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Review

Green Synthesis of Magnesium Oxide Nanoparticles and Nanocomposites for Photocatalytic Antimicrobial, Antibiofilm and Antifungal Applications

Marzieh Ramezani Farani [1], Majid Farsadrooh [2], Iman Zare [3], Amir Gholami [4] and Omid Akhavan [1,*]

[1] Department of Physics, Sharif University of Technology, Tehran P.O. Box 11155-9161, Iran
[2] Renewable Energies Research Laboratory, Department of Chemistry, Faculty of Science, University of Sistan and Baluchestan, Zahedan P.O. Box 98135-674, Iran
[3] Research and Development Department, Sina Medical Biochemistry Technologies Co., Ltd., Shiraz P.O. Box 7178795844, Iran
[4] Student Research Committee, Kurdistan University of Medical Sciences, Sanandaj P.O. Box 6618634683, Iran
* Correspondence: oakhavan@sharif.edu; Tel.: +98-21-6616-4566

Abstract: Magnesium oxide nanoparticles (MgO NPs) have emerged as potential materials for various biomedical applications due to their unique physicochemical properties, including biodegradability, biocompatibility, cationic capacity, high stability and redox properties. MgO NPs have become an attractive platform to combat microbes and may be a promising alternative to overcome challenges associated with eliminating microbial biofilms and antibiotic resistance. Hence, due to the increasing use of MgO NPs in biomedicine, new synthetic strategies for MgO NPs are necessary. MgO NPs synthesised using green methods are non-toxic, eco-friendly and have high stability for a wide range of biological, medical and catalytic applications. This review presents the recent advances in biosynthesis strategies of MgO NPs by diverse bio-templates, such as plant, bacterial, fungal and algal extracts. Its photocatalytic properties show a suitable inhibitory function against pathogenic agents, such as microbial proliferation, biofilm formation and fungal growth. Furthermore, MgO NPs and relevant nanocomposites are comprehensively discussed regarding the mechanisms of their effect on microbes, biofilms and fungal strains, as well as challenges and future perspectives.

Keywords: bio-template; green synthesis; magnesium oxide; photocatalytic properties

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1. Introduction

Nanomaterials are a subclass of materials that have an extraordinary surface and at least one dimension with an average size of 1 to 100 nm [1]. Special structures, high surface-to-volume ratio and a large number of atoms residing on their surfaces have distinguished nanomaterials [2]. The properties of bulk materials can improve or be created by engineered nanomaterials in terms of strength [3], conductivity [4], catalytic [5], antibacterial [6] properties, etc. [7,8].

Metal oxide and metal NPs have wide applications in different biochemical and medical applications, such as catalysis [9], tissue engineering [10], antibacterial and anticancer agents [11–13]. Among metal oxide NPs, MgO NPs have attracted attention for their high stability, biocompatibility and their antipathogenic (antiviral, antimicrobial and antifungal) activity [14,15]. MgO is an eco-friendly, economically viable and industrially important NP that stands out for its unique physicochemical properties, including outstanding refractive index [16], excellent corrosion resistance [17], high thermal conductivity [18], excellent optical transparency [19], mechanical strength [20], dielectric resistance [21], stability [22], flame-resistance [23], physical strength [24,25] and low electrical conductivity [26–28]. According to the mentioned characteristics, MgO is used as a refractory material, photocatalyst, electrochemical biosensor, semiconducting material, absorbent of organic and

inorganic pollutants from wastewater and catalyst in organic transformations [29]. Photocatalytic properties of MgO NPs play a significant role in removing the environmental pollution and antibacterial behaviour of these NPs [30]. MgO is an insulating metal oxide that has a wide gap with a band energy of 5–6 eV [31]. The bandgap energy of MgO is high (i.e., >5 eV), which makes it optically active for photocatalytic performance [32]. Moreover, MgO can generate reactive oxygen species (ROS) that can cause oxidative stress in in vitro or in vivo cell lines, leading to antipathogenic activity [33].

The synthesis methods, dimensions and shapes of nanomaterials can affect their properties, such as photocatalysis [34], anticancer activity [35], antibacterial function [36], biocompatibility [37] and other biomedical applications [38,39]. Nanomaterials are usually synthesised utilising a variety of chemical and physical processes (e.g., sol-gel, hydrothermal, sputtering, etc.) [40]. These methods require high temperatures, chemical additives, complex instruments and vacuum conditions [41,42]. Chemical synthesis methods of nanomaterials include reducing agents, solvents, metal salts, capping agents, growth, aggregation, nucleation, characterisation and stabilisation. Physical synthesis methods of nanomaterials mainly involve crushing and difficult physical conditions, which require advanced devices with high energy consumption [43]. Current advances in chemical techniques for the synthesis of nanomaterials have increased biological and environmental hazards due to the use of toxic chemicals and heavy metals, including Ti, attached to the synthesised nanomaterials [44–46]. Compared with physical and chemical methods, biological synthesis of nanomaterials has significant advantages, including high biocompatibility and no high energy consumption for synthesis processes [47]. Therefore, the use of biological sources for the synthesis of nanomaterials has increased significantly, such as plants [48], algae [49] and bacteria [50]. These natural sources include vitamins, polyphenols, polysaccharides, amino acids, biomolecules and phytochemicals that can act as chemical stabilisers and reducing agents to replace hazardous chemicals [51,52]. Green methods have been widely developed for the synthesis of MgO NPs using energy-efficient, cost-effective and eco-friendly manners [53,54].

This study was conducted to review and classify recent reports of green synthesis of MgO NPs into four main methods, including extract-based synthesis methods based on plants, bacteria, fungi and algae. First, photocatalytic antimicrobial, antibiofilm and antifungal performances of MgO NPs were compared. Next, different mechanisms of MgO NPs for anti-pathogenic functions such as antimicrobial, anti-biofilm and antifungal activities were investigated. Finally, applications of MgO nanocomposites from photocatalytic treatment were introduced to improve their stability and properties. Biosynthesis of MgO nanoparticles can obtain utilizing plants and microorganisms, as illustrated in Figure 1.

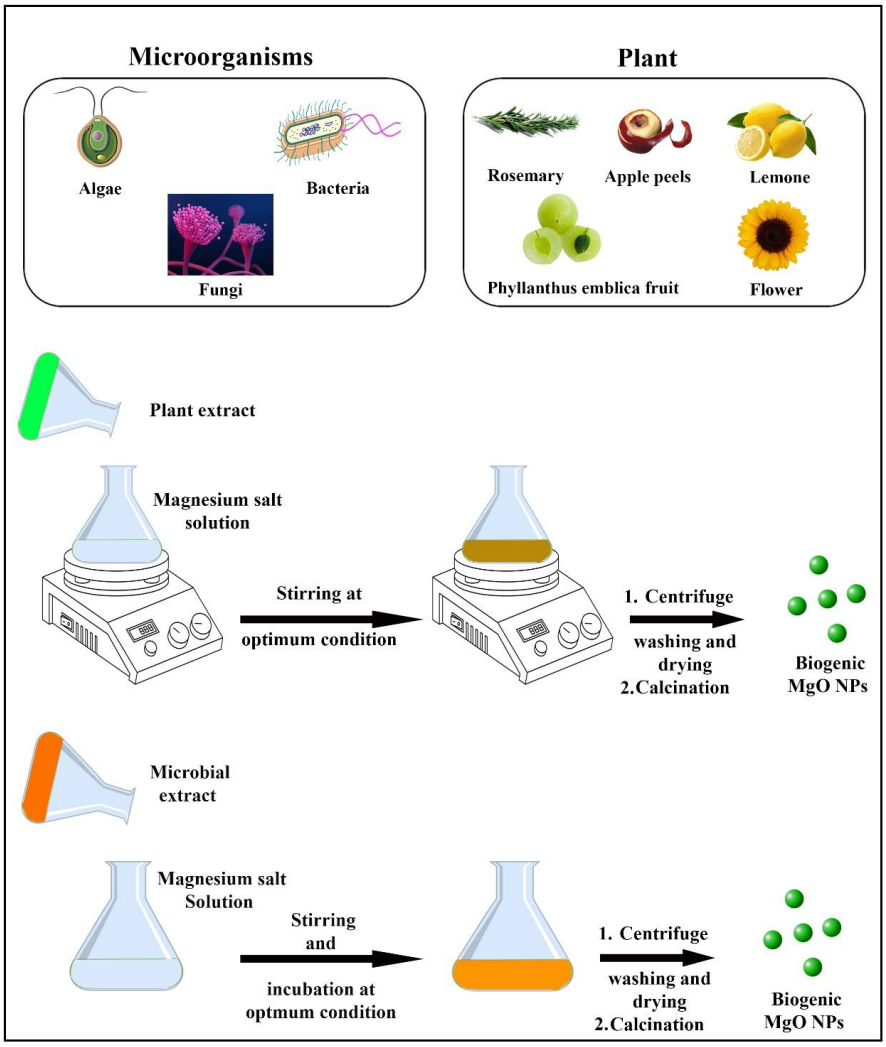

Figure 1. Biosources for the preparation of MgO NPs, and green method for preparation of MgO NPs.

2. Bio-Templates-Mediated Synthesis Strategies

2.1. Plant Extracts

Plants are the most commonly used biological substrates for the green synthesis of NPs because they are biocompatible and readily available [55,56]. It is possible to reduce and stabilise ions using a combination of biomolecules such as proteins, amino acids, flavonoids, citric acid, various enzymes, polysaccharides, phenols, saponins, terpenoids and vitamins found in plants extracts [57–60].

There are several methods of extraction in plants, including soaking, brewing, digestion, boiling, percolation, Soxhlet extraction, microwave extraction, ultrasound extraction and super/subcritical water [61,62]. The solvent used to extract bioactive compounds from plants is known as menstruum. The type of menstruum used for extraction depends on the type of plant and the location from which it is to be extracted. In general, polar solvents such as water, ethanol and methanol are used for polar compounds, while non-polar solvents such as hexane and dichloromethane are used to extract non-polar substances [63,64]. Several factors should be considered for choosing a solvent. The viscosity of the solvent should be as low as possible to allow easy penetration. On the other hand, the ability to recover the solvent should be considered, as it can be easily separated from the extract. Another important parameter in choosing a solvent is its selectivity and ability to extract the active substance and leave the inactive substance [65,66].

There are some interfering factors in the synthesis of plant-based NPs that affect the final shape and size of the NPs. It has been reported that pH plays a significant role in the

biosynthesis of NPs. Muthu and Priya [67] reported the biosynthesis of metal NPs and the increasing size of NPs with decreasing pH. It has also been reported that with the increase in pH, the reaction rate of plant extracts with NPs increases and causes the production of smaller NPs in larger numbers [68].

Another factor affecting the morphology and size of NPs in biosynthesis methods is temperature. The size of NPs decreases with increasing temperature and their morphology becomes more uniform; conversely, with decreasing temperature, the size of NPs increases [69,70].

Different factors affect the synthesis of MgO NPs using plant extracts (Figure 1). For example, Jeevanandam and colleagues investigated that the shape and size of MgO NPs are affected by pH changes, such that their size decreases and the shape changes to hexagonal at pH = 3 [71]. In addition, temperature affects the preparation of MgO NPs; if the temperature is inappropriate, it can affect phytochemicals by stabilising and reducing NPs. Likewise, the ratio and concentration of precursors and extracts also affect the size and shape of MgO NPs. In particular, a large number of core chains may be formed during the synthesis due to the high concentration of the precursor, leading to the production of large-sized NPs. Phytochemicals are sufficient to chelate, reduce and coat NPs due to their high extraction rate. Moreover, well-dispersed NPs can be synthesised by phytochemicals in plant extracts, as they can interfere with the aggregation of NPs during synthesis. Recent reports show that the use of plant extracts for the synthesis of MgO NPs is more compared with that of algae, fungi and bacteria. Table 1 summarises the types of plant species for the synthesis of MgO NPs that affect their dimensions and morphology.

Table 1. Reported methods of the biosynthesis of MgO NPs via plants.

No.	Green Source	Magnesium Source	Particle Size (nm)	Morphology	Ref.
1	*Bauhinia purpurea* leaf extract	Magnesium chloride	10–11	Nanoflakes	[72]
2	*Prosopis farcta* leaf extract	Magnesium nitrate	30−40	Nanowires	[73]
3	*Pisonia alba* leaf extract	Magnesium nitrate	<100	Roughly spherical	[74]
4	*Matricaria chamomilla* L. extract	Magnesium nitrate	18.2	Disc-shape	[75]
5	*Emblica officinalis* fruits	Magnesium nitrate	27	Spherical	[76]
6	*Limonia acidissima* fruits	Magnesium nitrate	4−8	Different shapes (flower-shaped, spherical and flake-shaped)	[77]
7	*Citrus aurantium* fruit peels	Magnesium nitrate	50−60	Spherical	[78]
8	*Annona squamosa* seeds	Magnesium nitrate	27 and 68	Irregular	[79]
9	Banana peel	Magnesium nitrate	15−20	Nanopowder	[80]
10	*Artemisia abrotanum* herb extract	Magnesium nitrate	10	Spherical	[81]
11	*Camellia-sinensis leaves* extract	Magnesium nitrate	Up to 80	NPs	[82]
12	*Aloe barbadensis* leaf latex extract	Magnesium nitrate	Liquid extract = 25−35, Solid extract = 39−60	Spherical	[83]
13	*Trachyspermum ammi* leaf extract	Magnesium nitrate	78.48	Spherical	[84]
14	*Hibiscus rosa sinensis* leaf extract	Magnesium sulphate	27.72	NPs	[85]
15	*Pterocarpus marsupium* heartwood extract	Magnesium nitrate	5−20	Spherical	[86]

Various mechanisms have been proposed to elucidate the biosynthesis of MgO NPs from plant extracts, as shown in Figure 2. Some studies indicated that covalent bonds can be formed between metal ions and bioactive compounds and then decomposed using

heat treatment to produce MgO NPs. Plant extracts contain a variety of phytochemicals and biomolecules, such as polysaccharides, alkaloids, enzymes, proteins, methylxanthines, saponins, phenolic acid, terpenoids and flavonoids, which can act as masking, chelating, stabilising and reducing agents.

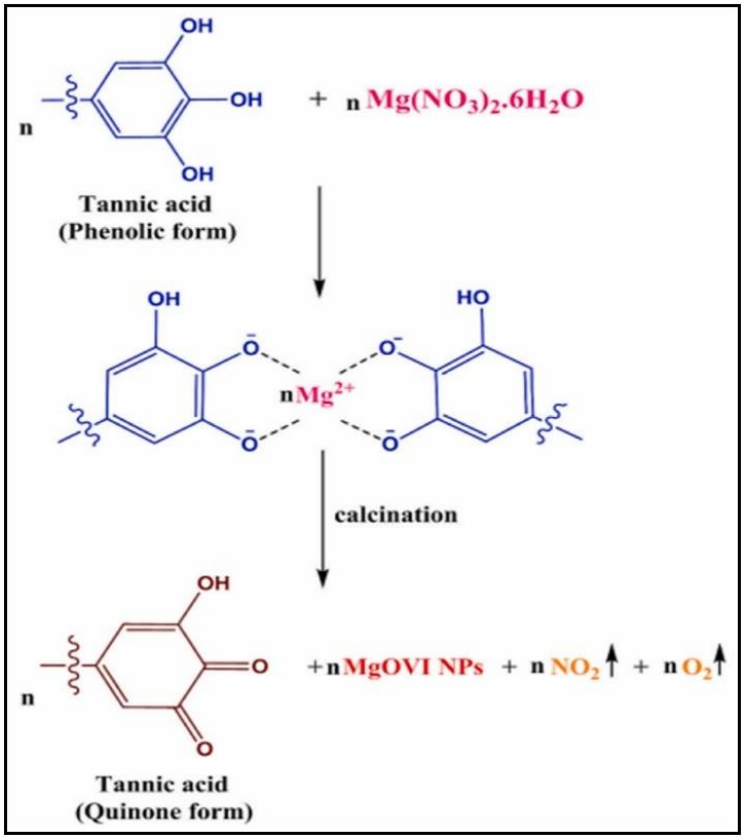

Figure 2. A plausible mechanism for MgO NP synthesis from plant extracts. Reprinted with permission from Ref. [54]. Copyright 2022, Elsevier.

The proposed mechanisms for the synthesis of MgO NPs from plant extracts are as follows: (1) Interaction occurs between $Mg(NO_3)_2$ in the precursor salt solution and tannic acids in the plant extract. (2) Mg–tannic acid complexes are formed as a result of this interaction. (3) MgO NPs are formed following the calcination of magnesium tannic acid complexes at a high temperature of 400 °C. It is widely accepted that plants synthesise MgO NPs through these mechanisms. Biosynthesis of MgO NPs by the reaction of 3 g magnesium sulphate and 50 mL *Hibiscus rosa-sinensis* leaf extract was introduced by Khan and co-workers [85]. Moreover, Abdallah et al. [87] investigated the antibacterial properties of NPs and the synthesis of MgO nanoflowers using rosemary extract without the intervention of chemical catalysts. They reported that MgO nanoflowers have antibacterial properties against *Xanthomonas oryzae pv*. It was declared that NPs significantly inhibited bacterial growth, biofilm formation and motility. In another example, Poonguzhali and co-workers used lemon juice to synthesise MgO nanostructures, as citric acid in lemon juice can act as a reducing agent [88]. In their study, MgO NPs were synthesised for use in gas sensing by a combustion technique using lemon juice as a natural source of citric acid. The exothermic reaction between lemon juice and metal nitrates and the very rapid self-combustion nature were influenced by the acidic lemon juice, which resulted in the production of NPs. A large amount of gas was produced during the combustion process, which prevented the aggregation of NPs. The results showed that lemon juice as a natural fuel plays an important role in the production of MgO nanorods with a smaller size.

In another intriguing study, Amina et al. [89] investigated the effect of MgO NPs synthesised by the green method using *Saussurea costus* plant roots on cancer. Their findings show that the synthesised NPs have high toxicity on MCF-7 breast cancer cells and cause cell death. Moreover, Ammulu et al. [86] used *Pterocarpus marsupium* heartwood extract to synthesise MgO NPs due to its richness in polyphenolic compounds and flavonoids; additionally, its antibacterial effects against *Escherichia coli* and *Staphylococcus aureus* were evaluated.

The majority of the studies supported the use of plant extracts in the production of NPs. However, based on our analysis of the literature, we found that many limitations and challenges hamper plant-based synthesis. These limitations include defects in technical and engineering approaches. The ratio of plant extract to chemical solution was the primary factor that affected the size and stability of NPs. Furthermore, there are economic limitations related to the purity of plant extracts, chemical solutions, obtaining consistent product with good performance and stoichiometric ratio of reagents. In addition, operational sustainability, limitations in process engineering and lack of life cycle assessment are considered major problems.

2.2. Bacterial Extracts

Bacteria have been found to be favourable candidates (as nanofactories) for the biological synthesis of NPs because they extracellularly produce NPs using the reduction of metal ions to metal particles. Furthermore, the amazing potential of NP production by bacteria may be remarkable due to their faster reproduction rate and easy cultivation [90]. The mechanism of synthesis of all NPs by bacteria remains the same, which typically refers to the synthesis of reductase enzymes by bacteria that contribute to the bioreduction mechanism [91]. The schematical mechanism of microbial synthesis of metal NPs is shown in Figure 3.

Microbial growth can occur in intracellular and extracellular environments, as it reduces magnesium ions to oxide NPs [92]. The extracellular mechanism of biomolecules is based on the release of proteins and enzymes from microorganisms that can reduce and stabilise metal salts [93]. Additionally, metal ions are internalised into cells in an intracellular mechanism, where they are reduced by several proteins and enzymes to produce NPs [93]. In Table 2, the types of microorganisms for the biosynthesis of MgO NPs are listed, which are synthesised based on magnesium source, different sizes and forms.

The biosynthesis of MgO NPs using *Lactobacillus plantarum* and *Lactobacillus sporogenes* was reported by Mohanasrinivasan et al. [94]. In their study, magnesium nitrate added to a diluted bacterial culture with the addition of a small amount of NaOH (0.2 M) thermally decomposed to yield MgO NPs. Interestingly, Kaul et al. [76] synthesised the MgO NPs using *A. fumigatus* and *P. chlamydosporium*. Metal ions at very high concentrations were reduced by enzymes secreted bymicroorganisms, leading to the production of intracellular and extracellular sizes.

The synthesis of NPs based on the bacterial method provides an excellent result, but this process has some disadvantages in the synthesis of NPs. In this regard, purification of NPs from bacteria requires time-consuming methods with several mechanistic aspects that are not well-understood. Meanwhile, the key problem in all of the preceding investigations was to control the shape and size of the NPs to achieve the suspension solution phase. In turn, the production and processing of NPs on an industrial scale is the most important challenge. For rational and economic development of NPs, more reliable information is needed in this field.

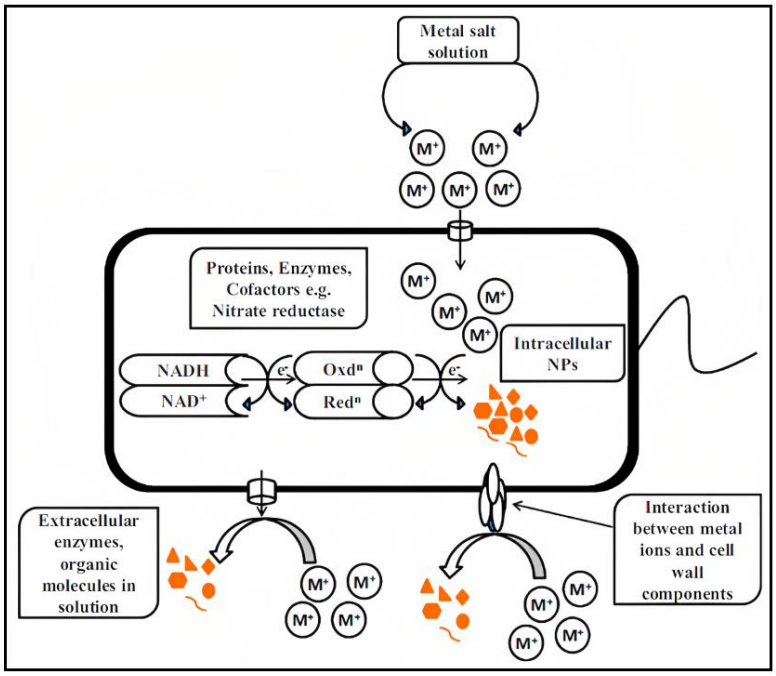

Figure 3. Plausible mechanisms of extracellular and intracellular biosynthesis of NPs from bacteria. Reprinted with permission from Ref. [95]. Copyright 2017, Taylor & Francis.

Table 2. Reported methods of the biosynthesis of MgO NPs via microorganisms.

No.	Biological Source	Magnesium Source	Particle Size (nm)	Morphology	Ref.
1	*Lactobacillus plantarum Lactobacillus*	Magnesium nitrate	<30	Spherical and oval	[94]
2	*Penicillium chrysogenum*	Magnesium nitrate	7−40	Spherical	[96]
3	*Sargassum wightii*	Magnesium chloride	68.6	Cubic	[97]
4	*Streptomyces* sp.	Magnesium nitrate	25	Spherical	[98]
5	*Acinetobacter johnsonii*	Magnesium nitrate	18−45	Spherical	[99]
6	*Trichoderma aureoviride*	Magnesium nitrate	13.7	Spherical	[100]
7	*Aspergillus carbonarious D-1*	Magnesium nitrate	20–86	Spherical	[101]
8	*Rhizopus oryaze*	Magnesium nitrate	20.38 ± 9.9	Spherical	[102]
9	*Burkholderia rinojensis*	Magnesium nitrate	26.70	Roughly spherical granular	[103]

2.3. Fungi Extracts

Green synthesis of nanomaterials through microorganisms such as fungi, compared with chemical synthesis methods, not only does not have the problem of using toxic and harmful materials, but can also give useful properties to NPs.

Using microorganisms such as fungi, nanomaterials can be synthesised, which have useful properties compared with other synthesis methods [104]. The advantages of the biosynthesis method using fungi include metal NPs, cost-effectiveness and biological safety, high concentration of extracellular redox enzymes and capping agents to stabilise the synthesised metal NPs, smaller size of the synthesised NPs compared with bacterial synthesis methods and easier control of the NP scale [105].

Biosynthesis methods of MgO NPs based on fungi extracts have been presented in previous studies [106]. Microorganisms such as fungi naturally have the property of reducing and oxidising metal ions to metal NPs or metal oxides and can participate in the synthesis of metal NPs [107]. Microscopic filamentous fungi (fungi and ascomycetes) and other fun-

gal species have been reported to produce approximately 6400 bioactive substances [108]. Fungi are widely used as stabilising and reducing agents due to their high tolerance to the bioaccumulation of heavy metals and metals. Fungi can be easily cultivated on a large scale and NPs with controlled morphology and size can be produced [109]. The biosynthesis mechanisms of MgO NPs using fungi are divided into two general categories: intracellular and extracellular. The metal precursor is added to the mycelium culture in the intracellular synthesis method and enters the biomass [110,111]. Extracellular synthesis involves the addition of a metal precursor to an extract containing only fungal biomolecules, resulting in the formation of free NPs. The use of this method is more common because it does not require a secondary method to release the synthesised NPs [112,113]. Figure 4 demonstrates the process of extracellular and intracellular synthesis of NPs by fungi extracts.

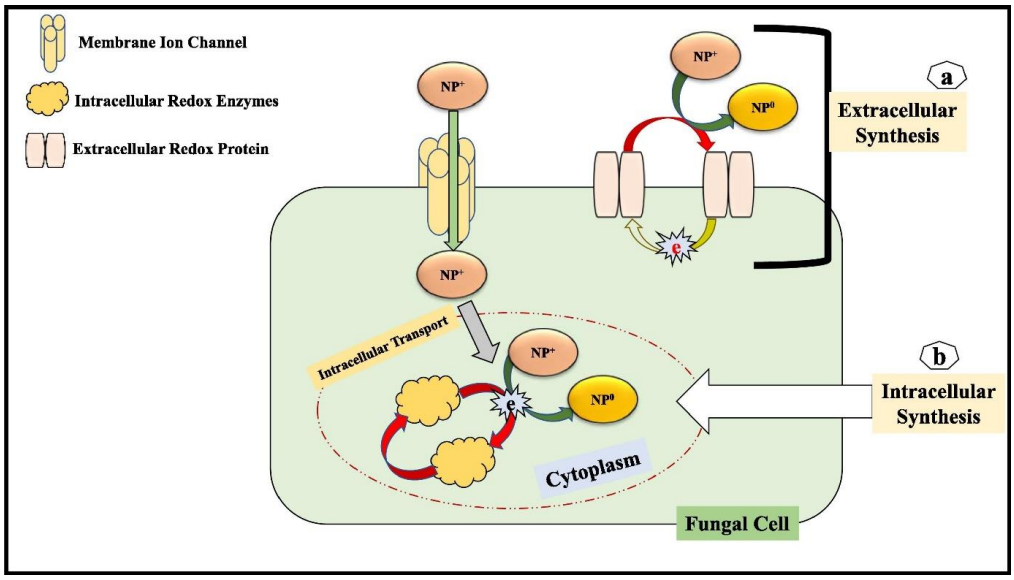

Figure 4. Proposed mechanisms of intracellular and extracellular pathway biosynthesis of NPs in the typical fungal cell. (**a**) In the extracellular pathway, metallic ions (M^+) are reduced to their neutral state (M^0) by membrane redox proteins. (**b**) In the intracellular pathway, metallic ions first enter the cell through membrane ion channels, which then by activation of intracellular redox proteins (NADPH) reduced to their neutral state. Reprinted with permission from Ref. [114]. Copyright 2021, Elsevier.

Various factors, such as precursor concentration, incubation temperature, contact times and pH can affect the biosynthesis of MgO NPs by fungi. Saied and co-workers biosynthesised MgO NPs using the fungal strain *Aspergillus terreus* S1 to remove chromium ions, decolourise tannery effluent and inhibit the growth of pathogenic microbes [115]. In their study, some operating parameters affecting the synthesis of MgO NPs were optimised as follows: incubation temperature (35 °C), pH 8, contact time (36 min) and concentration of $Mg(NO_3)_2$. Evaluations demonstrated that the inhibitory property of MgO is dependent on its concentration, and they were able to remove chromium ion concentration at 97.5%. The antimicrobial activity of MgO was evaluated and the maximum inhibition of pathogen growth was obtained at 200 µg/mL of MgO.

Temperature is one of the factors affecting the synthesis of metal NPs in this method [112]. However, studies on other metal NPs have shown that the smallest dimensions are optimally achieved at moderate temperatures [116]. At moderate temperatures, electrons can be transferred from proteins to metal atoms and lead to the synthesis of NPs, but at very high temperatures, such as 80 to 100 °C, proteins are denatured and this phenomenon causes an increase in the size of NPs [117].

The culture medium is another effective factor in the synthesis of metal NPs. Changes in the culture medium led to the synthesis of different metabolites and proteins by fungi [118]. Different environments were tested for fungi cultivation and different behaviours were

observed in the synthesis of nanomaterials [119]. Fouda et al. [96] synthesised MgO NPs using a cell-free extract of *Penicillium chrysogenum* with fugal metabolite. The activity of MgO NPs as an antimicrobial agent against the pathogens *Candida albicans*, *S. aureus*, *E. coli*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* was investigated. It has been reported that the antimicrobial activity decreases with a decreasing concentration of MgO NPs. Pugazhendhi et al. [97] synthesised antibacterial MgO NPs using aqueous extract of *Sargassum wightii*. The analysis results showed that NPs have a high antibacterial potential on Gram-negative and Gram-positive bacteria in a dose-dependent manner. Additionally, the anticancer properties of NPs on human lung cancer cell line A549 were investigated. Morphological changes, cell wall damage and loss of membrane integrity were observed. Since fungi can secrete more functionally active substances than bacteria, the method of synthesising MgO NPs from the isolation and culture of fungi is more effective. However, the use of fungi in the production of NPs requires precise and complete cultivation conditions. Moreover, the elimination of fungal residues and impurities after the production of NPs should use methods such as ultracentrifugation, dialysis and filtration. Because of this disadvantage, this method is more expensive and time-consuming than synthetic plant-based activities.

2.4. Algae Extracts

Algae are a diverse group of aquatic microorganisms that contain various phytochemicals such as polyphenols and flavonoids, similar to the phytochemicals found in the extracts of plants. In addition, algae extract contains various bioactive substances such as tocopherol, polyphenols, proteins, carbohydrates and pigments (e.g., phycobilin, chlorophyll and carotenoids). The synthesis process of MgO NPs by algae is performed as follows:

To take the place of the biological reduction reaction, the mixture was incubated under the proper conditions (time, temperature, and pH). In detail, a reaction occurs between the metal precursor salt and the algal extract; an alteration in colour indicates when nucleation has begun. At first, the nucleation of metal ions from metal salts in aqueous solution, and then M^+ ions were converted to M^0 by phytomolecular redox [120]. After that, the fusion and growth of nuclei close to each other leads to the synthesis of MgO NPs [54]. Furthermore, the formation of small- and large-sized metal NPs is attributed to Ostwald ripening, coalescence and oriented attachment [120]. To accumulate the resulting NPs, the algal biomass was dried and the resulting powder was incubated after washing with distilled water for several hours (8−16 h), and then the obtained product was filtered. MgO NPs were obtained after the filtration process. In this method, alkaloids, terpenoids, phenols, flavanones, amines, amides, proteins and pigments in the extracts contribute to reduce and stabilise the metal [121]. Algae has a good potential for algae-dependent synthesis methods due to its ability to absorb metals and change their shape [122]. Two intracellular and extracellular methods are the main methods of NP synthesis through algae [123]. The intracellular method refers to a process that occurs inside the cell and requires specific preparation steps, because this process depends on metabolic pathways that are responsible for synthesis, including respiration, nitrogen fixation and photosynthesis [124]. Extracellular synthesis is a process that occurs in extracellular media and is mostly supported by cellular metabolic products (e.g., enzymes, proteins, ions, RNA, antioxidants, etc.) [125].

The biosynthesis of MgO using marine brown algae *S. wightii* is reported as the following steps: (1) Extraction of *S. wightii* was performed using water at 80 °C for 30 min, and then the extract was filtered; (2) *S. wightii* extract and magnesium nitrate were mixed with a molar ratio of 9:1 and incubated at 90 °C for 6 h [97]. The bioactive component of the extract contributes to the synthesis of NPs. The controlling factors involved in the synthesis processes are generally pH, time, concentration and temperature. Acidic conditions and low pH cause the aggregation of NPs, and thus increase the size of the particles, while increasing the pH is associated with a decrease in the size of the NPs [126]. Increasing the concentration of the extract up to a certain amount leads to the decrease in the final size of the synthesised particles, but an overly concentrated increase has the opposite effect [127].

Fouda et al. [128] investigated MgO NP synthesis using metabolites produced by the brown alga *Cystoseira crinita*. Examining the antimicrobial properties of NPs demonstrated the high effect of produced NPs on Gram-negative and Gram-positive bacteria (Figure 5). According to the findings, the most important antibacterial effect of MgO NPs was the reaction and production of ROS [128]. Although the synthesis process of NPs from algae is safe and eco-friendly, it is time-consuming and multi-step, and its synthesis efficiency is lower than that of plants.

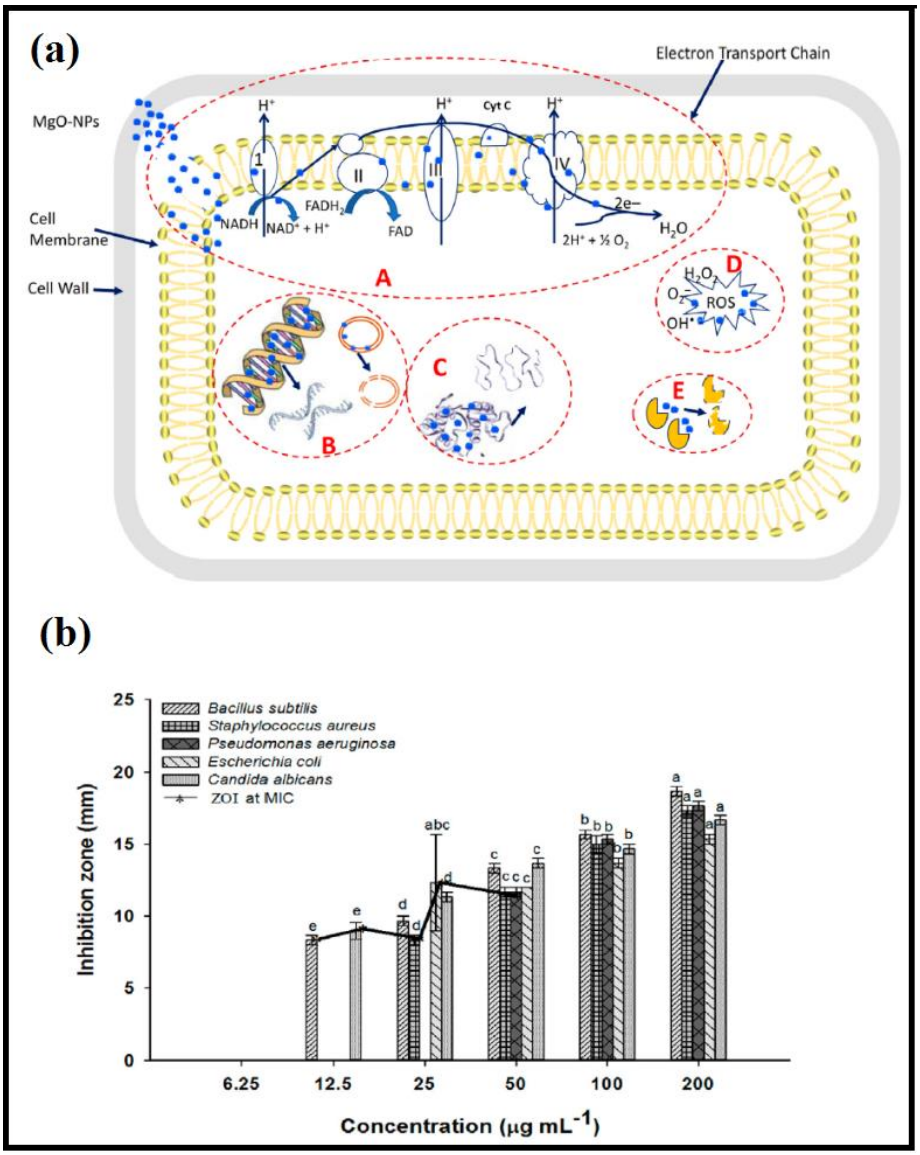

Figure 5. (**a**) Schematic illustration of the antibacterial mechanisms of MgO NPs. Adhesion of MgO NPs to the cell wall disrupts the selective permeability process via electron transport chains. After entering MgO NPs inside the cell, they react with plasmids and DNA, causing genotoxicity. MgO NPs react with proteins, leading to denaturation and enhancing the ROS that results in macromolecule disruption. These mechanisms also indicate that reactions with MgO NPs block and change active sites in enzymes. (**b**) The antibacterial activity of MgO NPs at different concentrations against *C. albicans*, *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis* and inhibition zones at MIC. Different letters (a, b, c, d, and e) at the same concentration indicate significant values ($p \leq 0.05$). Reprinted with permission from Ref. [128]. Copyright 2022, Frontiers.

3. Applications of Photocatalytic Treatment

3.1. Antimicrobial Activity

The photocatalytic antibacterial process is the reaction between ROS and bacteria. As an intermediate product, ROS mainly results from an incomplete reduction reaction between light-excited electrons/holes and oxygen (O_2) or water during the photocatalytic reaction process that produces ROS with higher activity. These species mainly include hydroxyl radicals ($^{\bullet}OH$), superoxide anions ($O_2^{\bullet-}$), singlet oxygen ($^1O_2$), etc. When the light energy irradiated to the photocatalyst is equal to or greater than its band gap, an electron is excited from the valence band (VB) to the conduction band (CB). This excitation process leaves a positive hole in the valence band (h^+). Consequently, electron–hole pairs (e^-/h^+) are produced. In VB, holes created by photons react with water and produce $^{\bullet}OH$ radicals. Since OH radicals are powerful oxidising agents, they can easily oxidise a variety of organic substances indiscriminately. While in CB, the electrons excited by oxygen are used to generate other ROS (e.g., $O_2^{\bullet-}$). After the protonation of the produced $O_2^{\bullet-}$, a hydroperoxyl radical (HO_2) is formed and subsequently decomposes into highly reactive $^{\bullet}OH^-$.

The mechanisms by which metal oxide NPs affect bacteria are complex and not fully understood. The main antibacterial mechanisms attributed to nanomaterials are generally classified as follows: (1) physical damage to the bacterial cell wall as a result of the electrostatic interaction of the sharp edges of nanomaterials with the cell wall membrane [129], (2) production of ROS [130] even in the dark [131], (3) entrapment of bacteria in aggregated nanomaterials [131], (4) oxidative stress [131], (5) disruption of bacterial glycolysis [131], (6) DNA damage [131], (7) visible light photocatalysis [132], (8) perturbation of proteins and cell structure leading to the release and interaction of metal cations and alkaline effects [103,133], (9) metal ion release [132], and (10) involvement in nanobubble generation/explosion [134]. The antimicrobial activity of MgO NPs depends on several factors, such as NP size, high surface charge, high thermal stability, biodegradability, shape and surface adsorption capacity, which cause significant toxicity of MgO NPs to biological systems. For example, Khan et al. [16] biosynthesised MgO NPs using *Dalbergia sissoo* extract and investigated their photocatalytic activity and antibacterial effect. In their study, the photocatalytic activity of MgO NPs was investigated by photolysis of methylene blue (MB) dye. The synthesised MgO NPs with lower band gap energy (4.1 eV) showed a maximum photodegradation efficiency of 81% against MB dye. In addition, the disc diffusion method was used to evaluate the antibacterial activity of MgO NPs. Evaluations revealed that MgO NPs with lower bandgap values have better antibacterial potential against *E. coli* and *R. solanacearum* bacterial strains compared with MgO NPs with higher bandgap values.

Green synthesis of MgO NPs (named MGN1, MGN2, MGN3, and MGN4 with different fuel ratios) using *Phyllanthus emblica* and evaluation of its photocatalytic and antimicrobial activity was reported by Jayanna et al. [135]. To determine the photocatalytic activity of MgO NPs, Evans blue dye decomposition was performed under UV light irradiation. Some different operating parameters, such as MgO NP concentration and pH effect, were optimised to achieve maximum degradation. Evaluations showed that MgO NPs under UV radiation can degrade Evans blue dye up to 90% of wastewater. The antibacterial activity of MgO NPs was evaluated against several human pathogenic strains of *S. aureus*, *P. aeruginosa*, *E. coli*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae*. Notably, the excellent inhibition of human pathogenic strains is strongly related to the possible mechanism of NPs promoting the production of free radicals, which interfere with the degradation of the bacterial cell wall and other cell organelles, leading to the death of the pathogen. They found that the activity of MgO (MGN3) was greater than or similar to the antibiotic used in this study against these bacteria, indicating the effectiveness of using MgO NPs for any clinical application.

The electrostatic interaction of MgO NPs with bacteria causes damage to the bacterial wall and their antibacterial activity. Metal oxide NPs are attached to the cell membrane

through van der Waals and electrostatic interactions. After binding to membrane proteins, MgO NPs disrupt the function of bacteria, and a strong electrostatic interaction with spores leads to cell death [136,137]. In addition, the defective nature of the surface and the positive charge of MgO NPs allow them to absorb halogen gases, which leads to their strong interaction with negatively charged bacteria [138].

It has been reported that the antibacterial activity of MgO NPs induces the generation of ROS-like $O_2^{\bullet -}$, which causes lipid peroxidation in bacteria [139,140]. An increase in the surface area of MgO NPs increases the concentration of $O_2^{\bullet -}$ in the environment and thus causes further destruction of the bacterial cell wall. When the size of MgO NPs is less than 15 nm, the agglomeration effect becomes critical due to the very high surface energy of the particles. The large size of accumulated MgO NPs prevents interaction with bacteria and reduces their antibacterial properties [141].

The alkaline mechanism is probably another antibacterial mechanism of MgO NPs. The formation of a thin layer of water around the particles occurs due to the absorption of moisture by the surfaces of the MgO NPs. The local pH of this thin layer of water formed around the NPs may be significantly higher than the equilibrium value of its solution. The high pH of this thin layer of surface water in the interaction of NPs with bacteria causes damage to the membrane and destroys the cells [142]. For example, Nguyen et al. [143] investigated the inhibitory, antifungal and antibacterial effects of MgO NPs on nine common pathogenic microorganisms, including four yeasts with drug-resistant strains, three Gram-positive bacteria with drug-resistant strains and two Gram-negative bacteria. As shown in Figure 6, different concentrations of MgO affected the morphology and adhesion of the target bacteria and yeasts. In general, with increasing MgO concentration, Gram-negative and Gram-positive bacteria and yeast adhesion density decreased. According to the scanning electron microscopy micrographs, MgO NPs had different effects on the morphology of each microorganism. MgO NPs disrupted the morphology of *E. coli* more than other microorganisms [143].

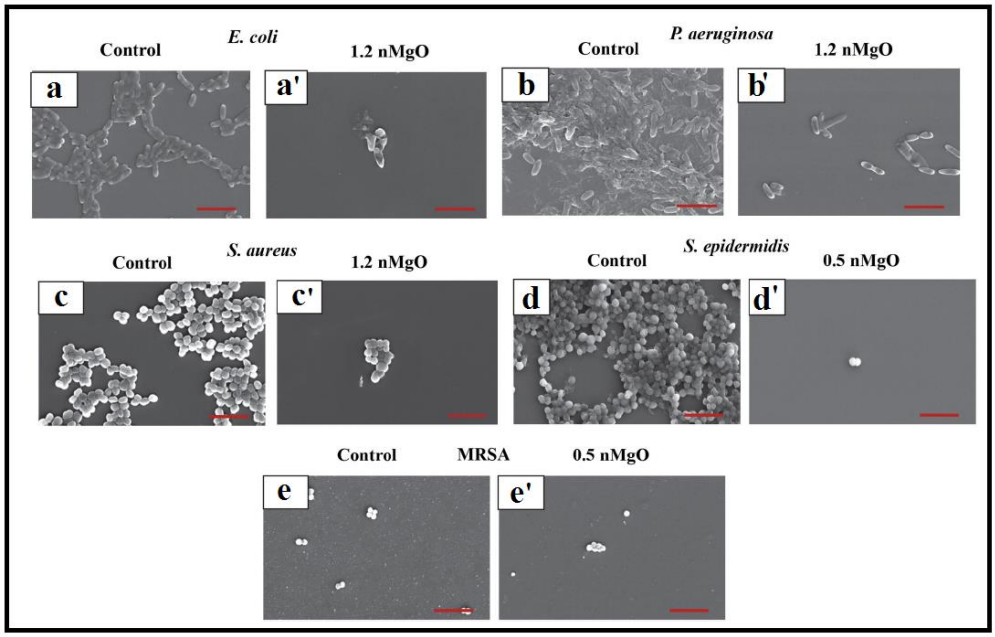

Figure 6. SEM images of *E. coli* (**a**,**a′**), *P. aeruginosa* (**b**,**b′**), *S. aureus* (**c**,**c′**), *S. epidermidis* (**d**,**d′**), and MRSA (**e**,**e′**) were cultured with 0.5 mg/mL of nMgO for 24 h. Scale bar: 5 µm with a magnification of 5000×. Reprinted with permission from Ref. [143]. Copyright 2018, Springer Nature.

The results of the investigations discussed above clearly show that biogenic MgO NPs can positively affect pathogenic bacteria to damage membrane and cell integrity and lead to the death of the bacterial pathogen. As a result, these NPs can be effectively used as potential candidates against a variety of pathogenic bacterial strains.

3.2. Antibiofilm Activity

Biofilms are populations of microorganisms that adhere to a surface and make the complex more resistant to external antimicrobial agents [144]. The biofilm matrix is composed of substances such as proteins, fibrin and polysaccharides [145]. Biofilms protect communities from adverse chemical, physical and biological factors in the environment such as biocides, UV radiation, desiccation, temperature and humoral and cellular immune factors [146]. Usually, the amount of antibiotics needed to kill free-floating bacteria or isolated bacteria is a thousand times less than the amount of antibiotics needed to kill bacterial biofilms [147]. Antibiotic resistance in biofilms leads to the continuous spread of their infection [148]. Furthermore, due to the gradient of oxygen and nutrient concentrations in the biofilm, antibiotics whose action is associated with metabolic disorders due to slow or stopped metabolism are much less effective against bacteria [149]. For example, Nguyen et al. [143] selected *S. epidermidis* as a model bacterium to investigate the effects of MgO NPs against biofilms. The plausible mechanisms of MgO NPs against biofilms are shown in Figure 7 [143]. The green synthesis of MgO NPs and their antibiofilm, antiaging and antioxidant activities were reported by Younis and co-workers [150]. Compared with ciprofloxacin, MgO NPs demonstrated favourable antibacterial activity against three skin pathogens: *P. aeruginosa*, *Streptococcus pyogenes* and *Staphylococcus epidermidis*. Moreover, MgO NPs inhibited biofilm formation with minimal biofilm inhibitory concentrations against the aforementioned strains. For instance, Shankar et al. [151] biosynthesised MgO NPs conjugated with silk sericin and then evaluated its ability in antioxidant, antiaging and anti-biofilm activities. Three concentration levels of MgO NPs (i.e., 10, 20, and 40 μg/mL) were investigated against *B. cereus* and *P. aeruginosa*. Their evaluation showed that biofilm formation in both *B. cereus* and *P. aeruginosa* strains was significantly reduced when treated with MgO NPs. It has also been reported that the reduction of biofilm formation in *B. cereus* was significantly higher than that of *P. aeruginosa*. The results showed that the significant inhibition of *P. aeruginosa* and *B. cereus* biofilm formation by MgO NPs was due to a dose-dependent manner [151].

S. epidermidis is one of the infectious agents that cause biofilm formation in medical devices and implants [152]. As the concentration of MgO NPs increased, the growth rate of *S. epidermidis* decreased and the death rate increased. This increase also affects the adhesion and morphology of bacteria [143].

3.3. Antifungal Activity

In addition to antibacterial and antibiofilm properties, MgO NPs also show antifungal properties. The effects of MgO NPs on fungal pathogens and complex antifungal mechanisms have been reported in previous studies, including physical damage and interactions between NPs and fungal cells, cell membrane destabilisation, oxidative stress reactions, etc. [153–156]. The cell wall of the fungus has a negative electrostatic charge due to the presence of glycoprotein [155]. This causes electrostatic interaction and binding of positively charged MgO NPs to the cell wall, which leads to changes in the charge and zeta potential of the membrane and loss of membrane stability and strength [157]. Investigations on bacteria have shown that NPs can oxidise bacterial cell wall lipids by generating free radicals [158]. Meanwhile, the possible antifungal mechanism for biogenic MgO is schematically presented in Figure 8.

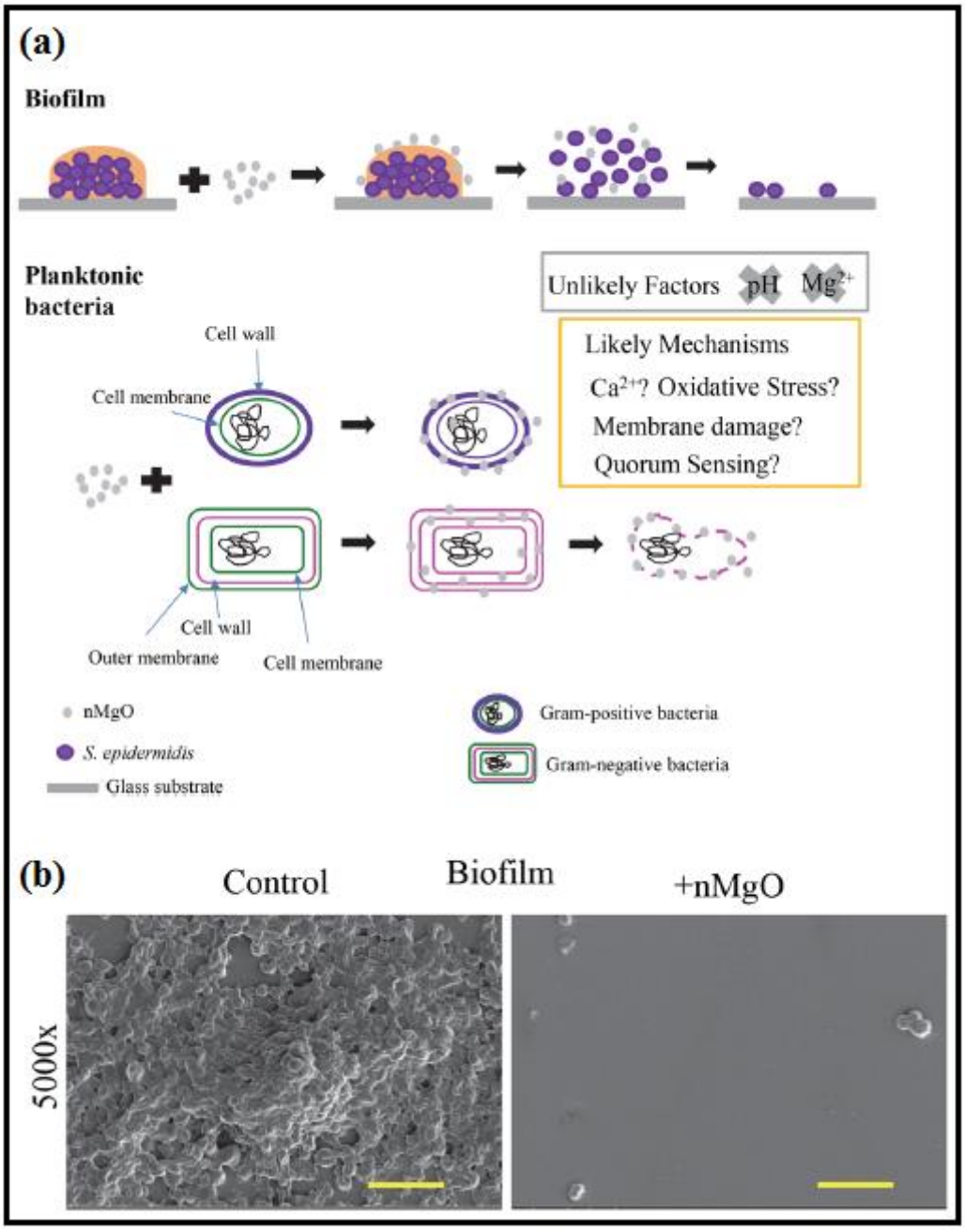

Figure 7. (**a**) A schematic illustration of the antibacterial and antibiofilm mechanisms of nMgO. Possible mechanisms of planktonic bacteria are included Ca^{2+} ions, oxidative stress, membrane damage, and quorum sensing, but alkaline pH 7 to 10 or concentration of Mg^{2+} ion from 1 to 50 mM have no inhibitory or destructive effects on *S. epidermidis*. (**b**) SEM images show disruption of *S. epidermidis* biofilm after 48 h of culture and additional 24 h of culture. Scale bar: 5 µm with a magnification of 5000×. Reprinted with permission from Ref. [143]. Copyright 2018, Springer Nature.

In an interesting study, Chen et al. [157] found the fungicidal effect of MgO NPs on *Phytophthora nicotianae* and *Thielaviopsis basicola*. They reported that MgO can inhibit fungal growth and spore germination after a direct interaction with fungal cells. In another report, Wani and Shah evaluated the antifungal properties of MgO and ZnO NPs on *Mucor plumbeus*, *Rhizopus stolonifer*, *Fusarium oxysporum* and *Alternaria alternata*. Their study exhibited the appropriate antifungal properties of MgO NPs at different concentrations, but the inhibition rate of spore germination was higher at higher MgO concentrations [160]. In this regard, for the first time, Abdel-Aziz et al. [103] synthesised MgO NPs using cell filtrate

of endobacterium *Burkholderia rinojensis*. Then, the antifungal activity of biogenic MgO NPs against *F. oxysporum* at different concentrations (i.e., 0.96, 1.92, 3.84, 7.68, and 15.36 µg/mL) was investigated. They found that MgO NPs at a concentration of 15.36 µg/mL have a strong inhibitory effect against fungal mycelium growth (Figure 9). A simple eco-friendly green synthesis of MgO NPs using *Pisonia alba* leaf extract was reported by Sharmila et al. [74]. The antioxidant activity of MgO NPs derived from *P. alba* leaf extract was evaluated by DDPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging and FRAP (ferric reducing antioxidant power) assay. The fungicidal activity of biosynthesised MgO NPs was also investigated against two fungal strains, *Aspergillus flavus* and *Fusarium solani*. The evaluations showed that the possible mechanism of the antifungal behaviour of MgO NPs is due to the electrostatic interaction between cell membrane proteins and MgO NPs. Based on the obtained results, it seems that MgO NPs strongly adsorbed on the microorganisms that produce ROS are absorbed inside the fungal cell, causing oxidative stress and leading to cell death.

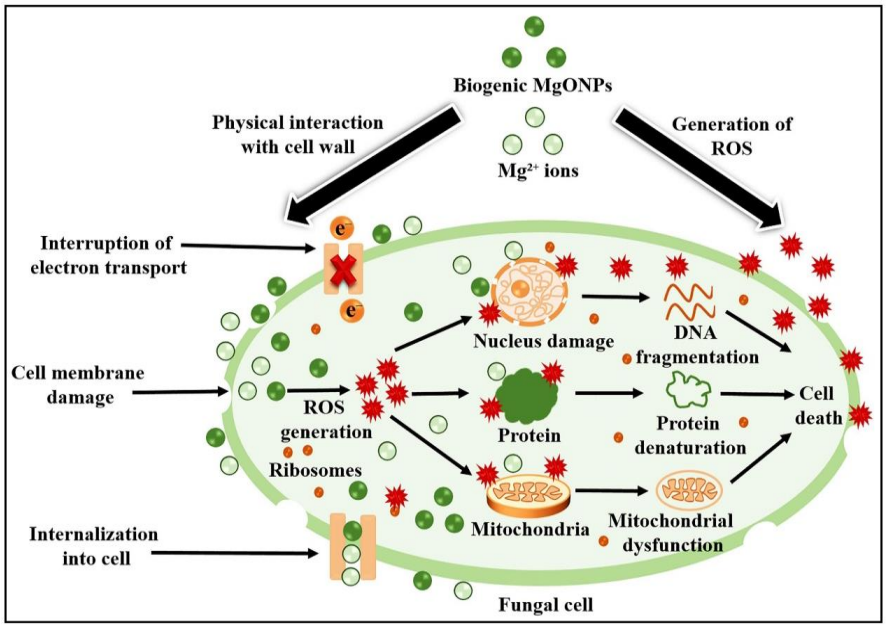

Figure 8. A schematic illustration of the antifungal mechanisms of MgO. Reprinted with permission from Ref. [159]. Copyright 2022, Elsevier.

In another study, Pavithra et al. [161] bioengineered 2D ultrathin sharp-edged MgO nanosheets using *Achyranthes aspera* via a green route without any surfactants. An in vitro antibacterial and antifungal assay for the 2D nanostructure of ultrathin MgO nanosheets mediated by *A. aspera* was investigated. Compared with the positive control of ciprofloxacin, MgO nanosheets exhibited significant antimicrobial activity in contact with both the bacterial and fungal strains. The key finding of this study supported the view that the antibacterial and antifungal potential of MgO nanostructures relies on the presence of oxygen vacancy on the surface of MgO nanosheets, which leads to lipid peroxidation and ROS production. This study indicated that the bacterial susceptibility of nanomaterials depends not only on the cell wall structures of Gram-positive and Gram-negative bacteria, but also on cellular enzymes and biochemical events. For this reason, biosynthesised MgO nanosheets show a better inhibition halo around bacterial strains compared with fungal strains. On the other hand, Podder et al. [162] investigated the effect of morphology and concentration on the intersection between the antioxidant and prooxidant activity of MgO nanostructures. In their study, three MgO nanostructures such as NPs, nanosheets and nanorods were synthesised. MgO nanorods produced the highest levels of $O_2^{\bullet -}$, while NPs scavenged $O_2^{\bullet -}$ to the highest extent (60%). These nanostructures exhibited the highest antibacterial (92%) and antibiofilm (17%) activity against *B. subtilis* ATCC 6633 in the dark.

Biofilm inhibition w similarly affected. The concentration-dependent cross-linking also showed morphology-independent DPPH scavenging behaviour at lower concentrations. Additionally, the nanosheets showed the highest protective ability against DPPH radicals due to their high surface area. In accordance with the aforementioned, it can be concluded that biogenic MgO NPs can be used as an efficient antifungal agent against various fungal strains, and the antifungal activity of green MgO NPs can be achieved mainly through two potential pathways: (1) Direct physical interaction of MgO NPs and Mg^{2+} ions with fungi can cause damage to their cell wall (electrostatic interaction); (2) ROS production causes oxidative stress, which leads to cell death.

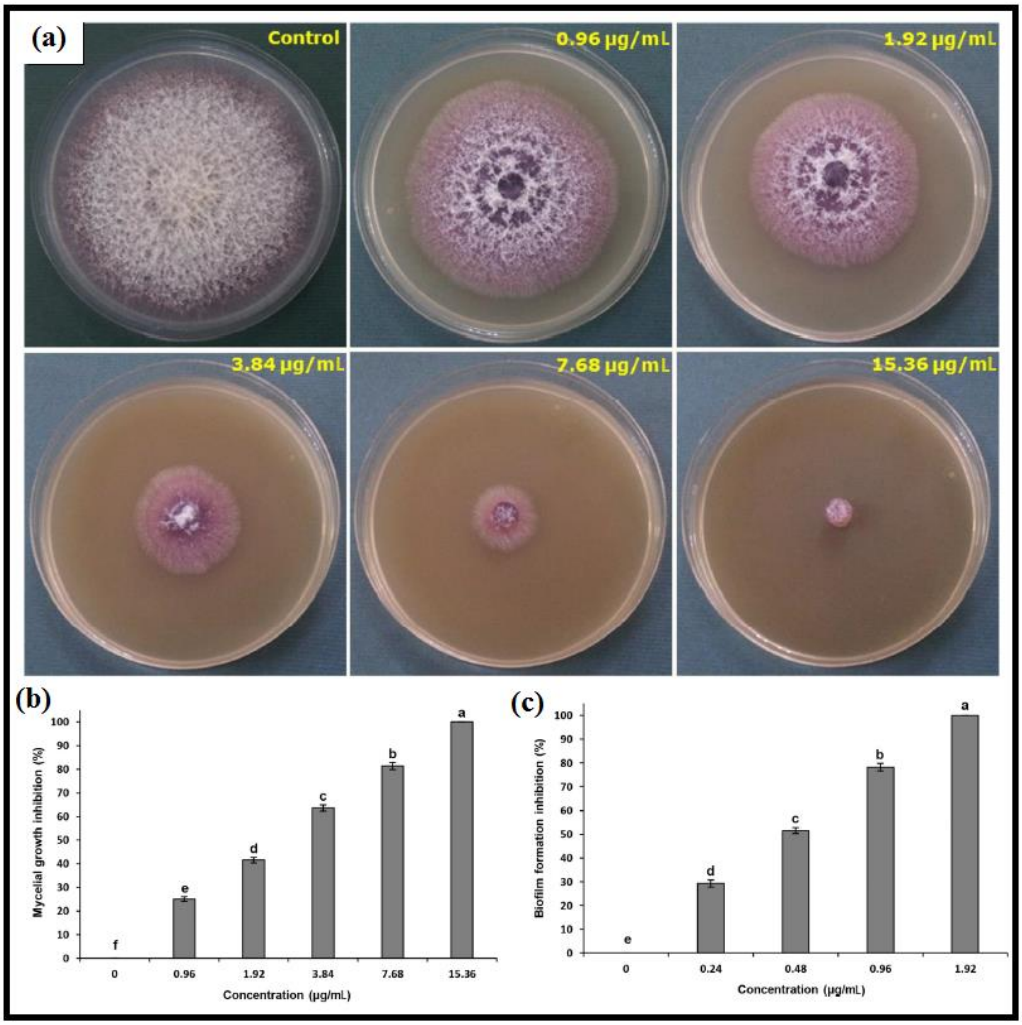

Figure 9. (**a**) Effect of *B. rinojensis* synthesised MgO NPs on mycelium growth of *F. oxysporum* f. sp. *lycopersici* at different concentrations. Effect of *B. rinojensis* synthesised MgO NPs on mycelial growth (**b**) and biofilm formation (**c**) of *F. oxysporum* f. sp. *lycopersici*. The letter indicates significant differences according to Tukey's HSD test at $p < 0.05$. Reprinted with permission from Ref. [103]. Copyright 2020, Elsevier.

4. MgO Nanocomposite

The use of MgO NPs in combination with other materials (e.g., Ag, Zn, chitosan, graphene, etc.) can improve the stability and antibacterial properties of the composite. It has been reported that bacterial growth can be more effectively inhibited by CS-ZnO nanocomposite films [163]. Additionally, the addition of MgO NPs to the chitosan matrix improves antibacterial, bioactivity and strength properties [164]. Various studies have investigated the properties and applications of MgO composites. For example, Yamamoto

et al. [165] investigated the effects of $CaCO_3$-MgO nanocomposites on plaque removal, which improves oral hygiene. In this study, 20 nm MgO crystallite dispersed in $CaCO_3$ grain was synthesised by thermal decomposition. In this study, the effect of the produced nanocomposite on Gram-positive and -negative bacteria was investigated and the appropriate antibacterial property of the produced composite was reported. In another study, Wang et al. [166] synthesised carboxymethyl chitosan (CMCS)−MgO nanocomposite for food packaging applications. They reported that the composite improved thermal stability and UV protection compared with pure CMCS. Additionally, it was reported that it showed high antibacterial activity against *Listeria monocytogenes* and *Shewanella baltica*.

In another report, Sabbagh et al. [167] introduced acrylamide-based hydrogel drug delivery systems for the release of acyclovir from MgO−hydrogel nanocomposites. In their study, acyclovir was loaded by soaking, and this system was used for vaginal delivery and release of the drug. Drug release was investigated in two different environments using PBS and aqueous solutions of simulated vaginal fluid, and the amount of released drug was determined using HPLC. The findings showed that the incorporation of MgO nanofillers can significantly reduce the initial burst release. This trend was attributed to the fact that MgO acted as an additional cross-linking agent. In an interesting study, Zhu et al. [168] synthesised Ag−MgO nanocomposites by loading Ag NPs on MgO NPs as an excellent antibacterial agent against *E. coli*. Antibacterial tests showed that the composite had more antibacterial activity compared with pure Mg and AgNPs alone, indicating a synergistic effect between Ag and MgO. ROS detection tests showed increased ROS production and antibacterial activity.

5. Conclusions and Prospects

MgO NPs as metal oxide NPs have special photocatalytic properties for antimicrobial, antifungal and antibiofilm applications. One of the problems in the use of chemical synthesis methods for MgO NPs is their low biocompatibility and the production of dangerous and harmful substances for the environment. Hence, due to the desire for better eco-friendly approaches, the knowledge of greener chemistry and the use of greener routes for the synthesis of various NPs are expanding. MgO NPs are biosynthesised in four general ways based on plants, fungi, bacteria and algae. Although the mechanisms behind the long-term toxicity, diffusion, uptake and excretion of these NPs are still not fully understood, their potential biological applications make them a promising candidate to replace chemically produced NPs in the coming years. These methods mainly depend on the metabolites produced by biological material and their extracts, which cause capping and stabilisation of the particles, and thus the formation of NPs. Many variables such as extraction ratio, temperature and pH have a significant effect on the surface area, size, stability and morphology of MgO NPs. Synthesised NPs from green methods are biocompatible and eco-friendly and can be a suitable approach for biological applications. The antimicrobial properties of these NPs are mainly related to ROS and cell wall or membrane degradation, although other processes such as electrostatic interaction and physical damage are also contributing factors. In general, the green synthesis of MgO NPs as biocompatible and eco-friendly materials can be suitable candidates for antipathogenic activity with optimal performance for biomedical applications. The purpose of this review is to understand the progress of green synthesis to prepare efficient MgO NPs for future biomedical and industrial applications. Genetically engineered plant sources can also be used to control the morphology, monodispersity and stability of NPs. In vitro or in vivo studies are needed for a more detailed investigation of the antioxidant potential of biogenic MgO NPs.

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