AR under anaerobic condition is shown in Figure 4A.\textsuperscript{[192]} This silver-resistant strain possesses robust resistance to Ag\(^+\) ions because of the constitutive expression of the tripartite CusCBA copper/silver efflux pump system that traverses the outer membrane, periplasm, and inner membranes of the bacterium (Figure 4B).\textsuperscript{[203]} It has been suggested that after exposure to Ag\(^+\) ions, the CusCBA actively pumps Ag\(^+\) ions from the cells, during which these ions are concentrated within the periplasm.\textsuperscript{[192]} Within the periplasm, electron transfer occurs via the nitrate reductase enzyme complex, aided by the coenzyme NADPH, reduces the Ag\(^+\) ions to nanoparticles (Figure 4C).\textsuperscript{[202]} Nevertheless, the role played by nitrate reductase in the reduction of Ag\(^+\) has recently been challenged in experiments which demonstrates that NADPH may be used as the sole reducing agent for the synthesis of AgNPs (Figure 4D).\textsuperscript{[203]}

Copper nanoparticles (CuNPs) possess antimicrobial activity and have been used as biocides and antibiotic substitutes.\textsuperscript{[193]} They are capable of hindering the growth or killing bacteria such as *E. coli*\textsuperscript{[194]} and *Bacillus subtilis*.\textsuperscript{[204]} Although fungi and other biological systems can also be used to synthesize CuNPs, bacteria are easier to culture and can produce extracellular nanoparticles.\textsuperscript{[193]} In addition, the milder experimental conditions (pH, temperature), easier downstream processing and shorter generation time render bacteria more appropriate for synthesizing CuNPs.\textsuperscript{[195]}

The use of bacteria for synthesizing CuNPs is affected by parameters including pH, concentration of copper, volume of cell-free supernatant, and reaction time. *Shewanella loihica* PV-4 expresses cytochrome C, soluble proteins such as vitamins, and organic acids that donate electrons for the reduction of heavy-metal ions.\textsuperscript{[205]} Extracellular and intracellular vanadium (V) and chromium (VI) nanoparticles have been simultaneously synthesized using *Shewanella loihica* PV-4.\textsuperscript{[206]}

Because the ionic form of copper is toxic at high concentrations, synthesis of CuNPs by copper-resistant bacteria was reported to involve 5 steps.\textsuperscript{[206]} Cuprous ions that enter the bacterium through its outer surface (step 1) are presented to the

| Category of NPs | Species | Shape | Size [nm] | Location | Ref. |
|----------------|---------|-------|-----------|----------|------|
| AuNPs          | *Deinococcus radiodurans* | Spherical, triangular, and irregular shapes | Average size 43.8 | In the cell envelope, across the cytosol and in the extracellular space | [182] |
|                | *E. coli* | Circular | ≈50 | Extracellular | [183] |
|                | *Geobacillus sp. strain ID17* | Quasi-hexagonal | 5–50 | Intracellular | [184] |
|                | *Rhodopseudomonas capsulate* | Spherical | 20–40 | | [185] |
|                | *Salmonella enterica* | Quasi-spherical | 3.3–18 | Extracellular | [189] |
|                | *Marinobacter pelagius* | Spherical | 2–6 | | [187] |
|                | *Shewanella oneidensis MR-1* | Spherical | 15 | Intracellular | [188] |
| AgNPs          | Aqueous extract of *Oscillatoria limnetica* | Quasi-spherical | 3.3–18 | Extracellular | [189] |
|                | *P. aeruginosa* IQ983948 | Spherical | 13–76 | Extracellular | [190] |
|                | *Bacillus strain CS 11* | Spherical | 42–92 | Extracellular | [191] |
|                | *Vibrio alginolyticus* | Spherical | 50–100 | Intracellular and extracellular | [191] |
|                | *Bacillus sp. GP-23* | Spherical | 7–21 | Extracellular | [191] |
|                | *E. coli* 116AR | Spherical | 5–70 | Intracellular | [192] |
| CuNPs          | *Pseudomonas fluorescens* | Spherical and hexagonal | Average size 49 | Extracellular and intracellular | [193] |
|                | *Shewanella oneidensis MR-1* | Spherical | 2–6 | | [187] |
|                | *Morganella psychrotolerans* | Quasi-spherical | 3.3–18 | Extracellular and intracellular | [189] |
|                | *Shewanella oneidensis* | Spherical | 20–50 | Extracellular and intracellular | [196] |
| Zn\(^{2+}\)-substituted Fe NPs | *Geobacter sulfurreducens* | Spherical | 20–50 | Extracellular and intracellular | [196] |
silver/copper-resistant machinery within the inner cytoplasmic membrane of the bacterium (step 2). This triggers a cascade of events wherein a metal ion reductase or similar proteins bind to the Cu²⁺ ions (step 3), reducing them to metallic CuNPs (step 4). The nanoparticles are subsequently released from the bacterium via a cellular efflux system (step 5).

An example of extracellular and intracellular synthesis of CuNPs by the copper-resistant, gram-negative, metal-reducing γ-proteobacterium, Shewanella oneidensis MR-1, is shown in Figure 5A,B.[198] Similar to Ag⁺ resistance by silver-resistance microbes, the CusCBA copper/silver efflux system may be involved in copper homeostasis for intracellular detoxification of copper Figure 5C.[200] This multicomponent efflux complex is disassembled in the absence of Cu⁺ or Ag⁺ and is only assembled in the presence of increase metal stress Figure 5D.[208]

Synthesis of compound nanoparticles that consist of two metals has also been reported. The magnetic moments of magnetite are significantly improved by the addition of zinc in the biosynthesis process. This process is undertaken by the iron-reducing bacterium Geobacter sulfurreducens and involves the oxidation of Fe²⁺ to Fe³⁺ and the replacement of some of the Fe⁺⁺ by Zn⁺⁺ (ZnFe₂₋₋₋O₄). By controlling the content of Zn⁺⁺ in the magnetic nanoparticles, the improved nanoparticles perform much better in magnetic resonance imaging application.[197]

Magnetite nanoparticles have been intensively investigated for a plethora of technological applications, including MRI, ferrofluids for audio speakers, magnetically targeted drug delivery, magnetic recording media, water purification, oil spill clean-up, emulsion separation of crude oil, as well as the diagnosis and treatment of bacterial infections.[209,210] Dissimilatory Fe(III) reduction is the process by which some microbes transfer electrons to external ferric iron [Fe(III)], reducing it to ferrous iron [Fe(II)] without assimilating the iron.[211] This biologic electron transfer mechanism is utilized by the Gram-negative metal and sulfur-reducing bacterium, G. sulfurreducens, in extracellular reduction of Fe(III) iron oxides (Fe₂O₃; rust) to generate soluble Fe⁺⁺ and solid magnetite (Figure 6A). A porin-cytochrome pathway is involved (Figure 6B),[212] in which electrons are transferred from quinol in the inner cytoplasmic membrane, through multiple c-type cytochromes present in the periplasm and across the OmbB and OmbC porin-like proteins on the outer membrane to the bacterial surface for the extracellular reduction process.[213] Indeed, G. sulfurreducens is one of the very few microbes that have been exploited in industrial scale-ups for the production of magnetite nanoparticles.

4.2. Inorganic Nanoparticles Synthesized by Fungi

Representative examples of metal nanoparticles produced by fungi are listed in Table 3. Fungi such as Cladosporium oxysporum AJP03,[181] Yarrowia lipolytica,[223] Mariannaea sp.,[236] and Penicillium citrinum[237] have been used to synthesize AuNPs. The mechanism involves reduction of Au⁺⁺ and Au⁺ by intracellular redox mediators and proteins derived from the cell walls of those organisms.[218,219] These processes may occur intracellularly or extracellularly (Figure 7).[240–242] Nitrate reductases, which are expressed on the fungal cell surface or within the cytoplasm, are capable of reducing Au ions[241] or Ag ions into the corresponding nanoparticles.[239] Another mechanism for fungal-mediated biosynthesis of AuNPs involves the reaction of AuCl₄⁻ ions by glutathione-like compounds such as phytochelatins and metallothioneins derived from S. cerevisiae, S. pombe, and Candida glabrata.[243] After reduction, the AuNPs are stabilized and prevented from aggregation by extracellular proteins derived from fungi as capping agents.[244] Yeasts
such as *Magnusiomycetes ingens*, *C. albicans*, *Hansenula anomala*, *Fusarium oxysporum*, and *Yarrowia lipolytica* have also been used to synthesize AuNPs.

Compared with bacteria, fungi have several advantages in AuNP biosynthesis. An important property is that fungi secrete large amounts of extracellular proteins with diverse functions, including serving as the reducing medium and capping agents. Because AuNPs are capable of enhancing the sensitivity of surface plasmon resonance biosensors, they have been used in bioassay applications to detect different analytes through observation of the color change of the AuNPs.

Fungi such as *Pythium aphanidermatum*, *Fusarium oxysporum*, *Epichococcus nigrum*, and *Schizophyllum commune* have also been used for green synthesis of AgNPs.

Similar to AuNPs, the mechanism of fungi-mediated synthesis of AgNPs involves reduction of Ag⁺ to Ag⁰ by the metabolites released by the fungi. Stabilization of AgNPs to prevent their aggregation may be achieved via a protective coating. Humic acid has been reported to function as a reducing agent for the synthesis of AgNPs at room temperature and as a capping agent to enhance the colloidal stability of the nanoparticles by preventing them from aggregating into a larger mass of silver. Compared with chemically synthesized nanoparticles, biogenic AgNPs are generally less toxic to the human body.

Both CuNPs and copper oxide nanoparticles possess antibacterial and biocidal properties. They have great potential for biomedical applications because of their cost-effectiveness and relatively low toxicity to human cells. Biosynthesized CuNPs possess more potent antimicrobial activities than nanoparticles synthesized using chemical routes. Fungus-mediated synthesis of CuNPs has been reported using *Stereum hirsutum*, various *Penicillium* sp., *Trichoderma koningiiopsis*, and the dead biomass of *Hypocrea lixii*. Many parameters influence the CuNP synthesis process. The particle size and polydispersity index of fungus-mediated CuNPs are influenced by pH and salt concentration. Because heavy metals are generally toxic to the live cells, the dead biomass of fungi is often used for synthesizing CuNPs. Proteins secreted by the fungi are also utilized for stabilization of CuNPs to prevent them from aggregation. However, the types of protein involved in interactions with CuNPs remain to be determined.

Iron nanoparticles may also be produced by fungi. These nanoparticles have important biotechnological applications, including chemical/biochemical sensing, disease diagnosis, optical imaging, photothermal cancer therapy, drug delivery, and antimicrobial agents. Further studies are required to identify more cost-effective and scalable biosynthesis methods.

5. Applications

5.1. Environmental Applications

Pollutants such as toxic metals and radionuclides pose serious environmental threats. Bioremediation via the use of bacteria is an important strategy to address these problems. The global bioremediation technology market was valued at US$...
12.600 million in 2018 and will reach US$ 22.100 million by the end of 2025, growing at a compound annual growth rate of 7.3% between 2019 and 2025.\textsuperscript{256} Microbe-induced carbonate precipitation (MICP) has been used for immobilization and bioremediation of toxic metals such as strontium,\textsuperscript{257} nickel,\textsuperscript{258} chromium,\textsuperscript{259} lead,\textsuperscript{260} uranium,\textsuperscript{128} cadmium,\textsuperscript{261} and arsenic\textsuperscript{262} from contaminated soil and aquatic ecosystems.

High concentrations of heavy-metal ions are toxic to most bacteria, causing derangement of nucleic acids, disturbance in oxidative phosphorylation and osmotic imbalance. Nevertheless, some bacteria species have evolved intricate intracellular and extracellular heavy-metal tolerance mechanisms such as transportation of the metal ions across cell membranes, intracellular and extracellular entrapments/precipitations, metal sequestration by intracellular metallothioneins, conjugate formation, and redox reactions, to convert these toxic metal pollutants to nontoxic or less toxic species (Figure 8).\textsuperscript{261}

Although some heavy-metal-resistant genes are located in the chromosomes, most of the genes that determine heavy-metal resistance mechanisms are found predominantly on plasmids. Hence, resistant traits are transferable to other organisms. This opens vistas for the development of novel genetically modified bacteria via engineering of single genes or operons (gene clusters) for environmental heavy-metal bioremediation. A detailed treatise on the genetic control of bacteria resistance to different heavy-metal ions is beyond the scope of the present review; interested readers are referred to a recent publication on the genetic basis of bacterial heavy-metal tolerance.\textsuperscript{264}

Fungi may also be used for the precipitation of metal-containing carbonates and phosphates; they provide a new method of metal biorecovery and purification, including Cu\textsuperscript{2+}, Zr\textsuperscript{4+}, Pb\textsuperscript{2+}, As\textsuperscript{3+}, U\textsuperscript{6+}, and Cd\textsuperscript{2+}.\textsuperscript{71,79,260} As described previously, yeasts have been investigated extensively for bioremediation of heavy-metal ions.\textsuperscript{83–94} An example of the use of S. cerevisiae for the biomineralization of uranium (VI) is illustrated in Figure 9.\textsuperscript{265} Uranium exists predominantly in two environmentally important oxidation states, U(IV) and U(VI). Whereas U(IV) is only sparingly soluble and more stable, U(VI) (e.g., UO\textsubscript{2}\textsuperscript{2+}) is soluble and may be discharged into aquatic systems via weathering and leakage from radioactive wastes. When S. cerevisiae is exposed to a source of UO\textsubscript{2}\textsuperscript{2+}, these ions are adsorbed to the surface of the cell wall. In the presence of phosphate released by the yeast cells, the U(VI) reacts to produce an amorphous, lamellar U(VI) precipitate on the cell surface. Gradual depletion of phosphate over a period of 7 days results in gradual neutralization of the acidic environment in the vicinity of the yeast cells. This neutral pH is conducive to the conversion of the amorphous precipitate into extracellular tetragonal chernikovite (UO\textsubscript{2}HPO\textsubscript{4}·4H\textsubscript{2}O) crystallites, a U(VI) autunite-type mineral.\textsuperscript{265}

Magnetic nanoparticles have shown great potential in the removal of traces of Sr(II),\textsuperscript{266} perfluorooctane sulfonate,\textsuperscript{267} chloroauroic ions,\textsuperscript{268} and other organic and inorganic pollutants from contaminated water.\textsuperscript{269} Magnetotactic bacteria can easily move under a magnetic field because of their magnetosomes. Some MTB absorb heavy-metal ions, including Fe, Ni, As, Ni, Mg, Cd, and Cu. Once absorbed, these ions can be readily removed by magnetic separation.\textsuperscript{270} For example, an MTB strain UPB-MAG05 has been found to be highly tolerant of Cd ions and can absorb these ions from Cd-polluted waters.\textsuperscript{271}
Ground improvement involves the modification of soil properties or constructing inclusions within the soil to achieve a required performance. Mechanical compaction and chemical grouting are often used for ground improvement. However, these methods suffer from high cost, high energy consumption, and potential environmental pollution. Microbe-induced calcite precipitation by urease-producing bacteria is a potential solution to address these issues (Figure 10A). \[272\] Urease catalyzes the hydrolysis of urea into ammonium and carbamate. Carbamate is spontaneously hydrolyzed to carbonic acid and ultimately to bicarbonate by carbonic anhydrase. Urea hydrolysis increases the pH around the cell and induces precipitation of CaCO$_3$ in the presence of soluble Ca$^{2+}$ (Figure 10B). \[273\] Soil shows remarkable improvements in strength, rigidity, permeability, and liquefaction resistance after MICP treatment. However, issues such as the homogeneity and durability of MICP-treated soils still require further investigations. \[274\]

| Radionuclides distributed in the environment cause adverse effects to the ecosystem because they can easily enter food chains. \[275\] Many conventional techniques have been used to address radionuclide contamination, such as chemical precipitation and flocculation or membrane exchange. \[276\] These methods, however, are not cost-effective. Biologically mediated mineralization appears to be a better alternative for removing these pollutants. For example, urease-positive fungi isolated from calcareous soil are capable of reducing radioactive Sr by inducing SrCO$_3$ precipitation. \[73\] |

| Table 3. Examples of the use of fungi for the synthesis of inorganic nanoparticles. |
|--------------------------------|----------------|----------------|----------------|----------------|
| Category of NPs | Species | Shape | Size [nm] | Location |
| AuNPs | Alternaria alternata | Spherical, triangular, hexagonal | 12±5 | Extracellular | [214] |
| | Aspergillus sydowi | Spherical (at 3 × 10$^{-3}$ M Au$^{3+}$ concentration) | 8.7–15.6 | Extracellular | [215] |
| | Penicillium rugulosum | Spherical, triangular, hexagonal | 20–80 | Extracellular | [216] |
| | Rhizopus oryzae | Spherical | 16–25 | Extracellular | [217] |
| | Yarrowia lipolytica | Spherical | 20 | Extracellular | [218] |
| | Magnusomycetes ingens | Spherical | 10–80 | Extracellular | [219] |
| | Plate (triangular and hexagonal) | | 16–420 | Extracellular | [219] |
| | Cell-free extract of Candida albicans | Spherical | 4–10 | Extracellular | [220] |
| | Hansenula anomala | Spherical | 4–50 | Extracellular | [221] |
| | Fusarium oxysporum | Spherical, hexagonal | 22–30 | Extracellular | [222] |
| | Yarrowia lipolytica | Spherical | 20 | Extracellular | [223] |
| | Xylanases derived from Aspergillus niger and Trichoderma lanbrachiatum | Spherical, oval, irregular | 7–52.5 | Extracellular | [224] |
| Ag-AuNPs | Pythium mycelium | Spherical | 70–80 | Extracellular | [225] |
| AgNPs | Fusarium oxysporum | Spherical, needle-shaped | 3.4–53.6 | Extracellular | [226] |
| | Schizophyllum commune | Spherical | 25.3–29.8 | Extracellular | [227] |
| | Schizophyllum commune | Spherical | 35.2–50.8 | Culture-free supernatant | [218,227] |
| | Yarrowia lipolytica | Spherical | | Extracellular | [218,227] |
| | Cell-free extract of Candida albicans | Nonspherical | Average size 30 | Extracellular | [221] |
| | Saccharomyces cerevisiae | Spherical | 60–80 | Extracellular | [228] |
| | Cornitermes cumulans | Spherical | 2–10 | Intracellular and extracellular | [229] |
| | Filtrate from Trichoderma harzianum | Spherical | 57–82 | Extracellular | [230] |
| | Stereum hirutum | Spherical | 5–20 | Extracellular | [231] |
| CuNPs | Penicillium rugulosum | Spherical | 79–295 | Extracellular | [232] |
| | Trichoderma koningiopsis | Spherical | Average size 87.3 | Extracellular | [233] |
| | Hypocrea lixii | Spherical | Average size 24.5 | Extracellular | [234] |
| | Rhodotorula mucilaginosa | Spherical | Average size 10.5 | Extracellular | [235] |
| FeNPs | Aspergillus oryzae TFR9 | Spherical | 10–24.6 | Extracellular | [214] |
High levels of toxic metal ions in the soil are detrimental to human health. Excessive lead can damage the brain, kidney, and even result in anemia.\textsuperscript{[277]} Bioremediation of lead-contaminated soil has been achieved with the use of \textit{Rhodobacter sphaeroides} via the precipitation of inert lead sulfate and lead sulfide.\textsuperscript{[278]} Phosphate-solubilizing \textit{Bacillus} sp. have been used to remove lead ions by decomposing phosphate-containing organic compounds and generating phosphate groups on their cell surface. The lead ions are subsequently precipitated as stable \textit{Pb}_3(\textit{PO}_4)_2.\textsuperscript{[279]}

Polychlorinated biphenyls, commonly used as dielectric and coolant fluids in electrical apparatus, carbonless copy paper, and in heat transfer fluids, are considered definitive carcinogens in humans. These organic chlorine compounds are not broken...
down in the environment and are considered “high-priority pollutants” because of their impact on the reproductive, immune nervous, and endocrine systems in animals. A number of microbes belonging to the phylum Proteobacteria, Firmicutes, Basidiomycota, Bacteroidetes, Ascomycota, Actinobacteria, and Euryarchaeota have been reported to degrade polychlorinated biphenyls. Recent studies have also utilized genetic modification of bacteria catabolic genes to enhance biodegradation of these biphenyls.[280]

A genetically engineered microbe is one containing genetic material that has been altered using genetic engineering techniques inspired by natural genetic exchange between microorganisms. These techniques are generally known as recombinant DNA technology. The advent of novel recombinant technology has resulted in the generation of microbes with increased mineralization potential. Genetically engineered microorganisms have shown potential for bioremediation of soil, groundwater, and activated sludge, exhibiting the enhanced degrading capabilities of a wide range of chemical contaminants. For example, biodegradation of polychlorobiphenyls by bacteria is generally incomplete, resulting in the accumulation of chlorobenzoates as dead-end metabolites. With the use of genetic engineering, the bph locus of Burkholderia xenovorans LB400, which encodes one of the most effective polychlorobiphenyl degradation pathways, has been incorporated into the genome of the polychlorobiphenyl-degrading bacterium Cupriavidus necator JMP134-X3. This modified bacterium mineralizes polychlorobiphenyls without accumulation of chlorobenzoates.[281]

5.2. Industrial Applications

Microbes provide novel insights for the design of new materials for energy concentration, storage, and transportation. Biominalization mediated by microbes has been used in electrochemistry. Manganese carbonate precipitation induced by fungi has become a new method for synthesizing electrochemical materials. The carbonized fungal biomass–mineral composite exhibits high specific capacitance as a supercapacitor and demonstrates excellent electrochemical performance when employed in lithium-ion batteries.[282] Biominalization of PbS and PbS–CdS core–shell nanocrystals induced by Stenotrophomonas maltophilia are used in quantum-dot-sensitized experimental solar cells to harvest more energy.[283] Biominalization of γ-FeOOH using the Gram-negative iron-oxidizing bacteria, Acidovorax sp. BoFeN1 strain, has been used to prepare α-Fe2O3 bacteriomorphs.[284] The γ-FeOOH is confined between the two membranes of the bacterium cell wall, which on air-heating treatment, is converted to hematite (α-Fe2O3) nanocrystals. The hematite crystals are assembled to form hollow, porous shells that retain the bacterial size and shape. These bacteriomorphs exhibit enhanced electrochemical reversibility when reacted with lithium and a high rate capability when compared to nontextured α-Fe2O3 particles of similar size. This bacterial-induced biominalization strategy offers an ecoefficient and scalable synthesis for electrochemical energy storage.[284] Diatom frustules demonstrate photonic properties when titanium or germanium dioxide is incorporated into the nanostructures. These modified frustules may be utilized for fabrication of dye-sensitized solar cells, nanostructured battery electrodes, and electroluminescent display devices.[285] Highly dispersed palladium–gold alloy nanoparticles have been synthesized by Shewanella oneidensis MR-1, an electrochemically active bacterium, as experimental electrocatalysts.
for direct liquid fuel cells to address the low energy-conversion efficacy of commercial catalytic systems (Figure 11). The bacteria functioned simultaneously as a reducing agent, a supporting material, and a doping heteroatom source. The extracellular Pd and Au nanoparticles produced on the surface of the bacteria in the presence of H$_2$PdCl$_4$ and HAuCl$_4$ are coated with graphene oxide to prevent them from aggregating during the subsequent hydrothermal step. Because of the electrical conductivity of the cell materials, the hydrothermal reaction was performed to the hybrid materials (i.e., cells, PdNPs, AuNPs, and graphene oxide) to produce Pd-Au alloy nanoparticles, during which nanoparticle aggregation was protected by graphene oxide. The latter was incorporated into the porous hybrid material as reduced graphene oxide for supporting the alloy nanoparticles. The strategy represents a novel method of utilizing natural resources to design electrocatalysts with improved catalytic activity under acidic and alkaline conditions.

Microbe-mediated CaCO$_3$ and hydroxyapatite precipitation have been reported to be a promising method for the bioremediation and preservation of stone and marble monuments with weathered surfaces or surface/subsurface cracks (Figure 12A). A biodeposition procedure may be used

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**Figure 9.** Extracellular mineralization of uranium (VI) by *Saccharomyces cerevisiae*. A) SEM images showing the precipitation of UO$_2^{2+}$ ions by: a) virgin yeast cells to b) an amorphous precipitate on the cell surface, and finally to c) extracellular crystallites in the presence of phosphate ions released by the cells, during the course of pH change from acidity to neutrality. B) Schematic of the proposed U(VI) biomineralization process. i) Surface adsorption of UO$_2^{2+}$; ii) reaction of U(VI) with released phosphate to produce amorphous U(VI) precipitate; and iii) Disappearance of the cell surface precipitate, which undergoes crystallization into extracellular chernikovite. A,B) Adapted with permission.[265] Copyright 2018, Springer Nature.
by applying a “microbial mortar” (i.e., calcium carbonate producing bacteria and CaCl₂/urea reacting agent) as a surface coating for restoring deterioration surfaces (Figure 12B). When cracks are present, biocementation procedures are employed in which the “microbial mortar” is mixed with fillers such as sand or stone/marble grains for filling those cracks (Figure 12B) to encourage cementation of those filler particles produced by MICP (Figure 12C).

Calcium carbonate precipitation is induced by the fungus *Colletotrichum acutatum* in the presence of organic acid and low carbon conditions. This process prevents the degradation of CaCO₃ and may be used for preservation of deteriorated stone relics. Calcium carbonate precipitation induced by *Cupriavidus metallidurans*, a Gram-negative, motile, non-spore-forming, rod-shaped bacterium known for its ability to resist toxic heavy metals, may be used for the restoration and preservation of ornamental stones by deposition of a compact vaterite layer on the marble substrate.

Biomineralization of CaCO₃ found in aquatic organisms such as coccolithophores and corals has attracted a lot of attention because of the ability of these extensively porous mineral skeletons to carry other materials. A microalga named *Chlorella* sp. KR-1 has been used to synthesize porous CaCO₃ particles for nonreactive supports for AgNPs. The so-formed nAg/CaCO₃ biocomposite exhibits antibacterial capacity and may be used as an additive to paints to minimize microbe-mediated dye degradation and to increase the lifetime of the painted surfaces.

Although concrete is extensively used as a construction material, formation of cracks within the Portland cement-based matrix severely limits its lifespan. Microbe-induced carbonate precipitation is a promising way of mimicking nature's sustainability. Microbial CaCO₃ produced by MICP reduces water sorption, plug pores, and bind grains within concretes to improve their durability and strength. Sealing–healing bioconcretes constructed via the incorporation of microbes with MICP capability are more resistant to corrosion and permeability of both chloride ions and water, by sealing cracks with precipitated CaCO₃; such improvements are valuable to prevent deterioration in concrete (Figure 10C).

In self-healing bioconcretes, 0.5 mm wide cracks can be completely healed via CaCO₃ precipitation induced by microbes. Although the idea of self-healing concrete is excellent, there is concern on the survivability of bacteria exposed to harsh conditions within the concrete matrices. Concrete are constructed from hydraulic tricalcium silicate-based Portland cements which releases Ca(OH)₂ on hydration, reaching a pH of 12 with little moisture or oxygen. In addition, pores within a concrete matrix is plugged with the calcium silicate hydrate phase which reduces pore size to <1 µm. Considering that bacteria range from 1 to 4 µm in sizes, there is a reasonable chance that they will undergo lysis after compression during cement hydration. Current research focuses on the use of microencapsulation techniques for protecting bacteria and bacteria spores that are incorporated in concretes designed for self-healing over lengthy periods.

Microbe-induced calcite precipitation, being one of the most representative examples of extracellular mineralization by single-cell organisms, is also recognized as an emerging technology for subsurface engineering applications such as modification of the permeability of rock formations and sealing defects in wellbore cement using the ureolytic bacteria *Sporosarcina pasteurii* for MICP. A wellbore is a hole that is drilled to aid the exploration and recovery of oil or gas. Cementation is one of the most critical steps in the drilling process.

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**Figure 10.** Microbe-induced carbonate precipitation (MICP) represents the most representative example of environmental and industrial applications of extracellular biomineralization by single-cell organisms. A) Ureolysis-driven calcite precipitation by bacteria. B) Schematic of MICP formation mediated by urease and carbonic anhydrase. C) Overview of the MICP-mediated self-healing process of cracks within a concrete matrix. D) To maintain the integrity of the wellbore cement, MICP is used by injecting bacteria-containing biomineralization-promoting fluids into the channel where CaCO₃ forms to limit further fluid injection. Bacterium image in (A): adapted from a CLker-Free-Vector-Images image on Pixabay. B) Reproduced under the terms of the CC-BY Creative Commons Attribution 4.0 International license (http://creativecommons.org/licenses/by/4.0/). Copyright 2019, Frontiers Media SA. C) Reproduced with permission. Copyright 2018, Springer Nature. D) Reproduced with permission. Copyright 2018, Elsevier.
and completion of oil or gas wells. A cement is placed in the annulus between the metal casing and the wellbore to provide hydraulic seal, create zonal isolation, protect usable water, provide structural support for casing, protect the casing from corrosion, and isolate the casing seat for subsequent drilling. The presence of delaminations, apertures, fractures, or voids in the wellbore environment substantially reduces wellbore integrity. Compromised cement may also result in loss of zonal isolation, leading to deleterious fluid flow of between zones or to the surface. A potential solution to enhance wellbore integrity is the use of MICP to seal leaks along flow pathways (Figure 10D). 3D sealing of wellbore cement defects by S. pasteurii-mediated mineralization are more biocompatible and comparatively less toxic than those synthesized using physical and chemical methods.[305] This widens their applications in medicine.

5.3. Biomedical Applications

Metal nanoparticles produced by microbe-mediated mineralization are more biocompatible and comparatively less toxic than those synthesized using physical and chemical methods.[306] This widens their applications in medicine.

5.3.1. Antimicrobial Activity

Microbe-based metal nanoparticles exhibit potent antimicrobial activity and have important applications as antibacterial surgical dressings and linings and as coatings for medical devices such as implants.[307] Silver nanoparticles are the most extensively employed microbe-synthesized metal nanoparticles and will be used as the example for discussion. Silver nanoparticles synthesized by Bacillus pumilus possess potent antimicrobial activity against different Gram-positive and Gram-negative human pathogenic bacteria, including methicillin-resistant Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus bovis, E. coli, P. aeruginosa, Shigella sonnei, Klebsiella pneumoniae, and Salmonella typhimurium.[308] Fungi produce enzymes that act as both reducing and capping agents to produce stable and shape-controlled AgNPs.[309] Synthesis of AgNPs using different genera of fungi such as Fusarium, Penicillium, and Aspergillus is clean, inexpensive, eco-friendly, reliable, and does not produce toxic chemicals. Fungi are advantageous over other microbes in that they possess high tolerance against harsh environmental conditions and are easily amendable to industrial scale-up.[310] The marine endophytic fungus Penicillium polonicum has been used to synthesize AgNPs with antibacterial activity against multidrug-resistant Acinetobacter baumannii biofilms.[311] The nanoparticles are attached to the cell wall of A. baumannii after exposure to the virgin bacterial cells for 30 min. This is followed by extrusion of the cytoplasmic contents and subsequently cell lysis (Figure 13A).

Silver has high affinity to sulfur- and phosphorus-containing compounds. Adhesion of AgNPs on bacterial cell walls causes physical changes on the bacterial membrane, resulting in the collapse of the cytoplasmic membrane and loss of viability.
Figure 12. MICP-mediated restoration of deteriorated stone/marble surfaces. A) Examples of surface eroded or weathered marble monuments (left) and marble surfaces (right). B) Applications of MICP for restoration of stone/marble structure. a) Biodeposition—surface treatment applied via spraying or painting of surfaces. Biocementation: b) remediation of surface cracks using prepacked filler particles; c) remediation of surface cracks using paste with filler particles, and d) remediation of cracks with grains of deteriorated stone/marble extending into structure. C) 3D volume reconstruction of microcomputed tomography scans acquired from biocemented sand. Brown particles: sand grains; green particles: calcite bonds. A) Images adapted from polygontwist (left) and PRAIRAT FHUNTA (right) images on Pixabay. B) Reproduced under the terms of the CC-BY Creative Commons Attribution 4.0 International license (http://creativecommons.org/licenses/by/4.0/). Copyright 2018, Elsevier. C) Reproduced under the terms of the CC-BY Creative Commons Attribution 4.0 International license (http://creativecommons.org/licenses/by/4.0/). Copyright 2018, Springer Nature.
in leakage and cell death. Release of Ag$^+$ result in binding to proteins present in the cell membrane that are involved in transmembrane adenosine triphosphate generation.\[310\] Being much smaller than bacteria, AgNPs also infiltrate cells and cause damage to the respiratory chain, enzymes, lipids, and DNA via elevation of intracellular reactive oxygen species, ultimately resulting in cell apoptosis.\[311,312\] The antimicrobial mechanisms of ApNPs are summarized in Figure 13B.\[313\]

It is prudent to point out not all AgNPs exhibit the same antibacterial efficacy. As an illustration, five different fungi isolated from medicinal plants have recently been used for in situ synthesis of 5–20 nm diameter AgNPs on cotton fabrics (Figure 14).\[314\] When they were synthesized ex situ, solutions containing those AgNPs all created inhibition zones on agar plates cultured with Gram-positive S. aureus and Gram-negative E. coli. However, when AgNPs were synthesized in situ on cotton fabrics, not all the “antimicrobial” cotton samples possess antibacterial properties against those bacteria.

5.3.2. Medical Imaging Devices and Diagnostics

Magnetic resonance imaging (MRI), which has excellent spatial resolution and soft tissue contrast, is a commonly used imaging
Iron oxide nanoparticles can be used as MRI contrast agent to enhance the image contrast between normal and diseased tissues.\cite{315} Magnetic nanoparticles increase proton magnetic resonance absorption and consequently enhance the sensitivity and specificity of MRI.\cite{316} Magnetosomes, which are magnetic nanoparticles of biological origin, provide better contrast for MRI than current magnetic iron oxide nanoparticle formulations possibly because of their larger size and high monodispersity.\cite{317}

Using a genetic engineering technology known as “directed evolution”, genetically modified bacteria have been produced with increased iron-sequestering ferritin protein. This results in the acquisition of mutated bacteria strains with significantly enhanced cellular magnetism, which, in turn, leads to improved physical attraction of ferritin-expressing cells to magnets and augmented contrast for cellular MRI.\cite{318} More recently, repeating segments of silaffin proteins derived from diatoms have been displayed on E. coli surfaces through genetic manipulation for regulating the synthesis of nanostructured TiO$_2$ anatase (Figure 15). The displayed surface proteins catalyze the hydrolysis of TiO$_2$ precursors through hydrogen bonding interaction on the cell surface. The genetically modified bacteria provide a framework for producing rod-shaped TiO$_2$ nanoparticles as well as providing an in situ carbon source. The synthesized TiO$_2$ anatase finds use as anode electrodes of lithium-ion batteries, with excellent lithium storage performance.\cite{319}

Bioengineered MTB are used to produce magnetosomes with diverse shapes and narrow distribution of crystal size as well as specific functions, which make them ideal as image contrasting agents. These custom-tailored magnetosomes have been intensively investigated for MRI-based detection of cancerous cells in experimental tumor models.\cite{320–324} Bioengineering of MTB may be achieved using methods that include direct chemical modification, genetic engineering, and hybrid modification (Figure 16A). With the advancement of nanotechnology, magnetosomes may be functionalized with different ligands such as enzymes, proteins, nucleic acids, green-fluorescent protein, antibodies, and drugs (Figure 16B)\cite{325} for multifunctional applications (Figure 16C). Among these applications, enhancement of imaging contrast is one of the best-known examples.\cite{326}

Chemical modification is realized by proteins in the magnetosome membrane including magnetosome-associated membrane (Mam) proteins or magnetic particle membrane-specific (Mms) proteins. These proteins may be modified through their amino groups or carboxyl groups. For example, chemical modification of magnetosomes with peptide P75 produces functionalized magnetosomes that bind to human epithelial growth factor receptor and epithelial growth factor receptor-2.\cite{327} Indirect interaction between magnetosome membrane and antibody may be achieved using a fusion tag. Staphylococcal protein A (SPA), which is an immunoglobulin G-binding protein and serves as fusion tag, fuses with MamC or MamF and binds to the fragment crystallization (Fc region) of immunoglobulins. Consequently, the magnetosome-SPA complex is capable of binding with antibodies.\cite{328} Chemical modification may also be achieved through the reaction between –NH$_2$ group on the bacterial magnetosome membrane and the –NH$_2$ or –SH groups of antibodies via coupling with various crosslinkers for immunoassays\cite{329} and antibody delivery.\cite{330} Magnetosome membrane may also be modified with biotin/streptavidin for protein and nucleic acid detection. Apart from the functionalization induced by crosslinkers, phospholipids on the magnetosome membrane carry negative charges, enabling them to interact...
with positively charged molecules. For example, the link between magnetosomes and the anticancer recombinant plasmid heat shock protein, 70-polo-like kinase 1-short hairpin RNA, as well as doxorubicin are based on charge interactions.\[331\] Because short-interfering ribonucleic acid (siRNA) is capable of silencing homologous gene expressions, they have been conjugated to magnetosomes via polyethyleneimine for the intracellular delivery of siRNA to cancer cells to inhibit their growth in a dose- and time-dependent manner (Figure 16D).\[332\]

Genetic engineering modification of magnetosomes enables expression of functional proteins on the magnetosome membrane. The gene of the functional protein is fused to the genes of proteins expressed on the membrane, such as Mms 16, Mam 13, and Mag A. The fusion gene is subsequently transferred into the MTB and coexpressed on the membrane with original proteins. For example, magnetosomes have been incubated of emerald green-fluorescent protein (EmGFP)- or biotin-decorated TMV particles to generate
magnetic nanostrands that express these proteins. Overexpression of magnetosome membrane proteins may be achieved via sequential chromosomal insertion by transposition to control the size and number of magnetosomes. This method provides possibility for diverse proteins to be expressed on the magnetosome membrane according to practical requirements.

Because chemical modification may introduce toxic substances to magnetosomes and that genetic manipulation is complicated to introduce active foreign proteins, a new hybrid...
method has recently been reported. Affibodies are small proteins with similar binding sites as antibodies. They are characterized by low immunogenicity, high biocompatibility, and excellent biodegradability, and can be harvested from E. coli at low cost. The anti-human epidermal growth factor receptor-2 (HER2) fused with MamC protein may be expressed in E. coli through genetic manipulations. Membrane proteins in the phospholipid bilayers of the magnetosome membrane are then removed to facilitate anchoring of this hybrid anti-HER2 bearing MamC protein via physical sonication for detection of HER2-positive breast cancer. Theoretically, many kinds of affibodies and other targeting proteins may be displayed on the magnetosome through this technology.

Nanometer-sized semiconductor cadmium telluride quantum dots (CdTe QDs) produced by yeasts and proteobacteria are biocompatible and the proteins capping these QDs can guide their entry into cells. Their strong, two-photon excitation luminescence enables them to be used for bioimaging without interference with cell fluorescence. The CeTe QDs may be used for bioimaging and biolabeling applications. For example, chromatophores containing CeTe QDs prepared from Rhodospirillum rubrum and the antibodies against the β-subunit of FOFO1–ATPase may be employed as a sensitive detector for the avian influenza virus subtype A/H5N1. Salt-stable, fluorescent, hexagonal quantum dots produced by the photosynthetic halophilic bacteria Halobacillus sp. DS2 retain their fluorescence in the presence of elevated salt concentrations, a condition that affects most QDs available to date. These QDs have potential use in fluorescence bioimaging.

5.3.3. Antitumor Therapy

Magnetic hyperthermia is a process employed for killing cancer cells. Iron oxide nanoparticles are administered to tumors and heated under the application of an alternating magnetic field to deposit an extremely localized dose of thermal energy. The heat produced by those nanoparticles induces antitumor activity. In the presence of an external magnetic field, magnetosome suspensions harvested from MTB can get into the tumor cells and inhibit their proliferation through controlling the heating of the magnetic nanoparticles. This strategy has been tested successfully in a clinical trial on patients with prostate cancer. The magnetosomes can be rendered fully biocompatible by removing potentially toxic organic bacterial residues such as endotoxins at the magnetosome mineral core surfaces, and by coating the magnetosomes with poly(lysine). Poly(lysine)-coated magnetosomes have been used successfully for experimental magnetic hyperthermia treatment in a murine model of glioblastoma, an aggressive, fast-growing brain tumor. Similarly, magnetosomes extracted from Magnetospirillum gryphiswaldense MSR-1 culture have been utilized, after purification, as theranostic agents in an in vivo murine subcutaneous model of colon carcinoma as well as an in vivo murine xenograft model of glioblastoma. Instead of using bioengineered harvested magnetosomes, a recent paper reported the use of live M. gryphiswaldense MSR-1 as magnetic hyperthermia agents for cancer treatment. The live MTB can be internalized by human lung carcinoma cells A549, and proliferation of the cancer cells is strongly inhibited by the hyperthermia treatment. This is a representative example of the use of live microbes with intracellular mineralization as autopropelling micro/nanorobots for biomedical applications. Other examples will be elaborated in due course.

Angiogenesis plays a critical role in tumor growth and metastasis. Apart from their antibacterial properties, AgNPs synthesized by microbes possess antiangiogenic properties and are cytotoxic against tumor cells by impairing mitochondria, blocking cell cycle, and activating apoptosis (Figure 18A). For example, AgNPs synthesized by the extremophilic, radiation-resistant bacterium Deinococcus radiodurans are capable of inhibiting tumor cells from the mammalian breast cancer cell line MCF-7. Silver nanoparticles synthesized by the cyanobacterium Oscillatoria limnetica are capable of killing multi-drug resistant bacteria as well as killing tumor cells derived from the human breast cancer (MCF-7) cell line and the human colon cancer (HCT-116) cell line. Likewise, AgNPs synthesized extracellularly by the fungus Trichoderma viride trigger apoptosis of MCF-7 cells and prevent their propagation in a time- and concentration-dependent manner (Figure 18B). Silver nanoparticles synthesized using the endophytic fungus Talaromyces purpureogenus are cytotoxic to the human lung carcinoma A549 cell line.

Theoretically, nanoparticles may be used to treat tumorous tissues via passive or active targeting (Figure 18C). In passive targeting, “naked” nanoparticles injected into the blood stream accumulate passively in the tumor via the leaky vasculature that is characteristic of tumorous tissues, in a manner known as the enhanced permeability and retention effect. In active targeting, nanoparticles are used as drug delivery vehicles. They may be conjugated with specific ligands for cell-specific identification and binding. The nanoparticles can release cancer drugs in a controlled manner on reaching the tumorous tissue to induce death of the cancerous cells via autophagy.

The active targeting strategy in antitumor therapy is exemplified by a recent study that harnesses the ability of yeast cells (S. cerevisiae) to thrive in a hypoxic and low-pH environment to deliver an anticancer drug to the hypoxic regions of tumorous tissues (Figure 19). The yeast cells are first exposed to Ca²⁺- and PO₄³⁻-containing solutions to induce intracellular mineralization of hydroxyapatite nanoparticles (nHAP). These ≈3 nm diameter nanoparticles are stabilized by biomolecules present within the cytoplasm of the yeast cells. Loading of the anticancer drug doxorubicin (DOX) into the nHAP@yeasts is subsequently performed to create DOX-nHAP@yeasts. Interaction between the hydroxyl and carboxyl groups of DOX with nHAP results in a higher DOX loading capacity when compared with the DOX loading capacity of native yeast cells. The DOX-nHAP@yeasts are then functionalized with negatively charged folic acid (FA) to produce DOX-nHAP@yeasts-FA, by using positively charged poly(diallyldimethyl ammonium chloride) (PDDA) as a bridging agent to the negatively charged yeast cell wall. Because FA expression is low in normal cells but is over-expressed in cancer cells, the DOX-nHAP@yeasts-FA are preferentially taken up by human hepatocellular carcinoma (HepG2) cells. On consumption of the FA by the tumorous cells, the loaded DOX is released into tumorous environment. The DOX-nHAP@yeasts-FA exhibit dual responsive release...
Figure 17. Examples of how intracellular mineralization of live MTB (i.e., magnetosomes) may be harnessed for potential biomedical applications. 

A) Cancer cells reproduce quickly in tumors, resulting in hypoxic regions with low oxygen tension. Magnetotactic bacteria have a preference for thriving in water with low oxygen concentration and can autonomously seek hypoxic tumorous areas. Their small size enables these bacteria to navigate through blood vessels and penetrate tumors. Once arrived, the MTB may be heated by application of an alternating magnetic field with defined amplitude and frequency for magnetic hyperthermia of the cancerous cells. The temperature may be raised to a therapeutic window of 40–44 °C, where the cancerous cells are driven to apoptosis without affecting the adjacent healthy tissue, or above 50 °C, where more violent (and less safe) thermal ablation may be used to induce cell necrosis. B) Design and operation of magnetotactic bacterial cages (MBC) for release of loaded therapeutic agents (in this case, AuNPs functionalized with single-stranded DNA(ss-DNA)). a) ssDNA-AuNPs are engulfed by live MTB via endocytosis under anaerobic conditions; b) upon exposure to aerobic conditions, dead MBC attach themselves to the cell wall and are readily consumed by the target cells; c) application of hyperthermia radiation results in destruction of MBC and cargo release. C) By combining directional magnetotaxis and microaerophilic control of autopropelling MTB, a larger amount of therapeutics may be delivered via covalently bound nanoliposomes that surpass the diffusion limits of large drug molecules toward hard-to-treat hypoxic regions in solid tumors. D) Conceptual overview of magnetically controlled micropropellers for convention-enhanced transportation of nanoparticles. i) A swarm of MTB generating convective flow to improve mass transport; ii) schematic of magnetofluidic platform for nanoparticle mass transport using magnetically induced convection. The microfluidic chip is placed between the objective lens of an inverted optical microscope and the electromagnets (left). A schematic depicts the chip, consisting of an upper channel filled with nanoparticles (red) and a lower water channel (blue) that both border a collagen matrix (gray) along restricting trapezoidal posts made of poly(dimethylsiloxane) (PDMS). The nanoparticles passively diffuse into the collagen matrix along their concentration gradient toward the water channel (right). A) Images adapted from Clker-Free-Vector-Images, Arek Socha, and Manfred Richter images on Pixabay. B) Reproduced under the terms of the CC-BY Creative Commons Attribution 4.0 International license (http://creativecommons.org/licenses/by/4.0/). Copyright 2016, Elsevier. C) Reproduced with permission. Copyright 2014, American Chemical Society. D) Reproduced under the terms of the CC-BY Creative Commons Attribution 4.0 International license (http://creativecommons.org/licenses/by/4.0/). Copyright 2019, American Association for the Advancement of Science.
profiles because of the FA dependency of tumorous cells and the pH-dependency of HAP nanoparticles, and inhibit cancer cell proliferation more effectively than free DOX. When the DOX-nHAP@yeasts-FA are injected into mice with subcutaneous xenografts of human hepatocellular carcinoma, they reduce tumor volume more significantly than nHAP@yeasts, nHAP@yeasts-FA, DOX-nHAP@yeasts, or free DOX.

To date, most of the studies on the use of nanoparticles for antitumor therapy are performed using tumor cell lines and have never been tested in animal studies or human clinical trials. Accordingly, rigorous studies are required in the future to validate their clinical antitumor potential.

5.3.4. Pathogen Detection

The surface of magnetic nanoparticles may be functionalized for the detection of pathogens. A magnetic nanoparticle microarray was developed for the simultaneous and simple detection of food-borne pathogens such as E. coli O157:H7, Salmonella enterica, V. cholera, and Campylobacter jejuni. The method involves the use of an oligonucleotide array onto which 5'-biotinylated single-strand bacterial polymerase chain reaction products are hybridized with streptavidin-coated magnetic nanoparticles to visualize the results of the hybridization process. Staphylococcal protein A, an immunoglobulin-binding protein from the cell wall of S. aureus, has been attached to magnetosome transmembrane proteins MamC or MamF present in M. gryphiswaldense strain MSR-1 for detection of Vibrio parahaemolyticus, a common pathogenic bacterium in seafood. [352] These genetically engineered magnetosomes with fused protein A bind antibodies automatically, and are inexpensive, ecofriendly alternatives to the use of commercial immunomagnetic beads for pathogen detection. [353] Apart from using harvested and purified magnetosomes, MO-1 MTB has also been decorated with rabbit anti-MO-1 cell polyclonal antibodies to become magnetic-guiding, auto-propelling microrobots for detection and separation of S. aureus. [354] After binding of the polyclonal antibody-coated MO-1 MTB to protein A on the surface of S. aureus (e.g., in an infected wound), the bacteria are mechanically injured and eliminated via the application of a swinging magnetic field (Figure 20). [355]

Apart from the use of magnetosomes and MTB, fluorescent CdTe QDs have been developed for barcode assay detection of S. aureus, methicillin-resistant S. aureus (MRSA), and Klebsiella pneumoniae. [356] By preparing complementary oligonucleotides for the specific bacterial genes (fnbA for S. aureus, mecA for MRSA, and wucG for K. pneumoniae) and labeling them for simultaneous detection, the detection limit of this method is as low as 10^2 colony forming units mL^−1. Although the CdTe QDs in this work were synthesized chemically, it is likely that CdTe QDs produced by yeasts and proteobacteria may also be employed for the same purpose. Likewise, AuNPs and AgNPs have recently been conjugated with different viral DNA or antibodies to develop nanoprobe systems for detection of viruses such as the Ebola virus, severe acute respiratory syndrome (SARS) corona virus, Dengue virus, different types of hepatitis viruses, herpes simplex virus, human cytomegalovirus, Kaposi sarcoma-associated herpesvirus, human immunodeficiency virus and influenza virus. [357,358] Considering that these metallic nanoparticles can be synthesized by different bacteria and
fungi, there is immense potential in utilizing microbe-produced nanoparticles in the timely field of viral detection research.

5.3.5. Drug Delivery Systems

Recent studies have investigated the use of microbe-produced nanoparticles as drug delivery systems. Yeast cells can induce CaCO3 mineralization and the CaCO3 can in turn induce certain drugs to enter the intracellular milieu of host cells due to the interactions of Ca^{2+} ions with other functional groups.[359] Because of their excellent biocompatibility, low toxicity, and traits that can be controlled by an eternal magnetic field, magnetosomes demonstrate great potential as drug carriers. For example, bioconjugates of doxorubicin prepared using magnetosomes with their surfaces modified by poly(l-glutamic acid) exhibit potent antitumor ability.[360]

With the advent of gene therapy, MTB have been utilized as cages for guidable delivery of deoxyribonucleic acid (DNA)-functionalized AuNPs.[361] Cargo loading is achieved under anaerobic conditions while the magnetosome-containing bacteria are alive, with the AuNPs functioning as transmembrane protein mimics to facilitate endocytosis of the MTB. The bacteria are killed after exposure to aerobic conditions, but with the cell wall intact to provide a carrier cage for DNA delivery. Upon intake by the host cells, magnetic hyperthermia is used to disrupt the bacterial cage for release of the loaded cargo (Figure 17B).

Although nanoparticles are designed to alter the pharmacodynamics and biodistribution of conventional drug formulations, a major hurdle that hinders their effective delivery is the presence of physiological barriers at the diseased sites.[362] While this is salient for inflammatory sites, the situation is more critical within the tumor microenvironment, where the increase in peripheral angiogenesis causes augmentation of the tumor interstitial fluid pressure and prevents optimal diffusion of the therapeutic agents to the tumorous regions.[363] Hence, propelling micro/nanorobots driven by external power sources such as sound, light, magnet, enzymes, photocatalysts,
electricity, or temperature have been an intensively studied topic of late in the arena of cancer therapeutics.\[364–366\] For magnetically driven micro/nanorobots,\[367\] those constructed with MTB and magnetosomes have found novel uses as self-propelling, magnetically driven, controlled drug delivery systems in cancer therapeutics.\[368,369\] Nanoliposomes (≈70 nm diameter) have been covalently attached to the surface of the live, auto-propelling MTB *Magnetococcus marinus* MC-1 for the synthesis of self-propelled carriers of therapeutic agents (Figure 17C).\[370\] Further improvements involve the use of both magnetic fields and mechanisms for the sensing of low oxygen concentrations to generate magneto-aerotactic, two-stage sensing *M. marinus* MC-1 for delivery of drug-containing nanoliposomes to hard-to-treat, oxygen-depleted hypoxic regions in solid tumors.\[371,372\] To further enhance convective flow through blood vessels to the target tumorous tissues, an entire swarm of live MTB *Magnetococcus maneticum* strain AMB-1 has recently been utilized to generate ferrohydrodynamic pumping to increase the translational velocity and mass transport of the loaded therapeutic agent to the poorly perfused internal environment of tumors that are surrounded by abnormal blood vessel architecture (Figure 17D).\[373\] Although entirely experimental in nature,

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**Figure 20.** Identification and elimination of *S. aureus* by live, antibody-functionalized magnetotactic bacteria (MTB) via the application of a swinging magnetic field. A) MTB are coated with polyclonal antibodies to allow them to bind to *S. aureus* via staphylococcal protein A. A swinging magnetic field is subsequently applied to induce damage to *S. aureus*. B) TEM images illustrating MTB binding to *S. aureus*. i) A representative MO-1 MTB. Blue triangles: flagella of the MTB cell; red triangle: chain of magnetosomes. ii) Attachment of MTB to *S. aureus* after the MTB is coated by polyclonal antibodies. Black arrows: *S. aureus*; red arrows: MTB. C) Hypothetical principle for bound *S. aureus* via the MTB under a swinging magnetic field. When the direction of the magnetic field alters, the MTB may exert a mechanical force on *S. aureus* to injure the pathogen. A–C) Adapted with permission.\[355\] Copyright 2017, Elsevier.
these new ventures offer exciting venues to deliver cancer-fighting drugs via the use of live micropropellers that represent unprecedented advances in contemporary drug delivery.

5.4. Other Applications

Microbe-mediated mineralization is intrinsically related to the origin of life. Organic components (biosignatures) associated with biominerals are thought to play important roles in prebiotic and biotic reactions.\[374\] In astropaleontology, planetary sites with enriched silica are believed to be the most appropriate locations to search for extraterrestrial lifeforms. The opaline silica related with microbe silification has become a promising target to identify potential habitability beyond what has been identified on earth, such as in Mars.\[375\] Indeed, the primary mission of the planned launching of the Mars 2020 rover by the National Aeronautics and Space Administration in July 2020 is to search for evidence of habitability, taphonomy, and organic carbon as a proxy for preserved ancient biosignatures within accessible geological materials.\[376\]

6. Challenges and Future Prospects

Microbes have contributed to mineral formation since the advent of life on Earth. Microbe-mediated mineralization ranges from natural minerals to the formation of minerals within live organisms, the research on which has gained momentum since the turn of the century. Biominerals produced by microbes are used extensively in various fields ranging from removal of heavy metals and radionuclides, to remediation of building materials and diagnosis/treatment of difficult-to-treat diseases in humans. Metal nanoparticles synthesized by microbes are generally free from toxic contaminants, which is a prerequisite for biomedical applications. Biogenic routes of synthesis generally do not require an additional capping step for generating stable and pharmacological active nanoparticles which is otherwise essential for synthesis based on physical or chemical methods. Nevertheless, there is much to explore in order to bring this environmentally safe, cost-effective, and convenient technology from the laboratory to field scales.

A critical bottleneck is harnessing the benefits of microbe-mediated mineralization is the translation of advances in scientific knowledge, often performed in the laboratory scale, to tangible technological applications that have to be produced in the industrial scale. One of the problems associated with this issue is that the level of control that is exerted at the single-object level tends to wane with scale-up attempts to address a large number of objects. It is important to assure that the biological systems involved do not change significantly when moving from laboratory scale culturing conditions to an industrial-relevant production level. For example, production of biogenic magnetite nanoparticles by the proteobacterium \textit{G. sulfurreducens} at the 5 L scale industrial setting resulted in changes in the growth behavior of the microbes, a feature that was not apparent when culture was performed at the 100 mL benchtop scale. This problem was subsequently resolved by the inclusion of other microbe nutrient supplements in the industrial fermentor.\[377\] Only a carefully designed scale-up procedure can assure the quality of the nanoscopic product, cost effectiveness, and a timely product launch.\[378\]

Biocompatibility associated with bacterial microrobots that are designed for use in biomedicine is another issue of concern. Biomedical micro/nanorobots are designed for environments involving complicated biological events and changing physiological conditions. Currently, the interaction between these self-propelling micro/nanostructures and the human body is not fully known.\[379\] The use of MTB as microswimmers is at best investigated in animal models; these models provide only qualitative information on cytotoxicity based on histological assays. For precise targeted delivery, it is necessary to thoroughly comprehend how foreign materials accumulate throughout the body and how to minimize the distribution of the administered live bacterial microrobots to nontarget tissues, which may create nonanticipated undesirable effects. For example, self-propulsion of MTB through the blood stream may end up in the gastrointestinal tract and alter the composition and distribution of its microflora. In addition, little is known about the responses of the body’s innate and adaptive immune systems to these new microbial intruders.\[380\] To effectively perform their designated functions, these microrobots must evade the array of defenses the body employs against foreign substances. Whether these non-self-intruders will succumb the body’s natural defense mechanisms to infections is a critical knowledge gap that needs to be filled before the clinical use of micro/nanosystems that are designed to bridge biology and engineering.

In addition, it is anticipated that a humongous number of these microrobotic units are moving independently to target the disease site. Compared with a single microrobot, the coordinated action of multiple nanorobots is helpful for delivery of large therapeutic payloads or large-scale therapeutic processes. Adoption of group communication and synchronized coordination algorithms at the nanoscale for enhancing precision treatment capability is a challenging issue. Research on the complex task in tackling these issues is only at its infancy and needs to be expedited before practical applications of these systems can be realized.

Acknowledgements

W.Q., C.-y.W., and Y.-x.M. contributed equally to this work. This work was supported by grants 81722015, 81870805, and 81720108011 from the National Nature Science Foundation of China and by the Youth Innovation Team of Shaanxi Universities.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

algae, bacteria, fungi, microbe-mediated mineralization, viruses

Received: November 28, 2019
Revised: January 9, 2020
Published online: April 9, 2020
