Synthesis and Processing of Nanomaterials Mediated by Living Organisms

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Nanomaterials offer exciting properties and functionalities. However, their production and processing frequently involve complex methods, cumbersome equipment, harsh conditions, and hazardous media. The capability of organisms to accomplish this using mild conditions offers a sustainable, biocompatible, and environmentally friendly alternative. Different nanomaterials such as metal nanoparticles, quantum dots, silica nanostructures, and nanocellulose are being synthesized increasingly through living entities. In addition, the bionanofabrication potential enables also the in situ processing of nanomaterials inside biomatrices with unprecedented outcomes. In this Minireview we present a critical state-of-the-art vision of current nanofabrication approaches mediated by living entities (ranging from unicellular to higher organisms), in order to expand this knowledge and scrutinize future prospects. An efficient interfacial interaction at the nanoscale by green means is within reach through this approach.

1. Introduction

Nanomaterials (NMs) are defined as materials whose components or basic building blocks have at least one dimension in the nanoscale (1–100 nm). NMs offer excellent properties, new applications, and unique interactions with biological systems by exploiting nanoscale phenomena. Despite this potential, nanofabrication approaches still rely on highly complex procedures, often incompatible with green chemistry principles. Therefore, increased efforts should be directed to develop unconventional methods with a higher throughput and lower cost, going along with a sustainable profile.

The use of organisms is a clean and affordable alternative to synthesize NMs, since these methods can be scaled up and also tuned by chemical and genetic modification. Organisms have developed the synthesis of NMs as a means to adapt to their environment, in order to enhance their life expectancy; thus, they can be considered green factories of useful NMs. The intersection between biology and materials science has provided extraordinary materials that cannot be easily synthesized in the laboratory, but Nature does it at ambient conditions without hazardous chemicals. Some of these are actually NMs with outstanding properties. For instance, magnetotactic bacteria (MTB), already discovered in 1963, biosynthesize magnetic nanocrystals (MNCs) that allow their orientation and migration under geomagnetic fields (Figure 1). There is also the case of biotemplates, alluding to the use of biomolecules, viruses, or biological extracts as templates for the synthesis or deposition of NMs. This approach is not within the scope of this Minireview, as a living system is not directly involved.

This Minireview aims at presenting the current state-of-the-art on the use of organisms for the synthesis and processing (i.e. integration into a biomatrix) of NMs. The generation of NMs mediated by living entities is an example of a bottom-up process, from atoms/ions or molecules to NMs (Scheme 1). This strategy encompasses concepts such as chemical synthesis, catalysis, directed precipitation, and mineralization. The main example is the reduction of metallic salts to form zero-valent metal nanoparticles (ZMNPs), together with the synthesis of quantum dots, MNCs, or bacterial nanocellulose (BNC). On the other hand, the integration of NMs into a biomatrix represents also a bottom-up approach in which the components are blended from scratch, in an extremely efficient way, by the action of specific organs or organelles (Scheme 1). These nanocomposites...
possess improved (and even novel) properties due to enhanced interfaces between biomatrices and NMs.

2. Bionanofabrication through Living Entities

Living beings, from bacteria to plants, can be used to produce NMs in a bottom-up fashion. The cell morphology and physiology allow high control of the NM’s size and shape during the synthesis through the cooperative action of

Scheme 1. Summary of diverse bionanofabrication approaches. Red background: bionanofabrication through living entities; blue background: in situ biogenic processing of NMs into a biomatrix.
biomolecules, especially enzymes and biopolymers. In contrast to the classical chemical synthesis of NMs, those made by bionanofabrication end up embedded in large amounts of organic matter, requiring additional purification steps to release and isolate them. The most representative examples to date are described in this section.

2.1. Zero-Valent Metal NPs

ZMNPs are by far the most investigated NMs among those synthesized through bionanofabrication. The reduction of a metal cation to the zero oxidation state within a cellular environment leads to controlled precipitation of such ZMNPs. Ag and Au are the most commonly addressed metals, but there are other examples like Pd and Pt. The process is clean, easy to scale up, and requires mild conditions in comparison to the typical syntheses of ZMNPs. The basis of ZMNPs production originates from the defense mechanism of living entities upon exposure to metal salts. The synthesis mechanism varies depending on the organism and has not been fully unraveled, but it can be either intracellular or extracellular. General mechanism involves the trapping of metal ions on the membrane surface or inside the cells, followed by the subsequent reduction in the presence of different enzymes. Organisms control the growth of ZMNPs in two ways: by locally supersaturating the ion solution or through the production of organic polymers inhibiting or favoring particle nucleation, thus controlling the shape. The size can be controlled by diverse factors, whereby pH, temperature, precursor concentration, and exposure time are key parameters. The concomitant organic matter is removed by centrifugation and/or sonication and the mixture is washed with different solvents to release and to purify the ZMNPs. It is also possible to prepare intermetallic nanostructures by this approach, depending on the addition sequence of the different precursors leading to core@shell NPs, alloys, and heterostructures.

Different groups of bacteria have been proven useful to reduce metal salts into ZMNPs, but there are still some drawbacks, such as occasional excessive polydispersity, requiring further optimization of the culture conditions and bacterial strains. The same strain can be used for the synthesis of different ZMNPs through modification of the precursor. In one example, Reddy et al. used Bacillus subtilis for the synthesis of both Au and Ag NPs below 10 nm. The mechanism in the fungi-mediated reduction does not differ from the bacterial mechanism and it can also be intracellular using enzymes to reduce Ag or Au cations.

Not only small organisms can be used to synthesize ZMNPs, also higher plants can reduce different metals. The accumulation of metal salts in plants triggers a defense mechanism favoring the synthesis of ZMNPs, which can be collected afterwards, provided that these are separated from the surrounding organic matter. Marchiol et al. demonstrated the integration of Ag NPs in different parts (roots, stem, and leaves) of three different plants (Brassica juncea, Festuca rubra, and Medicago sativa) exposed to AgNO₃.

2.2. Inorganic Biominerals

Biominaleralization is the process of synthesizing or depositing inorganic biominerals through biological systems, be it living organisms or inert biotemplates. In this Minireview, we will focus on specific compounds, prepared successfully by bionanofabrication.

2.2.1. Magnetic Nanocrystals

MTB produce MNCs (usually magnetite (Fe₃O₄) or greigite (Fe₃S₄)) with regular size and shape by controlled biominaleralization. Salvatore Bellini discovered this unique behavior in specific bacteria in 1963, but his work wasn’t published until 2009. It was Richard Blakemore in 1975 who rediscovered the ability of Spirochaeta plicatilis, isolated from marine mash muds, to produce MNCs and set the basis of the research in this field. MNCs are synthesized and arranged into specialized organelles, called magnetosomes, forming intracellular chains. Their function is to align MTB with external magnetic fields, a behavior known as magnetotaxis (Figure 1). Magnetosomes are composed of an organic membrane that stabilizes the MNC’s inorganic core. There are several strains of MTB, with diverse magnetosome morphologies and numbers of echelons, and each MTB produces MNCs between 40 and 250 nm in size. The mechanism of biominaleralization is intracellular and is controlled by the biological medium. Although the mechanism is not fully unravelled, there is a consensus that Fe ions are accumulated in the surrounding environment of MTB and internalized. Fe is converted into high-spin species and MNCs precipitate in alkaline media inside MTB. MNCs can be purified using an applied magnetic field and ultracentrifugation. MTB can be modified by genetic manipulation or by chemical tuning to tailor the magnetic response in vivo. One promising biomedical application of MNCs is as magnetic hyperthermia agents for cancer treatment.

2.2.2. Transition Metal Chalcogenide NPs

Transition metal chalcogenide NPs (TMCNPs) are known as semiconductor materials with promising optoelectronic and catalytic properties due to quantum confinement effects. These emerging materials can also be bionanofabricated by bacteria, yeast, fungi, worms, and algae. As with other bionanofabrication approaches, this could be useful to valorize toxic effluents by conversion to functional NMs. The production is based on the exposure of living organisms to chalcogenide precursors and the metal. In analogy to the synthesis of metal NPs, the production of TMCNPs stems from a protective mechanism against these substances; it can be again intra- or extracellular and external factors (pH, temperature, precursor concentration, and exposure time) determine the size of TMCNPs. Different enzymes produce metal-reducing compounds and metal-binding agents controlling the biosynthesis of TMCNPs. Core@shell TMCNPs are very useful owing to their unique set of properties, and these NPs are easy to synthesize by sequential exposure of certain organisms to different metal salts. For
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example, Spangler et al. [24] generated a thin shell of CdS over a PbS core, which could be used for sensitized solar cells. Other TMCNPs without quantum dot behavior, like Cu₃S, [25] Ag₃S, [26] and FeS₅, [27] have also been bionanofabricated using different organisms. One example is the biosynthesis of 30 nm FeS NPs using Shewanella oneidensis bacteria to bio-reduce a metal-complex dye with Fe and S₃O₃⁻² (Figure 2a). [27]

Figure 2. a) Sectional electron microscopy image of S. oneidensis producing FeS nanoparticles (left, scale bar = 0.2 μm) and its mechanism of FeS biosynthesis (right) by the degradation of naphthol green B and S₃O₃⁻²⁻. OM: outer membrane; IM: inner membrane; PS: periplasmic space; MQ: menquinone. Reproduced with permission from ref. [27]. Copyright 2016, Elsevier. b) Photograph of E. arvenses (left) and different electron microscopy images (center, scale bar = 500 μm; right, scale bar = 100 nm) of silica NPs biosynthesized by E. arvenses. Reproduced from ref. [30], open access, 2016 Hilaris Publisher. c) Electron microscopy images (scale bars = 2 μm) with inset photographs (scale bars = 10 cm) of a BNC xerogel (left) and aerogel (right). Reproduced with permission from ref. [34]. Copyright 2020, Springer Nature.

2.2.3. Silica Nanostructures

Nanostructured silica cell walls or polysilicic acid skeletons are common in many organisms, for instance, diatoms and sponges. [28] These biomaterials can be used as a source of silica NPs for applications in sensors, drug delivery, fuel cells, and microrobotics. [29] Organisms control the deposition of these functional nanostructures through the use of biomolecules. Diatoms are single-cell microalgae with nanoporous frustules as outer thecae. They are the main biogenic silica source under research due to the diversity of sizes and structures, their ready availability, and their easy handling. [28] The biomineralization is controlled by a set of peptides called silaffins and linear long-chain polyamines. [28] These unique structures can be purified by removal of the organic matrix such that their morphology is maintained; the structures can be modified in vivo during their formation or in vitro after synthesis. [28, 29] Biosilica from diatoms has been used as a valuable material for diverse applications, from photonics and electronics to health applications such as drug delivery and biosensing. [29] Sponges differ from diatoms by being bigger organisms, but they also produce nanostructured hierarchical silica, which is possible due to the use of specific proteins (silicateins). [21, 28] The other biomolecule involved in the growth of silicate sponges is collagen which controls the appositional growth. [21] Applications of this nanosilica in biomedicine (i.e. osteoporosis treatment or bone regeneration) have been developed. [21, 28] Plants are another biogenic source of nanosilica; Equisetum arvenses (commonly known as horsetail) is the main example. The process of extraction is based on acid washing and calcination to isolate SiO₂ NPs (Figure 2b). [30] This biogenic nanosilica has been used as catalyst support [31] and in controlled drug release. [32]

2.3. Bacterial Nanocellulose

In recent years, different cellulose NMs have been explored and are classified in three groups, depending on their synthesis process and morphology: nanocrystals, nanofibers, and bacterial nanocellulose (BNC). [33] In contrast to other nanocellulose materials, BNC is extruded by different aerobic bacterial strains, Komagataeibacter xylinus being the most common, in a bottom-up bionanofabrication synthesis from a carbon source, such as glucose, fructose, or glycerol. [33] BNC is made of nanosized fibers with a length from 100 nm to several micrometers. [33] Due to its dimensions and the absence of other biopolymers, BNC is more crystalline and more mechanically resistant, and can be processed more easily and cleanly than plant cellulose. BNC hydrogels are typically cleaned and sterilized with boiling water and dilute NaOH. [33–38] BNC is produced by Gram-negative bacteria through an enzyme complex located at their inner membrane. The catalytic complex includes subunits to generate the glycosyl transfer in the cytoplasm and subunits to ensure the ejection of the fibers as they grow, first to the periplasm and then outside the outer membrane. [39]

When dwelling in static conditions, bacteria generate a 3D network of BNC with high water content and crystallinity, at the air–water interface. In contrast, with stirring or agitation, an amorphous material is formed having an irregular shape. [33] In this way, distinct materials for specific final applications can be prepared by modulating the culture conditions. The temperature and pH are also determinant, and various studies have been devoted to their optimization. [31, 34, 35] The culture media in which bacteria grow have also a great influence on the production rate and the final properties of the BNC. Different carbon sources have been studied to enhance production and to revalorize agro-industrial residues. [35, 36, 39] A representative case authored by Tsouko et al. [40] compared
the yield of diverse carbon sources, in which sucrose provided five times higher BNC concentration than glucose. The incorporation of additives to enhance the production or to modify BNC is also an important field of research. In one study Zywicky et al. [37] added vegetable oil, resulting in a 500% increase in the BNC production yield. The drying process, as well as the bacterial strain, has a strong influence on the BNC final properties, for instance, in the porosity or the water adsorption capacity (Figure 2c). [34, 38]

3. In Situ Biogenic Processing of NMs into a Biomatrix

3.1. Silk Biomatrices

Silk is one of the most valuable hierarchical materials in nature, potentially interesting for many industrial applications, and it comes from invertebrate animals such as silkworms and spiders. For some millennia, humankind has harvested silk from these animals. Silk is constructed of coated peptidic fibrillar ensembles (fibroin@sericin in silkworm silk, and siprodn@glycoprotein in spider silk) with excellent mechanical properties, exceeding those of high-performance synthetic polymers such as Nylon and Kevlar. [41]

Due to its particular composition and microstructure, spider silk is generally stronger, tougher, and more ductile than the silkworm silk; however, both are widely employed as structural and functional materials. There has always been interest in engineering silk’s native properties with the eventual aim of creating superior metamaterials. Two major approaches are available. [42] Creating transgenic species and supplementing the diet of the insects, whereby the latter is the only viable approach for addressing NMs. By contrast, the use of higher modified organisms has been mainly demonstrated in silk-secreting species for the processing of NMs. Silkworms, with their inherent capability of biosynthesizing silk, represent the spearhead of this approach, as these may integrate NMs into the biomatrix in a “living” bottom-up way. Due to the easy domestication of silk moths, Bombyx mori silk is the most widely used, and its production largely depends on the feeding during the larval stage. [43] Either way, supplying NMs to the silkworm larvae’s diet is a rapidly growing method, preferable to post-synthesis NM incorporation, as the latter entails more steps and the use of hazardous chemicals. Historically, the modification approach started when dyes were incorporated into the silkworm’s food, resulting in fluorescent and/or colored silks for the textile industry. [44] Subsequently, NM additives were used instead. The usual approach was wet impregnation (via spraying or spreading) of the natural food, mulberry leaves (Figure 3a,b). In some cases, a xenobiotic supplement is added to an artificial chow (made from mulberry leaf powder mixed with other vegetable powders) [44–47] since the blending with additives is simpler and more controllable. There is one noteworthy example in which the silkworms are fed by intravenous injection. [48] claimed as an exact intake and equal amongst all silkworms, without negative effects. This in situ and bottom-up silk nanocomposite fabrication is considered greener and easily scaled up. [49]

In the earliest reports from late 2013 authored by Wang et al. magnetic silk was fabricated by silkworms fed with Fe3O4 NPs [49] and high-strength silk was made by silkworms fed with carbon nanotubes (CNTs). [50]

Carbon-based NMs have been fairly explored in this context. CNTs in particular are the most studied supplements so far, with important improvements in the mechanical properties of the resulting silk (Figure 3c,d). [55–57] These reports underline the importance of a suitable dispersant for CNTs in the silkworm feed, since the intrinsic hydrophobicity of the latter requires finding solutions enabling their handling in water. All the attempts to incorporate CNTs into the silkworms’ diet have been conducted with Ca lignosulfonate as an adjuvant. Usually, CNTs or graphene is dispersed with Ca lignosulfonate in aqueous solution prior to mixing with the mulberry leaves or chow. Since Ca lignosulfonate is a natural compound with a composition related to that of mulberry leaves, [50] and it improves the midgut permeability of CNTs, control samples with only lignosulfonate feeding already lead to improved silk. [45] In contrast, an excess of this dispersant hinders the incorporation of CNTs into the silk. [52] and a purification step is needed to remove the surplus before feeding. Since there are no reports on the use of other dispersants for the same purpose, new adjuvants to address the NMs digestibility by silkworms remain unexplored. Other carbon-based NMs, such as graphene derivatives, have also been fed to silkworms resulting in improved silk. [45, 48, 51, 53, 54] While graphene nanoplatelets or graphene oxide (GO) lead to modest to poor mechanical improvements (Figure 3e,d), graphene quantum dots (GQDs) have demonstrated their superiority in silk reinforcement, even at very low doses. [48, 53] Such is the increase in mechanical properties granted by some carbon-based NMs, that their feeding to silkworms has been regarded as a way to upgrade properties of silkworm silk to that of spider silk. [45, 50] Complementary to the mechanical upgrade, carbon-based NMs may also provide novel functional properties to the silk, such as electrical conductivity. [50] a better graphitization after pyrolysis [51, 52] or the emergence of a highly stable fluorescence. [46, 48, 55] Another recurrent family of NMs fed to silkworms are metal and metal oxide NPs. Regarding ZMNP s, the most investigated are Ag NPs [56–59] but there are also reports on feeding with Cu [56, 60] and Fe [60] NPs. While the mechanical improvements of the silk in these cases do not match those from carbon-based NMs (Figure 3c,d), the actual benefits lie in the conferral of new properties such as a potent antibacterial effect [55, 58] or the electricity harvesting from silk cocoons. [59] A similar trend is observed for feeding with metal oxide NPs, which in this case are mostly based on TiO2 [49] or MoO3 [61] or MoO2 [62, 63] NPs. For instance, the excellent UV-absorbing ability of TiO2 led to UV-resistant silks, [49] the magnetic properties of Fe3O4 provided intrinsically magnetic silk, [49] and MoO2 NPs led to an enhancement of specific capacitance in carbonized silk. [62, 63]

All the aforementioned breakthroughs are possible because NMs are actually incorporated into the silk by the action of silkworms. This has been corroborated by some authors using specific characterization techniques. For instance, CNTs and graphene have been detected within the silk
by Raman spectroscopy\cite{51,52} and metal NPs by ICP-MS and advanced electron microscopy techniques\cite{58,60,62}. However, most authors agree that most of the NMs fed to the silkworms are expelled in their excrement\cite{44,45,51,52,57,58}. Therefore, the real amount eventually integrated into the silk is still elusive and hardly controllable. These findings importantly mean that NMs go through the entire digestive tract, but just a small part is able to pass this barrier towards the hemolymph, ultimately reaching the silk glands (SG)\cite{52,57,64}. A fundamental question arises regarding the fate of NMs inside the silkworm (Figure 3b). Indeed, those NMs finally reaching the SG are extruded along with the spun silk. NMs that are the proper size and chemically compatible with hemolymph will reach the SG to some extent. Small-sized carbon dots (CDs) and GQDs (both below 5 nm diameter) have been clearly found in SG by virtue of their luminescent properties (Figure 3e)\cite{46,48}. Metal NPs with a broader range of sizes (from 5\cite{57} to 20 nm,\cite{56} 50\cite{58,60} and even up to 100 nm diameter\cite{58}) have also been found in the SG after histopathological examination. But the hydrophilicity of NMs is also a fact to bear in mind, possibly explaining why highly oxidized and ultrasmall NMs are the most successful in silk reinforcing (Figure 3c,d)\cite{46,48}.

As NMs have been detected in degummed (devoid of sericin) silk, this indicates that, once in the SG, NMs actually interact with the fibroin core. It is widely accepted that NMs incorporated in silk by silkworm feeding do not alter the basic silk structure but its secondary conformation\cite{55}. As the \(\alpha\)-helix/random coil structures confer ductility and toughness to the silk, and \(\beta\)-sheets provide stiffness\cite{45} specific physico-chemical interactions of NMs with fibroin (likely including electrostatic, \(\pi-\pi\) stacking, van der Waals and hydrogen bonding\cite{48}) would change the ratio of secondary motifs, resulting in upgraded mechanical performance. It seems that oxidized carbon-based NMs (GO, GQDs, CDs)\cite{46,48,54}, small-size Ag NPs\cite{57} and hydroxyapatite NPs\cite{65} lead to an increase in \(\alpha\)-helix and random coil structures by hindering their conversion to \(\beta\)-sheets during fiber spinning. This would explain the high ductility and improved toughness reported in most of these cases (Figure 3d). The opposite trend is observed for CNTs, which is coupled to the increase in \(\beta\)-turns\cite{52} responsible for the silk stiffening. However, little or no changes in the secondary structure were observed when silkworms were fed large-size Ag NPs\cite{58} or cellulose nano-fibers\cite{47}. Since some of the results are contradictory\cite{51,52}, and mechanical improvements may occur even without changes in the secondary protein structure\cite{47}, more in-depth studies are needed to unravel all the underlying factors affecting silk’s properties.

At this point, one may wonder: are nanomaterials safe for silkworms to ingest? It is worth noting that silkworms are widely used as a model animal to test NMs’ toxicity\cite{66}; hence, as a general consensus, low doses of NMs are deemed harmless. Surprisingly, there have even been reports of the healing effects of NPs fed to poisoned or diseased silkworms\cite{66} however, toxic effects arise beyond a certain threshold, strongly depending on the NP size and amount fed. The example of Ag NPs may be the most illustrative, as the lethality of different sizes (ranging from 20 to 100 nm) has

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Figure 3. a) The silkworm feeding strategy to fabricate reinforced silk with CNTs or graphene. Reproduced with permission from ref. [51]. Copyright 2016, American Chemical Society. b) The fate of fed NMs within a silkworm: Hemolymph (He), fat body (FB), silk glands (SG), digestive tract (DT), Malpighian tubule (MT). Adapted from ref. [64], subjected to a Creative Commons 4.0 license, 2016 Springer Nature. c) Best improvements in toughness and d) ultimate tensile strength for different nanocomposite silkworm silks resulting from the NM feeding strategy. e) Direct visualization of the fluorescent emission in degummed silk and SG from silkworms fed with CDs. Adapted with permission from ref. [53]. Copyright 2019, Springer Nature.

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been studied.\[58,59\] For silkworms fed with 60–80 nm Ag NPs Nambajjwe et al. observed an 83% survival rate at 1 wt% feeding ratio, but a feeding ratio of 0.4–0.5 wt% increased this to a 92%.\[59\] In similar studies, the silkworms’ survival slightly decreased with increasing NP size (20–100 nm diameter), but was always above 85% for a 1 wt% feeding ratio.\[58\] Similar conclusions were drawn from Cu, Fe, and TiO\(_2\) NPs (30–50 nm diameter), which showed no issues at a = 0.3 wt% feeding ratio.\[60\] The most lethal NMs seem to be MoO\(_2\) NPs (50 nm diameter), which lead to nearly full mortality at 1 wt% so a safety threshold of 0.5 wt% was set.\[62\] For carbon-based NMs, no toxic effects were observed with CNTs, graphene, CDs, or GQDs at 0.2–1.25 wt% feeding ratio.\[56,48,51,53\] In essence, any NM may be safe for silkworms if the doses are below 1 wt% and particle sizes are not larger than 50 nm.

One last word concerning the production of silk by spiders: Taking into account their savage and predatory nature and the fact that they cannot be tamed, it becomes clear that the mass production of their dragline silk does not constitute a realistic option. Thus, studies on feeding spiders to improve their silk are almost nonexistent. A landmark study by Lepore et al.\[67\] in 2017 reported the feeding of spiders (from the Pholcidae and Thericidae genera) with CNTs and graphene by spraying the inner corners of the rearing box with aqueous dispersions. This indirect feeding resulted somehow in the incorporation of these NMs into the silk with some noteworthy improvements in its mechanical properties; in isolated cases large improvements were reported, for example, a 663% increase in toughness, although much less in average terms. Importantly, the authors admitted that they could not exert any control on the NM uptake.\[67\] Three years later Kelly et al. challenged Lepore’s work by a conscientious replication (including equivalent carbon NMs adapted from Lepore’s work) on Nephila pilipes spiders, but in this case by direct feeding via pipette on their chelicera.\[68\] Kelly’s results were drastically different from those of Lepore, as no CNTs or graphene were detected in the silk and the mechanical properties were unchanged. It would be intriguing to understand if and how spiders could incorporate NMs into their silk upon feeding.

### 3.2. Cellulosic Biomatrices

Owing to the fact that \(K.\ xylinus\) bacteria fabricate BNC, many studies have addressed their in situ culture with NMs, which facilitates NM integration with high homogeneity, thus avoiding post-synthesis steps. The enhanced intimate contact between NMs and the BNC biomatrix provides new or improved features, but usually takes a toll on the bacterial growth rate and the BNC crystallinity.\[69\] One of the earliest successful attempts was published by Park et al.\[70\] who obtained by this way a CNTs-BNC hybrid scaffold exhibiting the excellent in vivo bone regeneration ability compared to its ex situ analogue.

The key point is to keep NMs stably dispersed in water as long as possible, letting bacteria do their task; this is the reason why a synthetic copolymer had to be used as a bind-

Figure 4. a) Spherical BNC hydrogels cultured in the presence of GO. Adapted with permission from ref. [71]. Copyright 2020, Elsevier. b) Fabrication of BNC hybrid hydrogels by in situ culture with reduced GO. Reproduced with permission from ref. [72], subjected to a Creative Commons (CC-BY) license, 2019 American Chemical Society. c) Photograph of an A. thaliana root after biogenic integration of CNTs. Scale bar = 5 mm. Reproduced from ref. [71], open access, 2020 Frontiers. d) Preparation of hybrid yeast-CNTs nanocomposites together with an optical image. Reproduced from ref. [75] subjected to a Creative Commons 4.0 license, 2016 Springer Nature.
3.3. Fungal Biomatrices

Thanks to the studies conducted by Valentini, Bon, and Pugno, some interesting results on the biogenic integration of carbon based NMs into yeasts have been reported.[74–76] In the context of spontaneous fermentation of single-cell *Saccharomyces cerevisiae* fungi, an inexpensive method to conform a nanobiobybrid with unique properties is feasible. When graphene is used, its attachment to the yeasts cell walls is observed.[74] This leads to hybrid biofilms with great mechanical properties, and most importantly, the unique ability to self-repair surface cracks when the biofilm is immersed into the culture broth for just 40 min.[74] Further, when CNTs are used with the same purpose, the bridging of yeast cells is observed, ascribed to the internalization of CNTs (Figure 4d).[75] Not only an enhancement of the mechanical and electrical performance is seen, but also the emergence of a photoconductive activity. The potential of yeasts to process NM was exploited deeper, by performing the hybridization in a gelling silicone rubber medium.[76] The resulting nanocomposite presented auxetic (negative Poisson’s ratio) and electromechanical behaviors. This paves the way towards smart structures with self-sensing and actuating properties through simple and affordable fungal action over specific NM.

4. Summary and Outlook

In this Minireview, the most significant advances in the bionanofabrication of NMs and nanocomposites via the sole action of single-cell or higher organisms are presented and analyzed. This revolutionary approach is still in its infancy but has already shown tremendous potential. By such means, the specific metabolic and physiological activity of living entities can convert ionic species into well-defined NPs, or integrate different NMs into biomatrices, with high precision and excellent results; the resulting materials outperform their equivalent abiotic materials and processes in many ways. Although the underlying chemical and biological mechanisms by which all this occurs are not completely understood, the current knowledge is encouraging. Deeper insights into the specific roles of reducing enzymes, native metal-binding species, organelles, and glands responsible for the creation of these unique NMs and nanocomposites will certainly enlighten this field. What seems clear today is that living organisms serve as more than mere templates, as their biogenic activity can tailor the shape, size, and reduction state of NPs, as well as the dispersion state and homogeneity of nanocomposites. This far surpasses the limits of human phenotypes and derived products is another must to achieve such a goal. We envision the most promising future directions in the fields of energy and catalysis for biogenic metal NPs, because of the fine tunability in composition, morphology, and properties, as well as in smart textiles and structures for biomatrix nanocomposites, which show record mechanical properties and novel functionalities (such as magnetic behavior, fluorescence, and self-healing ability). While the facts prove that small organisms might be the best tools for the full realization of this nanotechnology, the remaining challenge refers to their practical exploitation (Figure 5).

![SWOT analysis summarizing the bionanofabrication field.](image)

**Figure 5.** SWOT analysis summarizing the bionanofabrication field.

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The authors declare no conflict of interest.


Green living nanofactories: Nanomaterials (NMs) and nanocomposites are typically prepared by procedures that are incompatible with green chemistry and sustainability principles. But some living entities, from single cells to higher organisms, are able to synthesize and process NMs very efficiently under milder conditions. In this Minireview, the advances and prospects of this growing trend are discussed and summarized.