Green nanotechnology-based zinc oxide (ZnO) nanomaterials for biomedical applications: a review

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Abstract

The rise of nanotechnology has brought to the world a new potential and broader perspective of what humanity can achieve through material manipulation at the nanoscale. As a consequence, the use of different nanomaterials has revolutionized both the industrial and biomedical worlds. Metallic and metal-oxide nanostructures have shown great potential due to their high surface to volume ratio and high reactivity. Among them, zinc oxide (ZnO) has revealed wider applicability, including in nanomedicine, where ZnO nanomaterials have shown great potential leading to effective interactions with biological membranes and exhibiting antibacterial and/or anticancer behaviors. However, consistent with several other nanostructures, the synthesis of ZnO nanomaterials is not devoid of drawbacks, such as the production of harmful and toxic byproducts, the use of toxic reagents, the employment of expensive instruments, and the lack of biocompatibility, all of which need to be overcome before extensive use. As a solution, green nanotechnology has allowed the production of ZnO nanostructures using environmentally friendly and cost-effective methods, which are based on the use of living organisms, natural biomolecules and waste materials. Once produced, green-synthesized ZnO nanoparticles have shown enhancements in terms of their cytocompatibility and biomedical properties compared to their traditionally produced counterparts, becoming excellent antibacterial or anticancer agents. These ZnO nanoparticles have also proven to be valuable materials in combination with wound healing processes and biosensing elements in order to trace small amounts of biomarkers associated with different diseases. As a consequence, there is a synergy between green nanotechnology and ZnO nanomaterials, which is leading to an exciting flourishing in the field, presenting a wide variety of biomedical applications for these nanostructures. This review compares and contrasts recent approaches and examples of the use of green-synthesized ZnO nanomaterials with traditionally synthesized structures, demonstrating a remarkable potential for their use as a powerful biomedical agent.

1. Piezoelectricity at the nanoscale

Piezoelectricity, discovered by the Curie siblings, Jacques and Pierre, in 1880, is understood to be a reversible physical phenomenon since it has both direct and reverse piezoelectric effects. The direct piezoelectric effect is defined as the electric polarization produced by mechanical strain in crystals belonging to certain classes, with the polarization being proportional to the strain and altering its charge with it (Blushan 2012, Winter et al 2012). By contrast, the reverse (or inverse) piezoelectric effect is referred to as the straining of a
piezoelectric material when electrically polarized by an amount proportional to the polarizing field (Martin 1972). Therefore, piezoelectricity can be used to create mechanical action by applying an external voltage for sensors and actuators, or can produce a voltage by mechanical strain for energy conversion (Arnau and Soares 2008).

At the nanoscale level, alterations in size and shape occur due to the increased surface-to-volume ratio, which affects the physical properties of materials, including piezoelectricity (Agrawal and Espinosa 2011, Hadjesfandiari 2013, J. Zhang et al 2014, Nasajpour et al 2017). Indeed, atoms at the surface of a nanostructure have fewer ‘neighboring’ particles than the atoms in a bulky structure, which directly impacts their atomic polarization, and consequently the piezoelectric properties of nanomaterials (NMs). Furthermore, atoms at the surface are subject to surface stresses which in turn causes substantial strain, thereby leading to changes in the polarization of the surface even in the absence of any externally applied forces (Dai et al 2011).

Hence, extensive research has demonstrated that nanoscale piezoelectricity has substantial advantages over bulk piezoelectricity as the forces required to deform piezoelectric NMs are significantly lower compared to their bulk counterparts, with forces small enough to extract directly from natural sources (such as ambient noise, wind energy, body movements, and flowing water). Therefore, nanostructures can serve as building blocks for energy harvesting devices (R. Agrawal and Espinosa 2011, X. Wang 2012). Among these piezoelectric NMs, both zinc oxide (ZnO) and gallium nitride (GaN) materials with 3D wurtzite atomic structures are the most frequently produced and employed in a wide range of morphologies and sizes. Once characterized, these NMs are often used to build smart nanosystems, from nanosensors to nanogenerators, with a variety of applications in material and biomedical sciences (Yan and Jiang 2011, J. Zhang and Meguid 2017).

This review is focused on the use of ZnO, a well-known II–VI semiconductor with a large energy bandgap of 3.2 eV at the bulk scale. ZnO crystallizes into two possible crystalline structures, the hexagonal wurtzite or the cubic zincblende (figures 1(A) and (B)). These two structures have no inversion symmetry which consequently leads to a material displaying piezoelectric properties (Look 2001). The first paper discussing the piezoelectric properties of ZnO was published by Hutson from Bell Labs in 1960 (Hutson 1960). Then, publications experienced a hiatus in releasing more data about this material (between 1960 and 1990). It was not until the early 90s when the scientific community regained interest regarding its piezoelectric properties. Ever since, the interest from the scientific community for ZnO has only seen an upward trend, as can be seen in figure 1(C). In 2006, Wang and Song successfully converted nanoscale mechanical energy into electrical energy by using piezoelectric ZnO nanowire (NWs) arrays, with an efficiency estimated to be 17%–30%. Their pioneering approach revealed the potential of converting mechanical, vibrational, and/or hydraulic energy into electricity for powering nanodevices.
Despite the variety of applications for ZnO NMs, there is a greater diversity of processes that can be used for the production of ZnO nanoparticles (ZnONPs), from physicochemical approaches relying on the use of traditional physical and chemical synthesis to the employment of environmentally-friendly and cost effective green methods, which will be the focus of this review. After a detailed analysis of some of the most widely employed traditional synthetic approaches, the use of green nanotechnology as a tool for the production of ZnO nanostructures will be discussed, along with some of the most remarkable and novel examples of the biomedical employment of green-synthesized ZnONPs.

2. Traditional synthesis of ZnO nanomaterials

Metallic nanoparticles such as ZnONPs can be synthesized using two different traditional approaches: mecanochemical and chemical methodologies. Mecanochemical synthesis includes protocols that use laser ablation and high-energy ball milling techniques, while the chemical synthesis consists of sol–gel, hydrothermal, co-precipitation and microemulsion processes—all of which are considered traditional methods (Kolodziejczak-Radzimska and Jesionowski 2014, Shah et al 2015, Nadaroglu et al 2017, Rane et al 2018, Yadi et al 2018). The advantages and disadvantages of the traditional methods for the synthesis of ZnONPs along with some novel and remarkable examples are discussed in brief.

2.1. Sol–gel techniques

Sol–gel techniques are mostly used for the fabrication of metal oxide NPs (Kumar et al 2015), described as the transition of a sol (e.g. the solution containing inorganic metallic salts) gradually into a solid ‘gel’ phase, over a series of hydrolysis and polymerization reactions. Finally, the gel is subjected to the vaporization of the solvents and a heating process to obtain the final product (Sajjadi 2005, Sakka 2013). Figure 2(A) shows a schematic illustration of the sol–gel technique. The advantages of sol–gel processing include simplicity of the procedure, a suitable control on the chemical composition (Al Abdullah et al 2017), and the production of a very fine powder-like structure of ZnONPs (Carter and Grant, Norton 2007). On the other hand, this technique does have its shortcomings in possible shrinkage, cracking while drying, and an often difficult porosity control (Kumar et al 2015). Despite the drawbacks, this technique is one of the most widely used because of the feasibility of the protocol and the quick generation of the valuable material, often a reason why it is mentioned numerous times in relevant literature. For instance, Hasniadwani et al synthesized rod-shaped ZnONPs by a sol–gel method in the range of 81.28–84.98 nm, after mixing zinc acetate dehydrate (Zn(CH$_3$COO)$_2$·2H$_2$O) with ethanol used as a solvent (Hasniadwani et al 2016). Similarly, Jurablu et al generated ZnONPs by a sol–gel method with an average size of 28 nm and spherical morphology. In this process, an ethanol solution of zinc sulfate heptahydrate (ZnSO$_4$·7H$_2$O) was used in the presence of a diethylene glycol (C$_2$H$_{10}$O$_3$) surfactant (Jurablu et al 2015). Other examples include the production of ZnONPs with an average particle size of 12–30 nm using a methanol (CH$_3$OH) solution of Zn(CH$_3$COO)$_2$·2H$_2$O and ammonia (NH$_3$) (Al Abdullah et al 2017), and the production of spherical-shaped ZnONPs by a sol–gel method in the range of 50–60 nm using Zn(CH$_3$COO)$_2$·2H$_2$O as a precursor (Alwan et al 2015).

2.2. Hydrothermal technique

Hydrothermal processes take place in a specific closed reaction vessel commonly known as an autoclave, with high pressure and high temperature. The insoluble, or poorly soluble, materials under room temperature are dissolved and recrystallized under high pressure and a pre-set temperature (G. Yang and Park 2019). Different solvents can be used in such reactions: for instance, if water is employed, the process is known as a hydrothermal technique, while if organic solvents are used, such as ethanol or polyols, the approach is named as a solvothermal technique (Komarneni 2003, Li et al 2015). Figure 2(B) represents a schematic illustration of the hydrothermal technique. The advantages of hydrothermal techniques include high purity and a high degree of crystallinity of the product (Kolodziejczak-Radzimska and Jesionowski 2014), high control of the final nanostructure size, morphology and crystal phase (Li et al 2015), as well as minimum pollution owing to the closed system conditions. This is precisely why the approach is often considered an eco-friendly methodology, and why it is included as a green methodology for the production of ZnONPs. However, this technique is also prone to drawbacks, some of which include slow reaction kinetics at any given reaction temperature (Parhi et al 2008), need for expensive autoclaves, limitations in studying the reaction (for instance, the observation of the reaction process is not possible as the reactor cannot be kept open) (Sonawane et al 2018), and potential safety issues during the autoclave procedure (Rane et al 2018).
Figure 2. Schematic steps involved in the synthesis of metallic NPs such as ZnONPs using (A) sol–gel (Arciniegas-Grijalba et al 2017), (B) hydrothermal (N. Agrawal et al 2017), (C) co-precipitation (Sadraei 2016), (D) microemulsion, (E) laser ablation (Honda et al 2016), and (F) high-energy ball milling (Baheti et al 2012) techniques. Polyamide zinc oxide (PA-ZnO); neodymium-doped yttrium aluminum garnet (Nd:YAG).

As observed with the sol–gel approach, hydrothermal/solvothermal approaches are considered straightforward and easy to set-up. Notable examples include the work of Bharti and Bharati, who synthesized ZnONPs using a hydrothermal method, with a size range of 15.8–25 nm along with different shapes (Bharti and Bharati 2017). Additionally, Reddy et al synthesized hexagonal shaped ZnONPs comprised of cylindrical pores of diameters ranging from 9 to 12 nm using zinc nitrate hexahydrate (Zn(NO$_3$)$_2$·6H$_2$O) and sodium hydroxide (NaOH) as the starting precursors (Reddy et al 2015). Similarly, Wirunmongkol et al synthesized ZnONPs through a hydrothermal process using an autoclave unit, in which Zn(NO$_3$)$_2$·6H$_2$O and NaOH were added as the starting precursors. The fabricated NPs had the morphology of a short prism and flower-like shapes, sized between 30 and 80 nm in width and 0.5–1 µm in length (Wirunmongkol et al 2013).

2.3. Co-precipitation techniques
In this method, MNPs are fabricated by simultaneous nucleation, growth and finally agglomeration of the small nuclei. Figure 2(C) represents a schematic illustration of the co-precipitation technique. The advantages of this method include simplicity, no high temperature requirement, and overall energy efficiency (Rane et al 2018). Nevertheless, one of the major disadvantages of this method is that the fabricated NPs contain a large amount of adsorbed water molecules which may affect their properties (Pudovkin et al 2018). Additional drawbacks include challenges in batch-to-batch reproducibility (Rane et al 2018), poor particle size distribution, and extensive agglomeration (Wang et al 2013, Mostafavi et al 2015). Despite this, notable examples include the work of Costenaro et al who synthesized spherical ZnONPs with a particle size ranging...
from 2 to 10 nm through a co-precipitation method using a solution of zinc acetate in methanol (Costenaro et al 2013), while, Purwaningsih et al synthesized ZnONPs by a co-precipitation method using zinc acetate dihydrate, hydrochloric acid, and ammonia as reactants. The morphology of the ZnONPs was found to be approximately a pseudo-spherical shape with the average particle size in the range of 11–20 nm (Purwaningsih et al 2016). Likewise, Adam et al synthesized uniform ZnONPs using a co-precipitation method with an average size of around 140 nm (Adam et al 2018).

2.4. Microemulsion technique
In this method, the collision of water droplets in a microemulsion environment led to a fast reactant exchange, resulting in a precipitation reaction in nanodroplets and consequent nucleation growth, with NPs stabilized by the presence of a surfactant. Figure 2(D) shows a schematic illustration of the microemulsion technique. Simplicity, thermodynamic stability and minimal agglomeration are the advantages of this method. Unfortunately, microemulsion processes suffer from disadvantages such as the impact of external factors like temperature and pH on the microemulsion stability and the constant need for high concentrations of surfactants and/or cosurfactants that may generally cause irritation (Rane et al 2018). In the literature, some examples include the work of Wang et al who synthesized ZnONPs from microemulsions in a microchannel reactor system with an average size of 16 nm. The ZnONPs were obtained by drying the solid product at 130 °C for 2 hr, and consequent calcination at 550 °C for 3 hr (Wang et al 2014). Similarly, Li et al synthesized ZnONPs by a simple microemulsion process with different morphologies including columnar, needle-like, and spherical under 100 nm (X. Li et al 2009).

2.5. Laser ablation techniques
In a conventional laser ablation technique, the removal of metallic ions from a metal surface takes place while immersed in a small volume of liquid, such as methanol, ethanol, distilled water, using a laser beam. Figure 2(E) represents a schematic illustration of the laser ablation technique. The advantages of this method are simplicity and a highly safe protocol from the environmental perspective, leading to a method that is both efficient and easy to perform (Mintcheva et al 2018). Nonetheless, the role of pyrolysis byproducts (as a consequence of the laser ablation in the presence of organic compounds) on the stability of the colloidal system is not clear and needs to be elucidated (Amendola and Meneghetti 2009). Notable examples of this technique include the work of Al-Dahash et al who synthesized ZnONPs by laser ablation in a NaOH aqueous solution (with a particle size ranging from 80.76 to 102.54 nm) and spherical morphology (Al-Dahash et al 2018). Besides, Farahani et al synthesized ZnONPs by laser ablation from a zinc target in methanol and distilled water solution (with almost spherical morphology) and a particle size ranging from 1 to 30 nm (Farahani et al 2016). Similarly, Mintcheva et al reported the synthesis of laser-ablated rod-shaped ZnONPs with an average width of 30 nm, and length in the range of 40–110 nm (Mintcheva et al 2018).

2.6. High-energy ball milling techniques
A high energy ball milling technique is employed to produce fine metal NPs using stiff balls in a high-energy shaker mill (Hodaei et al 2015). Figure 2(F) shows a schematic illustration of the high-energy ball milling technique, whose main advantage is the significant potential for scale up in order to produce large quantities of material. On the other hand, the disadvantages of this method include the possibility of contamination from milling balls and/or the atmosphere (Edelstein 2001, L. Yang 2015), as well as the formation of irregular shaped NPs (Piras et al 2019). Despite this, important examples include the work of Prommalikit et al who applied commercial grade ZnO powder with an average particle size of 0.8 µm as the starting material to synthesize ZnONPs by the high-energy ball milling technique. The final particle size after the milling process was found in the range of 200–400 nm (Prommalikit et al 2019). Similarly, Mohammadi et al reported the synthesis of rod-shaped ZnONPs by a high-energy ball milling process in the range of 20–90 nm (Mohammadi et al 2015). Likewise, Salah et al used a microcrystalline powder of ZnO to produce ZnONPs by the aforementioned high energy ball milling process. The samples were ball milled for 2, 10, 20, and 50 h. The results showed a time-dependent particle size. The more the ball milling time, the less the particle size. The sample milled for 50 h showed spherically shaped ZnONPs with a particle size of around 30 nm (Salah et al 2011).

3. Green nanotechnology
As previously discussed, there are several traditional methods for the preparation of ZnONPs, each with different obtainable shapes, sizes and subsequent applicability. Most of these synthetic processes are often accompanied by drawbacks, some of which have a clear environmental implication, potentially compromising their biomedical use once they contact biological tissues inside the human body. As it was
explained, the most common synthesis methods include both the solvothermal and sol–gel approaches, in which a Zn$^{2+}$ precursor is precipitated as ZnO in the presence of surfactants or precipitating agents (Kolodziejczak-Radzimska and Jesionowski 2014). These chemical substances usually include triethanolamine (TEA) (P. Li et al. 2005) and CTAB (Yu de Wang et al. 2002), each of them being associated with potential toxic effects against the environment and living organisms (Stott et al. 2004, Alkilany et al. 2009, Libralato et al. 2010).

Over the past 15 years, attempts to address this issue have seen green nanotechnology emerge to synthesize NMs by using methods that either reduce or eliminate harmful substances used as solvents, reducing, or capping agents (Karn and Wong 2013). The use of living organisms, biomolecules isolated from them, waste materials and environment-friendly physical methods has led to a wide variety of processes which enable the generation of NMs in a completely green manner. Additionally, green nanotechnology also targets the removal of toxic by-products from the synthesis process, as well as the partial or total elimination of using extreme reaction conditions or harmful chemicals, which might leave a trace of their toxicity on the final applications of the nanostructures.

Consequently, the synthesis of ZnONPs using green nanotechnology principles has led to the appearance of three main types of processes, which can be classified as follows: (a) microbiological-mediated biosynthesis, often employing bacteria or bacterial extracts; (b) natural extract-mediated biosynthesis, with raw materials mainly from plants and different algae; and (c) biomolecule/biopolymer-mediated biosynthesis, using biomolecules isolated from living organisms, such as carbohydrates or structural biopolymers (Kalpana and Devi Rajeswari 2018, Singh et al. 2018, Jin and Jin 2019, Zhu et al. 2019). The main advantage of the former two methods is that the raw materials used are naturally rich in amino, carboxyl and hydroxyl groups that are often used as stabilizing agents in aqueous medium, triggering the formation of NMs (Jafarirad et al. 2016, D. Sharma et al. 2016, Bayrami et al. 2018, 2019). Moreover, the phytochemicals present in algae and plant extracts enhance the antibacterial, antifungal and anticancer properties of green-synthesized nanostructures (Gunalan et al. 2012, Crua et al. 2019); hence, leading to a successful generation of a synergetic effect where both the biomedical properties of the raw materials and the metallic nanoparticles (MNPs) are combined in a single nanostructure that can be used to target different biomedical approaches.

4. Biomedical applications of green synthesized ZnONPs

The combination of green nanotechnology and ZnONPs has led to significant enhancements in terms of the biomedical use of these NMs. While using natural raw materials and living organisms as reducing and capping agents for the formation of ZnONPs, they are able to exert better biocompatibility and responses to their interactions with biological tissue, which substantially enhances their performance. As a consequence, green-synthesized ZnONPs are considered effective and viable NMs that can be used to target bacterial infections, destroy the membrane of cancer cells or deliver different compounds to diseased tissue, as well as to measure the concentrations of different biomarkers inside the body.

In the following section, the biomedical applications of green-synthesized ZnONPs will be discussed in terms of their use: antibacterial, anticancer, tissue engineering and biosensing agents, showing some novel and remarkable examples which highlight the improved performance of green-produced NMs in relative comparison to their traditionally-produced counterparts. The article summarized below provides complete physicochemical characterization of the different nanostructures in terms of composition, size and morphology (through the employment of different characterization techniques such as transmission electron microscopy (TEM), scanning electron microscopy (SEM), energy-dispersive x-ray spectroscopy (EDX), x-ray photoelectron spectroscopy (XPS) or x-ray diffraction analysis (XRD) among others), as well as the potential of these materials to serve as biomedical tools.

4.1. Antibacterial applications of green-synthesized ZnONPs

A large array of diseases caused by bacterial pathogens and the origination of multidrug resistance provokes the need for developing new vectors or novel drug molecules for effective drug delivery and thus, better treatments. Nanotechnology has emerged as a novel approach to fight bacterial diseases. The use of NPs as an antibacterial agent has given rise to rich literature on the subject, with excellent studies showing how MNPs like silver (Ag), gold (Au), copper (Cu) or iron (Fe), and metal oxide nanoparticles (MoNPs) like ZnO, copper oxide (CuO), titanium oxide (TiO$_2$) and iron oxide (FeO) NPs, can be used for the effective treatment of pathogenic bacterial diseases (Chwalibog et al. 2010, Ivask et al. 2014).

The antimicrobial properties of ‘NMs’ arise from their inherent properties, such as high reactivity and large surface area to volume ratio, which allows them to bond to a large number of ligands on the NPs surface and hence, with receptors present on the bacterial surface (Brown et al. 2018, Medina Cruz et al. 2019, ...
Medina-Cruz et al, 2019, Lomelí-Marroquin et al, 2019). Among all the NMs, the effect of ZnONPs is remarkable when combined with green synthesis approaches. Different natural raw materials, such as bacteria or plants, have been used for the production of ZnONPs which show a powerful antimicrobial effect. However, among all the green synthesis approaches, plant-mediated or phytochemical production flourished in the literature, due to the wide range of raw materials, and the possibility of generating a synergetic nanostructure combining the antimicrobial properties of both the plant extract and the NPs themselves (Jalal et al, 2010, Gunalan et al, 2012, Agarwal et al, 2017).

Several studies have reported that ZnONPs are effective at inhibiting both Gram-positive and -negative bacteria. While the crystalline structure and particle shape have a very small effect, the concentration of NPs highly impacts the antibacterial behavior of the structures. Additionally, a number of mechanisms have also been proposed to interpret these experimental observations, including the production of reactive oxygen species (ROS) due to the presence of the NPs, damage of the membrane cell wall through adhesion on the cell membrane, penetration through the membrane cell wall and cellular internalization of the NPs. However, the dominant mechanisms have not been identified. Importantly, there is no substantial discussion about the green production of ZnONP suspensions where the presence of additives such as stabilizers and capping agents might affect the interaction between the NPs and the cells (Li et al, 2009, Liu et al, 2009, L. Zhang et al, 2010, Emami-Karvani and Chehrazi, 2011).

In the following section, remarkable examples of both bacteria and plant mediated ZnONPs are discussed, focusing on the improvements of these nanostructures compared to their physiochemically or traditionally produced NPs.

4.1.1. Microorganism-mediated synthesis of ZnONPs with antimicrobial properties
Bacteria were among the first organisms used for the production of NPs due to their feasibility of isolation and manipulation and the presence of natural mechanisms of detoxification of metal ions (Medina Cruz et al, 2018). Additionally, bacteria offer quick growth which allows for the easy scale-up of NP production (Jayaseelan et al, 2012). One of the most relevant and recent examples was presented by Saravanan et al who generated anisotropic ZnONPs with a defined shape and size using Bacillus megaterium cell free extracts as unique reducing and capping agents from Zn(NO$_3$)$_2$ as the raw material. The UV spectrum of the ZnONPs displayed the surface plasmon resonance (SPR) peak at 346 nm (which is a characteristic of these nanostructures), while the field emission scanning electron microscope (FESEM) analysis showed mostly rod and cubic shaped ZnONPs with a diameter ranging between 45 and 95 nm. The multidimensional effect of the NPs on _H. pylori_ strains was assessed, resulting in the inhibition of bacterial growth at a concentration range of 16–17 µg ml$^{-1}$. The authors concluded that the potential inhibitory mechanism could be explained by ROS released on the surface of the NPs, which bond to the bacterial surface and kills the bacteria through electrostatic forces. On the other hand, it was also hypothesized that free Zn$^{2+}$ partially contributed an antimicrobial effect through the mechanical contact between the bacteria and ZnO rod surface. Besides, the biosafety profile of the NPs was studied using normal human mesenchymal stem cells (hMSC) with no significant toxicity to the mammalian cells at a concentration equal to and below 12.5 µg ml$^{-1}$ (Saravanan et al, 2018). Similarly, Abinaya et al demonstrated a novel and effective approach to synthesize ZnONPs using the exopolysaccharides (EPS) from the probiotic strain Bacillus licheniformis Dahb1. The EPS acted as a reducing and stabilizing agent for the formation of NPs by a co-precipitation method, which revealed a crystalline structure and SPR centered at 375 nm, with a hexagonal shape and a size range between 10 and 100 nm. The antibacterial activity of the ZnONPs was demonstrated at a concentration of 100 µg ml$^{-1}$, which was significantly proven to inhibit the effective growth of several bacterial strains, such as _P. aeruginosa_, _Proteus vulgaris_, _Bacillus subtilis_, and _Bacillus pumilus_. Light microscopy and confocal laser scanning microscopy provided evidence that the antibiofilm activity of the NPs was higher against Gram-negative bacteria over Gram-positive bacteria (Abinaya et al, 2018).

4.1.2. Plant extract-mediated synthesis of ZnONPs with antimicrobial properties
Inarguably, the use of plants for the production of ZnONPs is among the most widely employed approaches for NP production (Senthilkumar and Sivakumar, 2014, Hussain et al, 2019, Ruddaraju et al, 2019), due to the accessibility of a huge variety of plant species and the formation of a synergetic effect between the antibacterial activity of the plants extracts and the NPs themselves (figure 3 and table 1) (Khalil et al, 2017, Akhter et al, 2018, Gupta et al, 2018, Cheminguil et al, 2019).

An interesting approach was shown by Suresh et al when they synthesized ZnONPs using _Cassia fistula_ plant extracts as capping agents, composed of reducing components such as polyphenols (11%) and flavonoids (12.5%), which allowed for easy production of ZnONPs with sizes between 5 and 15 nm and a hexagonal wurzite structure (with a clear absorption band at 370 nm), assigned to the intrinsic band-gap absorption of ZnO. Significant antioxidant activity was exhibited by NPs through scavenging of
Figure 3. Mechanisms for the formation of plant-mediated ZnONPs. (A) Schematic diagram for the biosynthesis of ZnO NPs using the leaf extract of Azadirachta indica (Bhuyan et al 2015); (B) schematic diagram of Fe–ZnO photocatalytic mechanisms on palm oil mill effluent POME photodegradation and antimicrobial activities (Chai et al 2019); and (C) synthesis of ZnO NPs from the Sechium edule leaf extract (Elavarasan et al 2017). Pam oil mill effluent (POME).

1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radicals, while excellent bactericidal activity was observed toward Klebsiella aerogenes, E. coli, Plasmodium desmolyticum and S. aureus (D. Suresh et al 2015). Similarly, the biological production of ZnONPs from zinc acetate was recently demonstrated using the flower extract of Trifolium pratense as a unique reducing and capping agent. The prepared ZnONPs, with a size range of 60–70 nm, showed antimicrobial efficacy against clinical and standard strains of S. aureus and P. aeruginosa, as well as a standard strain of E. coli. The inhibitory effect of ZnONPs increased with a rise in concentration, with comparable results to the ones obtained with the antibiotic gentamicin. However, the ZnONPs rendered a better antimicrobial effect against Psudomonas aeruginosa than gentamicin (Dobrucka and Długaszewska 2016). Alternatively, an aqueous extract of Boerhavia diffusa leaves was employed to generate stable ZnONPs with an average size of 140 nm. The antibacterial and antibiofilm properties were evaluated showing a significant antibacterial effect towards Methicillin resistant S. aureus (MRSA) strains (Joseph et al 2016).

In a different report, spherical ZnONPs were synthesized by an eco-friendly green combustion method using a citrate containing Artocarpus gomezianus fruit extract. TEM studies demonstrated that the particles
Table 1. Antibacterial applications of green-synthesized ZnONPs.

| Platform          | Raw material                                                                 | System | Size         | Targeted bacteria                                      |
|-------------------|-------------------------------------------------------------------------------|--------|--------------|--------------------------------------------------------|
| Bacteria-mediated | Bacillus megaterium (NCIM 2326) cell free extract (Saravanan et al 2018) | ZnONPs | 45–95 nm     | • H. pylori                                            |
|                   | *Bacillus licheniformis* Dabh1 exopolysaccharides (EPS) (Abinaya et al 2018) | ZnONPs | 10–100 nm    | • P. aeruginosa • Proteus vulgaris • Bacillus subtilis • Bacillus pumilus |
| Plant-mediated    | Cassia fistula plant extract (D. Suresh et al 2015)                          | ZnONPs | 5–15 nm      | • Klebsiella aerogenes • E. coli • Plasmodium desmolyticum • S. aureus |
|                   | *Trifolium pretense* flower extract (Dobrucka and Długaszewska 2016)         | ZnONPs | 60–70 nm     | • P. aeruginosa • E. coli • S. aureus                |
|                   | Boerhavia diffusa leaves (Joseph et al 2016)                                 | ZnONPs | 140 nm       | • MRSA                                                |
|                   | *Artocarpus gomezianus* fruit extract (Anitha et al 2018)                     | ZnONPs | 39, 35, 31 nm prepared with 5, 10 and 15 ml of 10% extract | • S. aureus                                       |
|                   | Securin edule leaf extract (Elavarasan et al 2017)                           | ZnONPs | 30–70 nm     | • Bacillus subtilis • Klebsiella pneumonia            |
|                   | Azadirachta indica (Neem) leaf extract (Bhuyan et al 2015)                    | ZnONPs | 9.6–25.5 nm  | • Streptococcus pyogenes • E. coli • S. aureus       |
|                   | Azadirachta indica (Neem) extract (Madan et al 2016)                          | ZnONPs | 9–40 nm      | • Klebsiella aerogenes • S. aureus • E. coli • P. aeruginosa |
|                   | Acalypha indica leaf extract (Karthik et al 2017)                            | ZnONPs | 20 nm        | • E. coli • S. aureus                                |
|                   | Tabernaemontana divaricata green leaf extract (Raja et al 2018)               | ZnONPs | 20–50 nm     | • E. coli • S. aureus • Salmonella paratyphi         |
|                   | Aqueous leaf extract of *Laurus nobilis* (Vijayakumar et al 2016)             | ZnONPs | 47.27 nm     | • P. aeruginosa • S. aureus                          |
|                   | *Ruta graveolens* aqueous stem extract (Lingaraju et al 2016)                 | ZnONPs | 28 nm        | • Klebsiella aerogenes • P. aeruginosa • E. coli • S. aureus |
|                   | *Aristolochia indica* extract (Steffy et al 2018b)                           | ZnONPs | 22.5 nm      | • Multi-drug Resistant Organisms (MDROs) isolated from pus samples of DFU patients |
|                   | Aqueous extracts of garlic (*Allium sativum*), rosemary (*R. officinalis*) and basil (*Ocimum basilicum*) (Steffy et al 2018b) | ZnONPs | 14 and 27 nm | • S. aureus • Bacillus subtilis • L. monocytogenes • E. coli • Salmonella typhimurium • P. aeruginosa |
|                   | Bauhinia tomentosa leaf extract (G. Sharmila et al 2018)                      | ZnONPs | 22–94 nm     | • E. coli • P. aeruginosa                             |
were highly uniform, spherical in shape and loosely agglomerated, with sizes of 39, 35, and 31 nm for the ZnONPs prepared with 5, 10, and 15 ml of 10% fruit extract, respectively. The antimicrobial effect was evaluated through the zone of inhibition method, showing that the NPs exhibited significant antibacterial activity against *S. aureus* at a concentration range of 10–500 µg ml$^{-1}$ (Anitha *et al* 2018). Another example reported the use of *Sechium edule* leaf extracts as a raw material, producing NPs with a specific bandgap at 362 nm, spherical-shape and a size between 30 and 70 nm. Furthermore, compared with chemically synthesized ZnONPs, the biosynthesized NPs showed significant cytotoxicity when cultured with *Bacillus subtilis* and *Klebsiella pneumonia* at concentrations of 10–40 µg ml$^{-1}$. Additionally, it was shown that the antibacterial behavior could be due to the chemical interactions between hydrogen peroxide and the membrane proteins, or between other chemical species produced in the presence of ZnONPs and the outer lipid bilayer of the targeted bacteria (Elavarasan *et al* 2017).

Another interesting result was found in the low-cost and green synthesis of ZnONPs using an *Azadirachta indica* (Neem) leaf extract, producing pure, predominantly spherical NPs with a size ranging from 9.6 to 25.5 nm. The antibacterial activity of the samples was determined towards *S. aureus*, *Streptococcus pyogenes* and *E. coli* using the shake flask method, with results revealing that the bacterial growth decreased with an increased concentration. In addition, Gram-positive bacteria seemed to be more sensitive to the NPs than Gram-negative (Bhuyan *et al* 2015). Similarly, a low temperature solution combustion mechanism was recently reported for the production of ZnONPs using Neem extracts. The NPs revealed a pattern which confirmed a hexagonal wurtzite structure, while SEM images indicated the transformation of mushroom-like hexagonal disks to bullets, buds, cones, bundles and closed pinecone structures all over the samples, with an increase in the concentration of the neem extract and sizes between 9 and 40 nm. The antibacterial studies indicated that the ZnONPs had a significant antibacterial activity on *Klebsiella aerogenes* and *S. aureus*, but not against *E. coli* and *P. aeruginosa*. Further, the NPs exhibited significant antioxidant activity against scavenging DPPH free radicals (Madan *et al* 2016). In addition, Karthik *et al* produced ZnONPs using an *Acalypha indica* leaf extract as a unique reducing and capping agent from zinc acetate. The prepared NPs were calcined at three different temperatures (100, 300, and 600 °C) and the results showed that those NPs calcined at 600 °C exhibited a higher surface area (230 m$^2$ g$^{-1}$) and smaller particle size (around 20 nm), proving to be effective against *E. coli* and *S. aureus* at a concentration of 100 mg ml$^{-1}$. The study concluded that optimizing the production of ZnONPs by varying calcination temperature may render an effective strategy to produce favorable antibacterial agents employing plants as raw materials (Karthik *et al* 2017). Alternatively, Raja *et al* reported the green synthesis of 20–50 nm spherical ZnONPs using an aqueous extract of a *Tabernaemontana divaricata* green leaf. The FTIR analysis showed that the NPs were stabilized through the interactions of steroids, terpenoids, flavonoids, phenyl propanoids, phenolic acids and enzymes present in the leaf extract. On the other hand, the NPs showed antibacterial activity against bacterial strains of *Salmonella paratyphi*, *E. coli*, and *S. aureus* (Raja *et al* 2018).

### Table 1. Antibacterial applications of green-synthesized ZnONPs. (Continued.)

| Platform | Raw material System | Size | Targeted bacteria |
|----------|---------------------|------|------------------|
| *Ulva lactuca* seaweed extract (Ishwarya *et al* 2018) | ZnONPs | 10–50 nm | • *Bacillus licheniformis*  
• *Bacillus pumilis*  
• *E. coli*  
• *Proteus vulgaris* |
| *Amaranthus spinosus* leaf extract (Aiswarya Devi *et al* 2017) | Undoped and Fe-doped ZnONPs | 243 nm undoped/197 nm 1%-Fe-ZnONPs | • *E. coli*  
• *Bacillus safensis* |
| *Hibiscus rosa-sinensis* leaf extracts (Chai *et al* 2019) | Fe-doped ZnONPs | 15–170 nm | • *E. coli* |
| *Gymnema sylvestre* (*G. sylvestre*) leaves extract (Karthikeyan *et al* 2019) | Lanthanum, cerium and neodymium doped ZnONPs | 138 nm, 52 nm, 59 nm, and 63 nm for undoped, La, Ce and Nd-doped | • *S. aureus*  
• *Streptococcus pneumonia*  
• *Klebsiella pneumoniae*  
• *Shigella dysenteriae*  
• *E. coli*  
• *P. aeruginosa*  
• *Proteus vulgaris* |
The aqueous leaf extract of Laurus nobilis was used for the co-precipitation synthesis of ZnONPs with a crystalline and flower like structure, which showed a hexagonal wurtzite structure with a mean particle size of 47.27 nm. The antibacterial activity of the NPs was greater against S. aureus than P. aeruginosa at a range of concentrations between 25 and 75 µg ml$^{-1}$, while the cytotoxicity studies revealed that NPs showed no effect on normal murine RAW264.7 macrophage cells; and hence, becoming biocompatible at the same range of concentrations (Vijayakumar et al 2016). Similarly, Lingaraju et al employed an aqueous stem extract of Ruta graveolens as a unique reducing agent for the formation of ZnONPs with sizes around 28 nm, alongside a hexagonal phase with a wurtzite structure. Significant antibacterial activity was observed on S. aureus and P. demolyticum and moderate activity was observed on K. aerogenes and E. coli, with concentrations of 200 and 400 µg/well, determined by the agar well diffusion method. Furthermore, the ZnONPs effectively inhibited the scavenging of DPPH free radicals (as an IC50 value of 9.34 mg ml$^{-1}$ suggests) (Lingaraju et al 2016). More recently, the bacterialic effects of ZnONPs generated from Aristolochia indica were evaluated against Multi-drug Resistant Organisms (MDROs) isolated from the pus of patients attending a tertiary care hospital in South India. The ZnONPs, with a size of 22.5 nm and a zeta potential of $-21.9 \pm 1$ mV, exhibited remarkable bacterialic activity with MIC/MBC ranging from 25 to 400 µg ml$^{-1}$ with a significant reduction in viable counts from 2 h onwards. Furthermore, the protein leakage and flow cytometric analysis confirmed that the bacterial death was associated with the presence of the NPs in bacterial media (Stefy et al 2018b).

Another interesting study presented the production of ZnONPs by co-precipitation using aqueous extracts of garlic (Allium sativum), rosemary (Rosmarinus officinalis), and basil (Ocimum basilicum). The X-ray diffraction studies revealed that all ZnONPs had a hexagonal wurtzite structure with a particle size between 14 and 27 nm, the variations of which were dependent on the synthesis method and the type of extracts.

Importantly, the green synthesized ZnONPs showed good bacterialic activity against S. aureus, Bacillus subtilis, L. monocytogenes, E. coli, Salmonella typhimurium, and P. aeruginosa bacterial strains at a standard concentration of 100 µg ml$^{-1}$. Moreover, the NPs were found to exhibit enhanced antibacterial and antioxidant activities as compared to chemically-produced ZnONPs (Stan et al 2016). More recently, a facile and eco-friendly synthesis of ZnONPs employing a Bauhinia tomentosa leaf extract was reported by Sharmila et al with a hexagonal morphology observed and a size range of 22–94 nm. The NPs showed significant antibacterial activity against P. aeruginosa and E. coli. The results of the study demonstrated that the leaf extract contained phytochemicals, such as alkaloids, terpenoids, flavonoids, tannins, carbohydrates, and sterols, which were utilized as unique reductant agents for the generation of the ZnONPs, revealing no need of artificial or chemical agents to stabilize the nanostructures, in advantage to traditional methods (G. Sharmila et al 2018). In a similar manner, the use of a Ulva lactuca seaweed extract as a reducing and capping agent was postulated as an efficient method for the production of ZnONPs with average crystallite sizes between 10 and 50 nm, as well as excellent bacterialic activity was shown by the NPs on Bacillus licheniformis, Bacillus pumilis, E. coli, and Proteus vulgaris bacteria at a concentration of 50 µg ml$^{-1}$, along with high antibiofilm potential (Ishwarya et al 2018).

Intriguingly, plant extracts can be used for the successful production of bimetallic nanostructures. For instance, in 2017, an innovative study showed the production of both undoped and Fe-doped ZnONPs, synthesized using Amaranthus spinosus leaf extracts as a unique reducing agent. The results revealed that the ZnONPs were rod shaped with hexagonal phase structure and crystal size of 243 nm and 197 nm for undoped and 1%-Fe doped ZnONPs, respectively. Further, the authors demonstrated that the dissolution and aggregation decreased with Fe doping of NPs under conditions that are difficult using traditional synthesis. The antibacterial activity of NPs was tested against E. coli and Bacillus safensis using disc diffusion, minimum inhibitory concentration, and growth curve methods, revealing a powerful bacterialic activity of Fe-doped ZnONPs. This effect was more prominent when cultured with E. coli than with Bacillus safensis bacteria, when compared to undoped ZnONPs, which showed a reduced inhibitory effect. Additionally, the cytotoxicity of the NPs was studied against MCF-7 cells by an MTT assay, with IC50 values for undoped and 1 wt% Fe-doped ZnONPs of 400 and 600 µg ml$^{-1}$, respectively. Therefore, the cell viability with Fe-doped ZnONPs was higher than that of undoped ZnONPs, revealing that the doping of these nanostructures helped enhance their biomedical applications (Aiswarya Devi et al 2017). Similarly, the green synthesis of magnetic Fe-doped ZnONPs was developed using Hibiscus rosa-sinensis leaf extracts, with sizes ranging between 15 and 170 nm. While the FTIR analysis confirmed the presence of phytochemicals in the leaf extracts (which helped the formation of NPs), the ferromagnetic behavior was assessed by a magnetization study. After characterization, the NPs were tested as antimicrobial agents towards E. coli, demonstrating excellent antibacterial activity, as compared to those made of pure ZnO and commercial ZnO (Chai et al 2019). Very recently, another interesting report showed the production of ZnONPs doped with rare earth metals (REM) such as lanthanum (La), cerium (Ce) and neodymium (Nd), synthesized by Gymnema sylvestre leaf extracts. The average particle size was observed to be 138 nm, 52 nm, 59 nm, and 63 nm for undoped, La, Ce, and Nd-doped samples, respectively. The synthesized NPs samples were tested against clinical pathogens such as...
Figure 4. Common anticancer mechanisms of green-synthesized ZnONPs. Possible mechanisms underlying the cytotoxic activities leading to cell death as caused by La doped ZnONPs (Karthikeyan et al 2019); (B) The design of DOX-FA-ZnO NS as a functional drug delivery system for combined chemo-photothermal targeted therapy of breast carcinoma (Vimala et al 2017).

Zinc oxide nanostructures (ZnO NS); polyethylene glycol (PEG); folic acid (FA); doxorubicin (DOX); near-infrared (NIR); lanthanum (La).

S. aureus, Streptococcus pneumonia, Klebsiella pneumoniae, Shigella dysenteriae, E. coli, P. aeruginosa and a Proteus vulgaris bacterial strain using a well diffusion method. Results revealed that La-coated ZnONPs had a higher antibacterial effect when compared to uncoated, Ce- and Nd-coated samples. In addition, the in vitro cytotoxicity effect was analyzed using a A498 (human kidney carcinoma) cell line and a normal Vero (African monkey kidney) cell line, revealing IC50 values of 41.74, 35.86, 40.05 and 63.08 µg ml$^{-1}$, respectively. On the other hand, IC50 values were quantified at 55.27, 49.69, 56.83 and 51.10 µg ml$^{-1}$ for undoped, La-, Ce- and Nd-coated ZnONPs samples (against A498 and Vero cell lines) (Karthikeyan et al 2019).

All these studies successfully revealed that the use of both bacteria and plants allow for the noncomplex, environmentally friendly, and cost-effective production of valuable ZnONPs with antimicrobial properties. These green ZnONPs displayed a comparative advantage over traditionally synthesized ZnONPs, with this behavior clearly making an impact in the research around piezoelectric NMs, shifting efforts towards the employment of these green approaches instead of physicochemical methods.
4.2. Anticancer applications of green-synthesized ZnONPs

Cancer is a conjunction of diseases characterized by the abnormal growth of tissue that might lead to the development of tumors that can spread into other tissues and cause severe effects in the patient, with complications and severities potentially causing death ((US) and Study 2007). In 2019, cancer was cataloged as the second leading cause of death in the US with approximately 2 million people being diagnosed every year (Siegel et al. 2019). Current approaches such as chemotherapy and radiotherapy, although functional, are not completely effective and present significant drawbacks in the form of severe side effects, such as immunosuppression, anemia, sickness, or even death. In fact, it has been reported in the literature that some cancer cells have become resistant to treatments, leading to the appearance of chemotherapy-resistant tumors, making those treatments no longer a viable option for this kind of patient (Pavlopoulou et al. 2016).

As a consequence, significant efforts have been made towards the development of new approaches. Consequently, the use of nanotechnology has become more popular, as it can be applied towards cancer treatment and overcome substantial drawbacks (of the traditional treatment options) without experiencing toxicity to healthy tissues (Blanco et al. 2015, Mostafavi et al. 2019). For instance, different NPs have been shown to effectively encapsulate chemotherapy drugs and deliver them directly to the tumor site, diminishing (or in some cases, completely eradicating) the possible side effects (Ahmed et al. 2018). Moreover, the use of NMs allows for a more efficient permeability to the tumor site, when compared to the free drugs (Su and Hu 2018, Asadi et al. 2018). NPs can also be functionalized to target specific cancerous cells by attaching different molecules, such as aptamers, proteins or antibodies, and the NPs can specifically bind to the carcinogenic structures (Vahed et al. 2019).

ZnO has been investigated in the past few years for anticancer treatments. Since Zn is present in the body as an essential trace element (in tissues such as brain, bones or skin), it often does not compromise cell viability (Jiang et al. 2018). On the other hand, ZnONPs are known to induce the production of ROS upon contact with the cells, which leads to mitochondrial damage, triggering cell death in cancer tissue (J. Wang et al. 2018). Moreover, ZnONPs can be used in combination with immunotherapy, since they are rapidly taken up by the immune cells because of their electrostatic properties. The surface charge of these NPs is normally neutral but can be modulated depending on the pH; hence at biological conditions, ZnONPs are positively charged. This fact allows for their easy digestion by negatively-charged immune cells (P. Sharma et al. 2019). Furthermore, ZnONPs have the ability to showcase luminescence, therefore they could be used as diagnostic tools or targeted drug delivery for cancer diseases. Commonly, ZnONPs can be doped with materials such as Mg or mixed with quantum dots (QD) in order to increase these luminescent properties (Martinez-Carmona et al. 2018). As an example, ZnO-QD NPs were loaded with doxorubicin (DOX) to deduce a pH-responsive drug delivery for lung cancer (Cai et al. 2016). From the variety of methods for synthesizing ZnONPs, green methodologies offer an ecofriendly and cost-effective approach that overcomes significant drawbacks of traditional synthesis. Different materials have been employed for a considerable amount of time for the production of green ZnONPs with anticancer properties, with plants and algae extracts acting as one of the most common reducing agents, owing to their high natural sugar concentration (Hameed et al. 2019). Once generated, the green-synthesized ZnONPs show different cell death mechanisms (figure 4) which are associated with the combination of the metallic ions present on the nanostructures and the natural compounds coming from the natural raw materials employed, leading to some interesting examples in the literature (table 2).

4.2.1. Fungi-mediated synthesis of ZnONPs with anticancer properties

Fungi are extremely versatile living organisms which have been also investigated for the production of ZnONPs with anticancer properties. For example, Pichia kudriavzevii (GY1) yeast-mediated ZnONPs showed anticancer activity against breast cancer cells, inducing sub-G1 phase apoptosis, by upregulating pro-apoptotic genes such as, p53, p21, Bax, and JNK, as well as downregulating anti-apoptotic genes like Bcl-2, AKT1, and ERK1/2 (Moghaddam et al. 2017). Similarly, Penicillium chrysogenum was used for the biosynthesis of extracellular ZnONPs, which were shown to be anticancer agents towards distinct cancer cell lines like breast and colon. An increased performance was also evidenced when the NPs were irradiated with gamma radiation (Housseiny and Gomaa 2019). Alternatively, biomass extracted from the fungus Aspergillus niger was used for the synthesis of ZnONPs following a co-precipitation method, with the NPs revealing a powerful anticancer activity when tested in hepatic cancer cells (while remaining cytocompatible to human embryonic kidney cells HEK-293). Furthermore, it was demonstrated that the NPs interfered in the G0/G1 cell cycle, hence depleting cell proliferation (Gao et al. 2019). Similarly, Majeed et al produced ZnONPs by the same fungus showing anticancer activity in a dose-dependent manner, targeting lung cancer cells. The NPs were able to induce DNA damage on the cancer cells, a mechanism of cell death, determined to be a mixture of both apoptosis and nuclear fragmentation (Majeed et al. 2018). Lastly, Aspergillus terreus was used for the production and isolation of the enzyme L-asparaginase, which, at the same time, was utilized for the synthesis
### Table 2. Anticancer applications of green-synthesized ZnONPs.

| Platform                        | Raw material                                           | System          | Size                  | Targeted cell line               |
|---------------------------------|--------------------------------------------------------|-----------------|-----------------------|----------------------------------|
| **Fungi-mediated**              | *Pichia kudriavzevii* Yeast (*Moghaddam et al. 2017*) | ZnONPs          | 10–61 nm              | MCF-7, breast                    |
|                                 | *Penicillium chrysogenum* Fungus (*Houssein and Gomaa 2019*) | ZnONPs          | 29–37 nm              | MCF-7, breast HCT-116, colon     |
|                                 | *Aspergillus niger* Fungus (*Gao et al. 2019*)        | ZnONPs          | 80–130 nm             | HepG2, liver                     |
| **Algae and plant-mediated**    | *Aspergillus niger* Fungus (*Majeed et al. 2018*)     | ZnONPs          | 11.8–17.6 nm          | A549, lung                       |
|                                 | *Aspergillus terreus* Fungus (*Baskar et al. 2015*)   | L-asparaginase—ZnONPs | 28–63 nm             | MCF-7, breast                    |
|                                 | *Sargassum muticum* algae extract (*Sanaeimehr et al. 2018*) | ZnONPs          | 50–100 nm             | WEHI-3, murine leukemia          |
|                                 | *Gracilaria edulis* Algae extract (*Mohamed Asik et al. 2019*)| ZnONPs          | 4.04 ± 1.81 nm; length 1.39 ± 0.6 nm; width | MG-63 bone |
|                                 | *Rehmanniae Radix* plant extract (*Cheng et al. 2020*) | ZnONPs          | 10–12 μm              | HeoG2, liver                     |
|                                 | *Myristica fragans* Plant extract (*Ashokan et al. 2017*) | ZnONPs          | 100–200 nm            | MCF-7, breast                    |
|                                 | *Albizia lebbeck* Stem bark (*Umar et al. 2018*)      | ZnONPs          | 66.25 nm              | MDAMB231, breast A549, lung      |
|                                 | *Mangifera indica* Leaves (*Rajeshkumar et al. 2018*) | ZnONPs          | 45–60 nm              | MG-63 bone                       |
|                                 | *Ziziphus nummularia* Leaves (*Padalia and Chanda 2017*) | ZnONPs          | 17.33 μm              | HeLa, cervical                   |
|                                 | *Laurus nobilis* Leaves (*Vijayakumar et al. 2016*)  | ZnONPs          | 47.27 nm              | A549, lung                       |
|                                 | *Nephelium lappaceum* Peel (*Yuvakkumar et al. 2015*) | ZnONPs          | 70–75 nm              | A549, lung                       |
|                                 | *Gymnema sylvestre, Plant extract* (*Karthikeyan et al 2019*) | ZnONPs          | 38 nm 33/27/23 nm     | A498, kidney                     |
|                                 | *Costus pictus D. Don, Leaves* (*J. Suresh et al. 2018b*) | ZnONPs          | 20–80 nm              | DLA, Daltons lymphoma ascites    |
| **Protein mediated**            | Collagen Protein (*Vijayakumar and Vaseeharan 2018*)  | ZnONPs loaded with curcumin | 9.3–13.7 nm | MCF-7, breast HeLa, cervical MDAMB231, breast MG-63, bone HeLa, cervical |
|                                 | *Milk casein protein* (*Somu and Paul 2019*)          | ZnONPs          | 20–50 nm              | HepG2, liver                     |
|                                 | *Tocopherol Lipid* (*Wu and Zhang 2018*)              | Chitosan coated ZnONPs | 100 nm              |                                |
of ZnONPs following the co-precipitation method. This nanobiocomposite showed anticancer activity against breast cancer cells, with the enzyme providing enhanced specificity and effectiveness to the NPs (Baskar et al 2019).

4.2.2. Algae and plants extract-mediated synthesis of ZnONPs with anticancer properties

Despite the promising use of fungi, plant and algae extracts rich in anticancer components that become a part of the nanocomposites once they are used as reducing and capping agents, have led to some interesting cases of synergetic effects for anticancer use. For instance, algae extract from Sargassum muticum was used to synthesize ZnONPs in an aqueous-based reaction. The NPs, when tested in concentrations from 175 to 2800 µg ml\(^{-1}\), showed decreased angiogenic effects, and induced apoptosis on human liver cancer cells (HepG2) (Sanaeimehr et al 2018). Moreover, the same algae was used as a unique reducing agent (mixed with zinc acetate as precursor) to obtain ZnONPs that were tested against murine cancer cells (WEHI-3). These cancer cells experienced increased efficiency in relative comparison to normal chemotherapy drugs, like paclitaxel (Namvar et al 2015). Similarly, it is common to conjugate ZnONPs to other molecules such as polymers, in order to increase their physical, chemical and biological properties as nanocomposites, while polymers are also known to reduce NPs recognition by the immune system. For instance, hyaluronan provided enhanced adhesivity, wettability and biocompatibility to Sargassum muticum-mediated ZnONPs. The nanostructures showed anticancer effects in a wide variety of cancer lines, especially producing effective results targeting acute promyelocytic leukemia (HL-60) cells (Namvar et al 2016). More recently, Gracilaria edulis was used for the extracellular synthesis of hexagonal-rod shaped ZnONPs, following a precipitation method. The structures were tested against cervical cancer showing no toxicity on healthy blood cells (PBMC). When compared to cisplatin, a drug used for cancer treatment that reported resistance to some patients, ZnONPs demonstrated enhanced performance in terms of its effectiveness (Mohamed Asik et al 2019).

Shifting towards the use of plant extracts, Rehmanniae Radix, a Chinese herb, was used as a raw material for the synthesis of rod-shaped ZnONPs by precipitation in basic media. The NPs were then used to treat bone cancer (MG-63: Human osteoblast-like cells) implying a dose-dependent effect and inhibiting cancerous proliferation at higher doses. Moreover, it was demonstrated that the nanostructures produced ROS and induced apoptotic signaling through the increased presence of Bax, caspase-3, and caspase-9 proteins, which are known to trigger apoptosis in cells (2020). Alternatively, Ashokan et al used Myristica fragrans as a unique reducing agent to produce ZnO nanorods, which were tested towards HepG2 cells. Although it was considered that the nanorods could release ZnO cations that could be toxic to the cells, the mechanism of cell death was associated with the production of ROS species. Moreover, membrane shrinkage and blebbing were also observed (Ashokan et al 2017). Alternatively, in an intriguing study, Huzaifa et al showed how Albizia lebbeck stem bark worked as an excellent chelating agent for the synthesis of ZnONPs, which were later tested on breast cancer cell lines, showing the production of ROS and inducing apoptotic effects on the cell membrane (Umar et al 2018). Furthermore, Mangifera indica (mango fruit) leaves were used as a unique material on the production of ZnONPs showing cytotoxic effects in lung cancer cells at low concentrations of 25 µg ml\(^{-1}\). The effects of these NPs were comparable to commercial but less biocompatible drugs, rendering significant advancements compared to traditional or commercially available treatments (Rajeshkumar et al 2018).

Seeds of Pongamia pinnata were used for the production of ZnONPs by a co-precipitation method in basic media, where the NPs were then able to inhibit and reduce the proliferation of breast cancer cells at a concentration of 50 µg ml\(^{-1}\) (Malaikozhundan et al 2017). In another study, different shapes of ZnONPs, such as hexagonal, rods, rectangles, and triangular, were obtained when mixed with an aqueous solution of Eclipta prostrata leaves. The conjunction of the NPs showed induction of apoptosis and dose-dependent effect against liver cancerous cells, with an elevated efficacy at a concentration of 100 µg ml\(^{-1}\) (Chung et al 2015). Similarly, a Borassus flabellifer fruit extract produced ZnO nanosheets when mixed with zinc nitrate, which was then loaded with DOX, functionalized with folic acid, and coated with polyethylene glycol (PEG) for targeting purposes. This system was used for photothermal therapy, releasing heat when irradiated with NIR light, and subsequently proving itself effective against human breast cancer cells (Vimala et al 2017).

In an elegant study by Padalia et al a Ziziphus nummularia leaf extract was used to produce spherical and irregular shaped ZnONPs. The structures were tested in a range from 2 to 200 µg ml\(^{-1}\), and evidently decreased the viability of cervical cancer cells by 60% (Padalia and Chanda 2017). Another example in the form of Laurus nobilis leaf extracts were employed in order to produce ZnONPs (following a co-precipitation method), resulting in the successful inhibition of lung cancer cell proliferation at concentrations of 80 µg ml\(^{-1}\) without compromising healthy murine RAW264.7 macrophage cells (Vijayakumar et al 2016). Alternatively, a Rambutan (Nephelium lappaceum) waste peel was used for the synthesis of ZnO nanochains. In this process, the presence of polyphenols on the peels allowed for the natural ligation of the ions and their further reduction. The ZnONPs were then tested against liver cancer cells, which resulted in a 60% reduction.
of cell viability, as well as changes in cell morphology (Yuvakkumar et al 2015). Furthermore, Tecomastanifolia, a flower with origins in Peru, served as the primary ingredient in the production of ZnONPs, owing largely due to its high presence of phytocomponents with reducing and capping potential. Moreover, the NPs showed enhanced anticancer activity against lung cancer (Govindasamy Sharmila et al 2019). In another study, Karthikeyan et al utilized Gymnema sylvestre as a novel reducing and capping agent for the production of undoped and rare-earth doped ZnONPs. Consequently, the doped structures showed enhanced activity against kidney cancer lines compared to the undoped NPs (Karthikeyan et al 2019).

Alternatively, Castus picts D. Don (insulin plant) leaves were demonstrated to generate ZnONPs by complex formation, with anticancer activity in a dose-dependent manner, from concentrations 10–50 µg ml⁻¹ against Daltons lymphoma ascites (J. Suresh et al 2018a).

4.2.3. Natural biomolecule-mediated synthesis of ZnONPs with anticancer properties

Animal proteins are largely present in the body and can be used to synthesize ZnONPs with properties such as high biocompatibility and stronger structural characteristics than their physiochemically synthesized counterparts. Besides, they are able to show significant anticancer activity. For instance, collagen was used as a unique reducing agent in the synthesis of ZnONPs by a precipitation method in basic media. The authors showed how a concentration of 75 µg ml⁻¹ of these particles was highly effective against hepatic cancer but not toxic on murine (RAW 264.7) macrophage cells (Vijayakumar and Vaseeharan 2018). On the other hand, milk casein protein was used to synthesize ZnONPs; the mixture of which was then loaded with curcumin to treat breast (MCF-7 and MDAMB231), cervical (HeLa: Henrietta Lacks), and osteosarcoma (MG-63) cancer cells. The NPs were capable to target specifically the cancerous cells as they were conjugated to a folic acid receptor (Somu and Paul 2019). Moreover, lipids can be utilized to synthesize ZnONPs, as demonstrated by Wu and Zhang. According to their method, zinc acetate was mixed with tocopherol, rendering NPs that were coated with chitosan, to enhance their interaction with cancerous cells. The ZnONPs were tested against cervical cancer showing the release of ROS that led to cell apoptosis (Wu and Zhang 2018).

Therefore, a variety of natural raw materials originating from fungi, algae or plant species, as well as natural biomolecules, can be used for the synthesis of ZnONPs, showing enhanced properties as anticancer agents by, commonly, promoting ROS generation and inducing cell apoptosis. These particles could be applied successfully to a wide variety of cancer cell lines without compromising healthy tissue, hence, offering the green synthesis of ZnONPs as an eco-friendly and cost-effective alternative for cancer treatment. In terms of their increased biocompatibility, these NMs offer added advantages over their traditional synthesized counterparts.

4.3. Tissue engineering and wound healing applications of green-synthesized ZnONPs

Apart from its antimicrobial and anticancer properties, ZnO nanostructures present antineoplastic, wound healing, ultraviolet scattering, and angiogenic properties, which are often employed in tissue engineering applications (Ghazali et al 2018, Kalantari et al 2020). Particularly, osteogenesis and angiogenesis effects from the ZnONPs have effectively been demonstrated in numerous studies, due to their remarkable thermostability, marked antimicrobial activity, and low cost (Manuja and Raguvaran 2015, Laurenti and Cauda 2017, Medina et al 2020).

Although the rise of ZnONPs has led to new and promising antibacterial and anticancer properties, there has been only select research published highlighted the role of ZnO nanostructures in promoting cell growth, proliferation, differentiation, and increasing the metabolic activity of different cell lines for tissue engineering and regenerative medicine applications (figure 5). Consequently, the implementation of green technology using ZnONPs in tissue engineering has turned into an even narrower field. Nevertheless, a few examples have emerged in the past few years introducing the idea that the pro-angiogenic properties of ZnONPs can be extremely useful enhancing the integration of the scaffolds to host tissue. However, a much better understanding for the mechanism of their selective cytotoxic action, as well as the lack of appropriate biocompatible dispersion protocols, is required in this regard.

For example, Shubha et al reported the use ZnONPs synthesized by gallic acid, as isolated from the Phyllanthus emblica aqueous extract. The physical, chemical, in vitro, and in vivo toxicity of their products were compared with a clinically advocated ZnONPs. The group used babl mice 3T3 fibroblasts in vitro as one of the key cells of connective tissue (which assist in tissue repair and regeneration). The in vitro and in vivo results revealed that at the highest concentration, green synthesized ZnONPs were less toxic than the commercially available nanostructures. This factoid effectively implies that ZnONPs could be a potential candidate to be applied in the vicinity of connective tissue cells (Shubha et al 2019). Alternatively, an environmentally friendly and affordable synthesis of ZnONPs was reported using Prosopis fructa and coffee extracts as unique raw materials with an average size 26 nm. In vitro tests revealed a strong antibacterial effect of these NPs when cultured with Acinetobacter baumannii and P. aeruginosa cultures. These ZnONPs were
Figure 5. The use of green synthesized ZnONPs in tissue engineering. (A) ZnONPs synthesized using Gallic acid isolated from a Phyllanthus emblica aqueous extract, designated as Pe ZnO, were studied in vitro with cytotoxicity assessment on balb 3T3 fibroblasts and in vivo with silkworm Bombyx mori larvae (Shubha et al 2019); (B) photographic representation of the wound healing process in the excision wound model, showing the control (Group 1), standard drug (Group 2), ZnOTP1\% treated (Group 3) and ZnOTP2\% treated (Group 4) at 0, 3, 6, 9, 15 and 17 days post wounding (Yadav et al 2018).

impregnated on cotton wound bandages, conferring patches with antimicrobial properties. This property can potentially be used for treating and covering infection-sensitive wounds, namely diabetic or burns wounds (Khatami et al 2018). Similarly, ZnONPs were synthesized using Barleria gibsoni aqueous leaf extracts, which acted as both a reducing and protective agent, because of the presence of polyphenols, flavonoids and amino acids in the extracts. The NPs were characterized as hexagonal wurtzite structures with a size range between 30 and 80 nm. Acting as an efficient and superior tropical antimicrobial formulation, they were tested for their antibacterial properties for healing burn infections. Moreover, the ZnONPs exhibited a remarkable wound healing potential in rats after an in vivo study (Shao et al 2018).

In a similar work, Kumar et al studied the phytochemical effect of a Raphanus sativus (white radish) root extract for the production of ZnONPs, with the aim to develop an effective antimicrobial agent towards E. coli for wound healing applications. While the ZnONPs were synthesized using the root extract, commercially available ZnONPs were bio-functionalized using the same extract, with the goal of analyzing any possible differences in their biomedical applications. Both NPs showed enhanced antimicrobial activity
Table 3. Biosensing applications of green-synthesized ZnONPs.

| Raw material                                      | Platform                                         | Target molecule | Lowest detection Limit | Linear range  |
|--------------------------------------------------|--------------------------------------------------|-----------------|-------------------------|---------------|
| ZnONPs synthesized using leaf extract of Ocimum tenuiflorum | ZnONPs coated on GCE                            | Glucose         | 0.043 µM                | 1–8.6 mM      |
| ZnONPs synthesized using Corymbia citriodora leaf extract | ZnONPs coated on GCE                            | H₂O₂            | 0.07 µM                 | 0.1 and 150 µM|
| ZnONPs synthesized using Carica papaya seed extract | ZnONPs integrated on MWCNTs on a glassy carbon electrode | Silymarin       | 0.08 mg l⁻¹             | 0.014–0.152 mg l⁻¹|
| Hydrothermally-synthesized ZnONPs                | ZnONPs embedded with nitrogen-doped carbon sheets and GOx on a glassy carbon electrode | Glucose         | 6.3 µM                  | 0.2–12 mM     |
| Thermally-decomposed ZnONPs                     | ZnONPs coated on GCE                            | Dopamine        | 1–300 µM                | 1 µM          |
| Focused sunlight synthesized ZnONPs             | ZnONPs-decorated MWCNTs–graphene hybrid composite coated on GCE | Paraoxon AChE   | 1 pM                    | 1–26 nM       |

Compared to their pure ZnONPs counterparts, after an extensive characterization, it was concluded that the NPs showed a high potential as wound healing agents, with the need of subsequent studies (Kumar et al 2019). Steffy et al reported the synthesis of ZnONPs using Strychnos nux-vomica extracts acting as a powerful agent capable of tackling antibiotic-resistant bacterial pathogens, and for treating a non-healing ulcer. The ZnONPs (with a size range of 10–12 nm) exhibited significant bactericidal potency at a concentration of 100–200 µg ml⁻¹, against MDR–Methicillin-resistant S. aureus, MDR–E. coli, MDR–P. aeruginosa, and MDR–Acinetobacter baumanii. These ZnONPs also exhibited significant bactericidal potency (at a similar concentration range) against the standard bacterial strains S. aureus, E. coli, P. aeruginosa and Enterococcus faecalis. The wound-healing properties were assessed by a scratch assay on a mouse L929 fibroblastic cell line, in order to quantify cell migration towards the injured area. Cytotoxicity was assessed using 3-[4,5-dimethyl-2-thiazol-yl]-2,5-diphenyl-2H-tetrazolium bromide (MTT) cellular viability assay on the L929 cell line and a human embryonic kidney epithelial (HEK-293) cell line. This experiment resulted in ZnONPs exhibiting wound-healing and reduced cytotoxic properties at active antimicrobially-favored concentrations (Steffy et al 2018a).

An impediment in the process of wound healing can be attributed to reactive oxygen species and inflammation. Yadav et al assessed the use of ZnONP synthesized by Trianthema portulacastrum Linn, with sizes of 10–20 nm. An in vitro anti-inflammatory activity study of the NPs was conducted by membrane stabilization and albumin denaturation (along with proteinase inhibitory assays), resulting in a significant wound contraction rate, epithelialization and histopathology of the healed tissues of rats, which confirmed the promising wound healing property of the nanostructures. In addition, inflammatory markers and the profile of antioxidant enzymes also support the wound healing potential of NMs, revealing a potential use in wounds via antioxidant and anti-inflammatory activity (Yadav et al 2018).

4.4. Biosensing applications of green-synthesized ZnONPs

Over the past few decades, ZnONPs have shown unprecedented performance as a biosensor, owing to a high isolectric point, biocompatibility and other multifunctional characteristics. This notion explains why ZnONPs have extensively been studied as a transduction material for biosensor development. Besides the fascinating properties of ZnO (already discussed), ZnONPs help retain the biological activity of the immobilized biomolecule, achieving enhanced sensing performance, and allowing for a successful label-free electrical detection of biomarkers (and other molecules of interest). The combination of green nanotechnology with ZnO at the nanoscale has rendered some outstanding examples (table 3 and figure 6).

Recently, a non-enzymatic glucose biosensor was developed by using a ZnONP-synthesized Ocimum tenuiflorum leaf extract. While the XRD analysis of the NPs revealed a crystalline and hexagonal wurtzite structure, the UV–vis absorption spectrum estimated the ZnONPs band gap of 2.82–3.45 eV with an average size ranging from 10 to 20 nm. Once characterized, a glassy carbon electrode (GCE) glucose sensor was fabricated by coating the electrode with the ZnONPs. The electrode showed superior electrocatalytic activity with a reproducible sensitivity of 631.30 µA mM⁻¹ cm⁻² and a linear dynamic range from 1 to 8.6 mM, coupled with a low detection limit of 0.043 µM. Moreover, the response time was found to be below 4 s.
Figure 6. Highly selective, sensitive and stable enzymatic glucose sensor was fabricated on glassy carbon electrode (GCE) using ZnONPs embedded in nitrogen-doped carbon sheets and coupled with GOx (Muthuchamy et al 2018). (Dayakar et al 2017). In a similar study, ZnONPs synthesized by a Corymbia citriodora leaf extract acting as a unique reducing and stabilizing agent were employed for the construction of an electrochemical H$_2$O$_2$ biosensor on a GCE. The sensor exhibited a stronger ability to reduce H$_2$O$_2$, contrasted with that of a bare GCE and a chemically modified GCE with ZnONPs. A linear relationship between the current response and H$_2$O$_2$ concentration was observed between 0.1 and 150 µM, with a lower detection limit at 0.07 µM. The study also concluded that there was no interference with other molecules, such as uric, ascorbic acids, and glucose, which are three electro-active molecules that commonly coexist in biological systems and could interfere with the electrochemical determination of H$_2$O$_2$. The authors were able to demonstrate that a 20-fold excess of the acids and glucose produced negligible current responses, suggesting that the electrode presented an excellent ability to detect H$_2$O$_2$, among species prone to interference (Zheng et al 2016).

Alternatively, ZnONPs, with an average size of 4–8 nm, were synthesized by using a Carica papaya seed extract, while using oleic acid as an important capping agent. Later, the authors investigated the electrochemical application of the ZnONPs as a sensor for silymarin, by integrating them with multi-walled carbon nanotubes (MWCNTs) on a GCE. While the electrochemical signals obtained from the electrode were 2-fold higher than both MWCNTs/GCE and bare GCE electrodes, the electrochemical detection could detect 122 mg of silymarin in the quoted concentration, confirming a detection efficiency of approximately 76%. At a molecular level, the authors found that the charge transfer from ZnO clusters to OH groups (of silymarin) strengthened the interactions between the silymarin molecule and ZnO clusters. The obtained energies indicated the most probable position and functional groups in silymarin, which led to its detection and binding with a linear relationship found between 0.014 and 0.152 mg l$^{-1}$, with a lower detection limit of 0.08 mg l$^{-1}$ (D. Sharma et al 2018). A stable enzymatic glucose sensor, of a highly selective environmentally friendly and sensitive nature, was fabricated on GCE using ZnONPs embedded in a nitrogen-doped carbon sheet (together with glucose oxidase (GOx)). The production of these sheets was achieved by a simple hydrothermal method, wherein Zn powder, aqueous ammonia, and peach extract served as predecessors for the ZnONPs, nitrogen and carbon, respectively. Once built, the fabricated biosensor exhibited a high and reproducible sensitivity of 231.7 µA mM$^{-1}$ cm$^{-2}$, a linear range from 0.2 to 12 mM, along with a correlation coefficient $R^2$ of 0.998 and the lowest detection limit of 6.3 µM. The authors found the biosensor to be generally stable and selective, which allowed for its successful application to human blood serum for quantitative monitoring (Muthuchamy et al 2018). On a similar note, an ecofriendly method for the synthesis of self-assembled hexagonal pyramids of ZnONPs was created through the use of thermal decomposition of zinc nitrate. These novel pyramids were thoroughly characterized, and tested for their features as a biosensor, towards the detection of dopamine at trace levels. These synthesized ZnONPs showed a linear range for...
dopamine up to 300 μM, with a detection limit of 1 μM. While efforts to reproduce results over periods of several months took place, its electrochemical performance indicated no significant deviation (Udayabhanu et al 2016). Correspondingly, the green synthesis of ZnONPs decorated MWCNTs–graphene hybrid composite was treated with focused sunlight (a clean energy source) in the form of a convex lens of 90 mm diameter (exposed for 1–2 min). Upon coating with a GCE, the composite was evaluated for its application as a transducer candidate as a organophosphorous biosensor. The hybrid composite showed noteworthy electrochemical activity, owing largely to its large electrochemically-active surface area, in comparison to the ZnONP-decorated graphene prepared by the same root. Later, this phenomenon was discovered due to the presence of MWCNTs between the graphene layers. These graphene layers effectively prevent its restacking, thereby increasing the likelihood of accessing the surface area of interest. Using this composite as a transducer candidate, this fabricated biosensor presented a high affinity to acetyl cholinesterase (AChE) enzyme with an evident Michaelis–Menten constant (Km) value of 0.8 mM. Further, a linear response for Paraoxon detection was exhibited from 1 to 26 nM, with a detection limit of 1 pM (Nayak et al 2013).

5. Conclusion

The rise of nanotechnology has brought about a tremendous enhancement in several scientific fields, especially for biomedical applications of materials. Metallic and metal-oxide NMs have been used as agents to fight diseases, such as bacterial infections and cancer, and to detect important biomarkers—the quantification of which might result in successful medical diagnosis. However, progress is not free of drawbacks, and the synthesis of these valuable NMs faces some disadvantages, on both the environment and society. This ultimately affects the biomedical performance of these materials. Therefore, to avoid the production of toxic by-products and unwanted reactions with different biological membranes, green nanotechnology makes a strong claim as a potential solution with the innovative idea of employing living organisms and biomolecules as unique reducing and capping agents for the formation of NMs. These green-synthesized nanostructures have been successfully implemented as biomedical agents, offering substantial performance enhancements, compared to their traditionally produced counterparts. These enhancements include a better cytocompatibility and clearance from the organisms, and a mild synthetic process which is both environmentally friendly and cost-effective. ZnO has been extensively studied as a valuable nanomaterial for biomedical applications, which has successfully met the green nanotechnology approaches and resulted in a synergetic effect, in which both the properties of the raw material and the inherent features of ZnO has led to strong antimicrobial features, anticancer properties, and biosensing capabilities. Despite the examples presented in this review, some limitations may arise from the use of green-synthesized ZnO nanomaterials. For instance, the heterogeneity of the nanomaterials is inherent to the synthetic protocols where the raw materials may lack homogeneity in composition and chemical distribution, while the potential attachment of toxic components coming from bacterial or fungi cells to the final nanoparticles might be another source of limitation for their biomedical use. Therefore, and although there exists some literature exploring the use of green-synthesized ZnONPs as biomedical agents, there still exists a lack of substantive knowledge of their selective cytotoxic mechanism, as well as their appropriate biocompatible protocols. However, more research should be done in terms of standardization of synthetic protocols, as one of the main drawbacks of green chemistry is the heterogeneity in the obtained products. Nonetheless, significant advancements in the field, as well as promising characteristics, are leading to optimism around the use of these nanostructures as powerful biomedical agents. Therefore, this review provides examples for the significance and wide-spread use for the use of green-synthesized ZnO nanomaterials as a powerful and promising biomedical tool.

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References

Abinaya M, Vaseeharan B, Divya M, Sharmili A, Marimuthu Govindarajan N S, Alharbi S K, Khaled J M and Benelli G 2018 Bacterial exopolysaccharide (EPS)-coated ZnO nanoparticles showed high antibiofilm activity and larvicidal toxicity against malaria and zika virus vectors J. Trace Elem. Med. Biol. 45 93–103
Adam R E, Pozina G, Willander M and Nur O 2018 Synthesis of ZnO nanoparticles by co-precipitation method for solar driven photodegradation of Congo red dye at different pH Photonics Nanostruct.-Fundam. Appl. 32 11–18
Agarwal H S, Kumar V and Rajeshkumar S 2017 A review on green synthesis of zinc oxide nanoparticles—an eco-friendly approach Resour.-Efficient Technol. 3 406–13
Agrawal N, Munjal S, Ansari M Z and Khare N 2017 Superhydrophobic palmitic acid modified ZnO nanoparticles Ceram. Int. 43 14271–6
Agrawal R and Espinosa H D 2011 Giant piezoelectric size effects in zinc oxide and gallium nitride nanowires. A first principles investigation Nano Lett. 11 786–90
Ahmed S E, Awad N, Paul V, Moussa H G and Husseini G A 2018 Improving the efficacy of anticancer drugs via encapsulation and acoustic Curr. Top. Med. Chem. 18 857–80
Aiswarya Devi S, Harshiny M, Udayakumar S, Gopinath P and Matheswaran M 2017 Strategy of metal ion doping and green-mediated ZnO nanoparticles: dissolution, antibacterial and cytotoxic traits Toxicol. Res. 6 854–65
Akhter S, Humayun M, Mahmood Z, Ahmad S and Mohammad F 2018 Plant-mediated green synthesis of zinc oxide nanoparticles using Svertia chrysis leaf extract, characterization and its antibacterial efficacy against some common pathogenic bacteria BioNanoScience 8 811–17
Al Abdullah K, Awad S, Zaraket J and Salame C 2017 Synthesis of ZnO nanopowders by using sol–gel and studying their structural and electrical properties at different temperature Energy Proc. 119 563–70
Al-Dahashi G, Mubder Khilkala W and Abd Alwahid S N 2018 Preparation and characterization of ZnO nanoparticles by laser ablation in NaOH aqueous solution Iran. J. Chem. Eng. 37 11–16
Alkilyan A M, Nagaria P K, Hxeel C R, Shaw T J, Murphy C J and Wyatt M D 2009 Cellular uptake and cytotoxicity of gold nanorods: molecular origin of cytotoxicity and surface effects Small 5 701–8
Alwan R M, Kadhim Q A, Sahah K M, Ali R A, Mahdi R J, Kassim N A and Jassim A N 2015 Synthesis of zinc oxide nanoparticles via sol–gel route and their characterization Nanosci. Nanotechnol. 5 1–6
Amendola V and Menegetti M 2009 Laser ablation synthesis in solution and size manipulation of noble metal nanoparticles Phys. Chem. Chem. Phys. 11 3805–21
Anitha R, Ramesh K V, Ravishankar T N, Sudheer Kumar K H and Ramakrishnappa T 2018 Cytotoxicity, antibacterial and antifungal activities of ZnO nanoparticles prepared by the Artocarpus gomezianus fruit mediated facile green combustion method J. Sci.: Adv. Mater. Dev. 3 440–51
Arciniegas-Grihalba P A, Patiño-Porcella M C, Mosquera-Sánchez I. P, Guerrero-Vargas J A and Rodríguez-Páez J E 2017 ZnO nanoparticles (ZnO-NPs) and their antifungal activity against coffee fungus erythricum salmonicolor Appl. Nanosci. 7 225–41
Arnau A and Soares D 2008 Fundamentals of piezoelectricity Piezoelectric Transducers and Applications ed Antonio Arnau Vives (Berlin, Heidelberg: Springer) pp 1–38
Asadi N, Annabi N, Mostafavi E, Anzabi M, Khalilov R, Saghi S, Mehrizadeh M and Akbarzadeh A 2018 Synthesis, characterization and in vitro evaluation of magnetic nanoparticles modified with PCL–PEG–PCL for controlled delivery of 5FU Int. J. Nanomed. 13 786–90
Ashokan A P, Paulpandi M, Dinesh D, Murugan K, Vadivalagan C and Benelli G 2017 Toxicity on dengue mosquito vectors through Myristica fragrans-synthesized zinc oxide nanorods, and their cytotoxic effects on liver cancer cells (HepG2) J. Cluster Sci. 28 265–26
Baheti V, Abbasi R and Militky J 2012 Ball milling of jute fibre wastes to prepare nanocellulose Mater. Sci. Eng. C 32 317–20
Bhushan B (Ed) 2012 Encyclopedia of Nanotechnology (Netherlands: Springer)
Bhuyan T, Mishra K, Khatuna M, Prasad R and Varma A 2015 Biosynthesis of zinc oxide nanoparticles from Azadirachta indica for antibacterial and photocatalytic applications Mater. Sci. Semicond. Process. 32 55–61
Blanco E, Shen H and Ferrari M 2015 Principles of nanoparticle design for overcoming biological barriers to drug delivery Nat. Biotechnol. 33 941–51
Brown C D, Cruz D M, Roy A K and Webster T J 2018 Synthesis and characterization of PVP-coated tellurium nanorods and their antibacterial and anticancer properties J. Nanopart. Res. 20 254
Cai X, Luo Y, Zhang W, Dan D and Lin Y 2016 PH-sensitive ZnO quantum dots–doxorubicin nanoparticles for lung cancer targeted drug delivery ACS Appl. Mater. Interf. 8 23442–50
Carter C B and Grant Norton M eds 2007 Sols, Gels, and Organic Chemistry Materials: Science and Engineering (New York, NY: Springer) pp 400–110
Chai H Y, Lam S M and Sin J C 2019 Green synthesis of magnetic Fe-doped ZnO nanoparticles via Hibiscus rosa-sinensis leaf extracts for boosted photocatalytic, antibacterial and antifungal activities Mater. Lett. 242 103–6
Chen H, Missaout T, Chérif Mzali J, Yildiz T, Konar Y, Smiri M, Saidi N, Hafiane A and Yatmaz H C 2019 Facile green synthesis of zinc oxide nanoparticles (ZnO NPs): antibacterial and photocatalytic activities Mater. Res. Express 6 10
Cheng J, Wang X, Qiu L, Yunkai L, Marraaki N, Elgorban A M and Xue L 2020 Green synthesized zinc oxide nanoparticles regulates the apoptotic expression in bone cancer cells MG-63 cells J. Photochem. Photobiol. B 202 111644
Chung I-M, Rahuman A, Marimuthu S, Kirthi A, Anbarasan K and Rajakumar G 2015 An investigation of the cytotoxicity and caspase-mediated apoptotic effect of green synthesized zinc oxide nanoparticles using Eclipta prostrata on human liver carcinoma cells Nanomaterials 5 1310–30
Chwalibog A, Sawosz E, Hotowy A, Szeliha J, Mitura S, Mitura K, Grodzik-M, Orlowski P and Sokolowska A 2010 Visualization of interaction between inorganic nanoparticles and bacteria or fungi Int. J. Nanomed. 5 1085–94
Costenan D, Carniato F, Gatti G, Marchese I and Bisio C 2013 Preparation of luminescent ZnO nanoparticles modified with aminopropyltriethoxy silane for optoelectronic applications New J. Chem. 37 2103–9
Crua V A, Medina D, Zhang B, González M U, Huted Y, García-Martín J M, Cholula-Díaz J L and Webster T J 2019 Comparison of cytocompatibility and anticancer properties of traditional and green chemistry-synthesized tellurium nanowires Int. J. Nanomed. 14 3155–76
Dai S, Gharbi M, Sharma P and Park H S 2011 Surface piezoelectricity: size effects in nanostructures and the emergence of piezoelectricity in non-piezoelectric materials J. Appl. Phys. 110 104305
Dayakar T, Venkateswara Rao, K, Bikshalu K, Rajendar. V and Park S H 2017 Novel synthesis and structural analysis of zinc oxide nanoparticles for the non enzymatic glucose biosensor Mater. Sci. Eng. C 75 1472–9
Kiran Kumar A B V, Saita E S, Narang P, Ashwarya M, Raina R, Gautam M and Shankar E G 2019 Biofunctionalization and biological synthesis of the ZnO nanoparticles: the effect of Raphanus sativus (white radish) root extract on antimicrobial activity against MDR strain for wound healing applications Inorg. Chem. Commun. 100 101–6

Kolodzieczak-Radzimska A and Jesionowski T 2014 Zinc oxide–surface engineering for applications: a review Materials 7 2833–81

Komarneni S 2003 Nanophase materials by hydrothermal, microwave-hydrothermal and microwave-solvothermal methods Carr. Sci. 85 1730–40 http://www.jstor.org/stable/24109979

Kumar A, Yadav N, Bhatt M, Mishra N, Chaudhary P and Singh R 2015 Sol–gel derived nanomaterials and its applications: a review Res. J. Chem. Sci. 5 98–103

Laurenti M and Cau da V 2017 ZnO nanostructures for tissue engineering applications Nanomaterials 7 E374

Li J, Hong R Y, Li M Y, Li H Z, Zheng Y and Ding J 2009 Effects of ZnO nanoparticles on the mechanical and antibacterial properties of polyurethane coatings Prog. Org. Coat. 64 504–9

Li J, Qingliu W and Ji W 2015 Synthesis of nanoparticles via solvothermal and hydrothermal methods Handbook of Nanoparticles, ed M Alliofkhazraei (Cham: Springer International Publishing) pp 1–28

Li P, Wei Y, Liu H and Wang X K 2005 Growth of well-defined ZnO microparticles with additives from aqueous solution J. Solid State Chem. 178 855–60

Li X, Gaehong H, Xiao G, Liu H and Wang M 2009 Synthesis and morphology control of ZnO nanostructures in microemulsions J. Colloid Interface Sci. 333 465–73

Libralato G, Volpi Ghiaradini A and Avezzù F 2010 Seawater ecotoxicity of monoethanolamine, diethanolamine and triethanolamine J. Hazard. Mater. 176 535–9

Lingaraju K, Raja Naika H, Manjunath K, Basavaraj R B, Nagabhushana H, Nagaraju G and Suresh D 2016 Biogenic synthesis of zinc oxide nanoparticles using Ruta graveolens (L.) and their antibacterial and antioxidant activities Appl. Nanosci. (Switzerland) 6 703–10

Liu Y, He L, Mustapha A, Li H, Hu Z Q and Lin M 2009 Antibacterial activities of zinc oxide nanoparticles against Escherichia coli O157:H7 J. Appl. Microbiol. 107 1193–201

Lonelli-Marroquin D, Cruz D M, Nieto-Aguiarrolla A, Crua A V, Chen J, Torres-Castro A, Webster T J and Cholula-Díaz J L 2019 Starch-mediated synthesis of mono- and bimetallic silver/gold nanoparticles as antimicrobial and anticancer agents Int. J. Nanomed. 14 2171–90

Look D C 2001 Recent advances in ZnO materials and devices Mater. Sci. Eng. B 80 383–7

Madan H R, Sharma S C, Suresh U D, Vidya Y S, Nagabhushana H, Rajanaik H, Anantharaju K S, Prashantha S C and Sadananda Mayia P 2016 Facile green fabrication of nanoscale ZnO plates, bullets, flower, prismatic tip, closed pine cone: their antibacterial, antioxidant, photoluminescent and photocatalytic properties Spectrochim. Acta A 152 404–16

Majeed S, Danish S and Norazmi F S B 2018 Fungal derived zinc oxide nanoparticles and their antibacterial and anticancer activities against human Alveoli lung cancer A-549 cell line Adv. Sci. Eng. Med. 10 551–6

Malakozhundan B, Vaseheban B, Vijayakumar S, Pandisveli K, Kalandian M A R, Murugan K and Benelli G 2017 Biological therapeutics of Pongamia pinnata coated zinc oxide nanoparticles against clinically important pathogenic bacteria, fungi and MCF-7 breast cancer cells Microb. Path. 104 268–77

Manjua A and Rugavaran R 2015 Zinc oxide nanoparticles: opportunities and Challenges in important sciences Immunome Res. 11

Martin R M 1972 Piezoelectricity Phys. Rev. B 5 1607–13

Martinez-Carmona M, Gun'ko Y and Maria V R 2018 ZnO nanostructures for drug delivery and theranostic applications Nanomaterials 8 E268

Medina Cruz D, Mi G and Webster T J 2018 Synthesis and characterization of biogenic selenium nanoparticles with antimicrobial properties made by Staphylococcus aureus, methicillin-resistant Staphylococcus aureus (MRSA), Escherichia coli, and Pseudomonas aeruginosus J. Biomed. Mater. Res. A 106 1400–12

Medina Cruz D, William T-S, Zhang B, Huang X, Crua A V, Nieto-Aguiarrolla A Cholula-Díaz J L et al 2019 Citric juice-mediated synthesis of tellurium nanoparticles with antimicrobial and anticancer properties Green Chem. 21 1982–98

Medina-Cruz D, González M U, Tien-Street W, Fernández-Castro M, Vernet-Crúa A, Fernández-Martínez I, Martínez L, Huttel Y, Webster T J and García-Martin J M 2019 Synergic antibacterial coatings combining titanium nanocolumns and tellurium nanorods Nanomed.: Nanotechnol., Biol., Med. 17 36–46

Medina-Cruz D et al 2020 Green nanoparticle-based drug delivery systems for osteogenic disorders Expert Opin. Drug Deliv. 17 341–56

Mintcheva N, Aljalaih A A, Wunderlich W, Kalinchin S A and Iwamori S 2018 Laser-ablated ZnO nanoparticles and their photocatalytic activity toward organic pollutants Materials (Basel) 11 1127

Mohaddam B, Amin M M, Azizi S, Rahim R A, Arif A B, Navaderi M and Mohammad R 2017 Eco-friendly formulated zinc oxide nanoparticles: induction of cell cycle arrest and apoptosis in the MCF-7 cancer cell line Genes B 281

Mohamed Asik R, Gowdhami B, Mohamed Jaibar M S, Archunan G and Suganthy N 2019 Anticancer potential of zinc oxide nanoparticles against cervical carcinoma cells synthesized via biogenic route using aqueous extract of Gracillaria edulis Mater. Sci. Eng. C 103 109840

Mohammad M, Mirhosseini M, Shirzad M, Dehghani Hamdan A and Yazdani N 2015 Synthesizing Zinc nanoparticles by high-energy milling and investigating their antimicrobial effect J. Shahid Sadoughi. Univ. Med. Sci. 23 2070–82 http://jsuu.ssu.ac.ir/article-1-2933-ep.html

Moshfaghi E, Babaei A and Atiae A 2015 Synthesis of nano-structured La0.6Sr0.4Co0.2Fe0.8O3 perovskite by co-precipitation method J. Ultrafine Grained Nanostruct. Mater. 48 45–52

Moshfaghi E, Soltantabar P and Webster T J 2019 Nanotechnology and picochemistry Biomaterials in Translational Medicine eds Lei Yang, Sarit Bhaduri and Thomas Webster (Elsevier) pp 191–212

Muthuchamy N, Atchudan R, Edison T N J, Perumal S and Lee Y R 2018 High-performance glucose biosensor based on green synthesized zinc oxide nanoparticle embedded nitrogen-doped carbon steel Chem. 816 195–204

Nadarōgha H, Gänger A and Ince S 2018 Synthesis of nanoparticles by green synthesis method Int. J. Innov. Res. Eng. 16 6–9

Namvar F, Azizi S, Rahman H S, Mohammad R, Rasedee A, Soltani M and Rahim R A 2016 Green synthesis, characterization, and anticancer activity of hyaluronan/zinc oxide nanocomposite Onco.Targets Ther. 9 4549–59

Namvar F, Rahman H S, Mohammad R, Azizi S, Tahir P M, Chartand M S and Yeap S K 2015 Cytotoxic effects of biosynthesized zinc oxide nanoparticles on murine cell lines Evid. Based Complement. Altern. Med. 2015 1–11

Nasajpour A, Manjua S, Shree S, Mostafavi E, Sharifi R, Khalilpour A, Saidzadeh S et al 2017 Nanosynthesized fibrous membranes with rose spike-like architecture Nano Lett. 17 6235–40
Nayak P, Anbarasan B and Ramaprabhu S 2013 Fabrication of organophosphorus biosensor using ZnO nanoparticle-decorated carbon nanotube–graphene hybrid composite prepared by a novel green technique J. Phys. Chem. C 117 13202–9
Padalia H and Chanda S 2017 Characterization, antifungal and cytotoxic evaluation of green synthesized zinc oxide nanoparticles using *Ziziphus nummularia* leaf extract Artif. Cells, Nanomed. Biotechnol. 45 1751–61
Parhi P, Kramer J and Manivannan V 2008 Microwave initiated hydrothermal synthesis of nano-sized complex fluorides, KMF3 (K = Zn, Mn, Co, and Fe) J. Mater. Sci. 43 5540–5
Pavloupolou A, Oktay V, Vougas K, Louka M, Vorgias C E and Georgakilas A G 2016 Determinants of resistance to chemotherapy and ionizing radiation in breast cancer stem cells Cancer Lett. 380 485–93
Piras C C, Fernández-Prieto S and De Borggraewe W M 2019 Ball milling: a green technology for the preparation and functionalisation of nanocellulose derivatives Nanoscale Adv. 1 937–47
Prommalikit C, Mekprasart W and Pecharapa W 2019 Effect of milling speed and time on ultrafine ZnO powder by high energy ball milling technique J. Phys.: Conf. Ser. 1259 12023
Pudovkin M S, Zelenikhin P V, Syhtreva V, Morozov O A, Koryakovtseva D A, Pavlov V V Osin Y N et al 2018 Coprecipitation method of synthesis, characterization, and cytotoxicity of Pr3+: LaF3(CPr3 = 3, 7, 12, 20, 30%) nanoparticles J. Nanotechnol. 2018 9
Purwaningish S Y, Pratapa S, Trivikantoro and Darmanto 2016 Synthesis of nano-sized ZnO particles by co-precipitation method with variation of heating time AIP Conf. Proc. vol 1710 (AIP Publishing) p 30040
Raja A, Ashokkumar S, Pavithra Marthandam R, Jayachandiran J, Chandra Prasad K K, Ganapathi Raman K R and Swaminathan M 2018 Eco-friendly preparation of zinc oxide nanoparticles using tabernanema divaricata and its photocatalytic and antimicrobial activity J. Photochem. Photobiol. B 181 53–58
Rajeshkumar S, Venkat Kumar S, Ramiaah A, Agarwal H, Lakshmi T and Roopan S M 2018 Biosynthesis of zinc oxide nanoparticles using Mangifera indica leaves and evaluation of their antioxidant and cytotoxic properties in lung cancer (A549) cells Enzyme Microb. Technol. 117 91–95
Rane A V, Kanny K, Abitha V K and Thomas S 2018 Chapter 5—methods for synthesis of nanoparticles and fabrication of nanocomposites Synthesis of Inorganic Nanomaterials, ed S S Bhagyaraj, O S Olufemi, N Kalarikkal and S Thomas (Woodhead Publishing and Elsevier) pp 121–39
Rauf A, Mohd M O, Rehman F U, Khan A R and Husain N 2019 Bougainvillea flower extract mediated zinc oxide nanoparticles and its bioconjugate with curcumin for antimicrobial and anticancer activity Biomed. Pharmacother. 116 108983
Reddy A R, Mallika A N, Sowi Babu K and Venugopal Reddy K 2015 Hydrothermal synthesis and characterization of ZnO nano crystals Strain (e) 10 10–14
Ruddaraju L K, Pammil S V N, Vijay Kumar Pallala P N, Padavala V S and Kolapalli V R M 2019 Antibiotic potentiating ability and anti-cancer competence through bio-mediated ZnO nanoparticles Mater. Sci. Eng. C 103 109756
Sadraei R 2016 A simple method for preparation of nano-sized ZnO Res. Rev.: J. Chem. 2319 9849
Sahajela S P 2013 Sol–gel process and its application in nanotechnology J. Polym. Eng. Technol. 13 38–41
Sakka S 2013 Chapter 11.1.2—sol–gel process and applications Handbook of Advanced Ceramics, ed S Somiya 2nd edn (Oxford: Academic Press) pp 883–910
Salah N, Habib S S, Khan Z H, Memic A, Azam A, Alfaraj E, Zahed N and Al-Hamedi S 2011 High-energy ball milling technique for ZnO nanoparticles as antibacterial material Int. J. Nanomed. 6 863–9
Sanaei-Mehr Z, Davadi I and Namvar F 2018 Antiangiogenic and antiapoptotic effects of green-synthesized zinc oxide nanoparticles using Sargassum muticum algae extraction Cancer Nanotechnol. 9 5
Saravanan M, Gopinath V, Chaurasia M K, Syed A and Purushothaman N 2018 Green synthesis of anisotropic zinc oxide nanoparticles with antibacterial and cytotoxic properties Microb. Pathog. 113 57–63
Senthilkumar S R and Sivakumar T 2014 Green tea (*Camellia sinensis*) mediated synthesis of zinc oxide (ZnO) nanoparticles and studies on their antimicrobial activities Int. j. pharm. pharm. sci. 6 204
Shah M, Fawcett D, Sharma S, Tripathy S K and Poinern G E J 2015 Green synthesis of metallic nanoparticles via biological entities *In Vitro* and *In Vivo* J. Mater. Sci. 50 1259–50
Shao F, Yang A J, Yu D M, Wang J, Gong X and Tian H X 2018 Bio-synthesis of *Barleria gibsoni* leaf extract mediated zinc oxide nanoparticles and their formulation gel for wound therapy in nursing care of infants and children J. Photochem. Photobiol. B 189 267–73
Sharma D, Sabela M I, Kanchi S, Bisetty K, Skilton A A and Honarpargar B 2018 Green synthesis, characterization and electrochemical sensing of silymarin by ZnO nanoparticles: experimental and DFT studies J. Electroanal. Chem. 808 160–72
Sharma D, Sabela M I, Suvardhan Kanchi P S, Mella G S, Stenström T A and Bisetty K 2016 Biosynthesis of ZnO nanoparticles using *Jacaranda mimosaefolia* flowers extract: synergistic antibacterial activity and molecular simulated facet specific adsorption studies J. Photochem. Photobiol. B 162 199–207
Sharma P, Jang Y, Lee J-W, Park B C, Kim Y K and Cho N-H 2019 Application of ZnO-based nanocomposites for vaccines and cancer immunotherapy Pharmaceuticals 12 1016
Sharmila G, Muthukumarana C, Sandiya K, Santhiya S, Sathiya Pradeep R, Manoj Kumar N, Suryanarayanan N and Thirumurugurum M 2018 Biosynthesis, characterization, and antibacterial activity of zinc oxide nanoparticles derived from Bauhinia tomentosa leaf extract J. Nanotechnol. Chem. 5 293–300
Sharmila G, Thirumurugurum M and Muthukumarana C 2019 Green synthesis of ZnO nanoparticles using *Tecoma castanifolia* leaf extract: characterization and evaluation of its antioxidant, bactericidal and anticancer activities Microchem. J. 145 578–87
Shubha P, Lakithi Gowda M, Namratha K, Manjunatha H B and Byrrapa K 2019 *In vitro* and *in vivo* evaluation of green-photocatalytic synthesized ZnO nanoparticles J. Drug Deliv. Sci. Technol. 49 692–9
Siegel R L, Miller K D and Jemal A 2019 Cancer statistics, 2019 CA: Cancer J. Clin. 69 7–34
Singh A, Singh N B, Afzal S, Singh T and Hussain I 2018 Zinc oxide nanoparticles: a review of their biological synthesis, antimicrobial activity, uptake, translocation and biotransformation in plants J. Mater. Sci. 53 185–201
Somu P and Paul S 2018 A biomolecule-assisted one-pot synthesis of zinc oxide nanoparticles and its bio conjugate with curcumin for potential multilayered therapeutic applications New J. Chem. 43 11934–8
Sonawane G H, Patil S P and Sonawane S H 2018 Chapter 1—nanocomposites and its applications Applications of Nanomaterials, ed S S Bhagayaraj, O S Olufemi, N Kalarikkal and S Thomas (Woodhead Publishing and Elsevier) pp 1–22
Stan M, Popa A, Toloman D, Silipas T D and Vodnar D C 2016 Antibacterial and antioxidant activities of ZnO nanoparticles synthesized using extracts of *Allium sativum*, *Rosmarinus officinalis* and *Ocimum basilicum* Acta Metall. Sin. (Engl. Lett.) 29 228–36
Stefly K, Shanthi G, Maroky A S and Selvakumar S 2018a Potential bactericidal activity of S. Nux-vomica–ZnO nanocomposite against multidrug-resistant bacterial pathogens and wound-healing properties J. Trace Elem. Med. Biol. 50 229–39
Steffy K, Shanthi G, Marovy A S and Selvakumar S 2018b Enhanced antibacterial effects of green synthesized ZnO NPs using aristolochia indica against multi-drug resistant bacterial pathogens from diabetic foot ulcer Int. Infect. Public Health 11 463–71

Stott W T, Radtke B J, Linscombe V A, Mar M H and Zeisel S H 2004 Evaluation of the potential of triethanolamine to alter hepatic choline levels in female B6C3F1 mice Toxicol. Sci. 79 242–7

Su Y-L and Hu S-H 2018 Functional nanoparticles for tumor penetration of therapeutics Pharmaceuticala 10 E193

Suresh D, Nethravathi P C, Rajanaike U H, Nagabhushana H and Sharma S C 2015 Green synthesis of multifunctional zinc oxide (ZnO) nanoparticles using Cassia fistula plant extract and their photodegradative, antioxidant and antibacterial activities Mater. Sci. Semicond. Process. 31 446–54

Suresh J, Pradhesh G, Alexramani V, Sundararajan M and Hong S I 2018a Green synthesis and characterization of zinc oxide nanoparticles using inulin plant (Costus pictus D. Don) and investigation of its antimicrobial as well as anticancer activities Adv. Nat. Sci.: Nanosci. Nanotechnol. 9 15008

Suresh J, Pradhesh G, Alexramani V, Sundararajan M and Hong S I 2018b Green synthesis and characterization of zinc oxide nanoparticles using inulin plant (Costus pictus D. Don) and investigation of its antimicrobial as well as anticancer activities Adv. Nat. Sci.: Nanosci. Nanotechnol. 9

Udayabhanu G N, Nagabhushana H, Basavaraj R B, Raghav G K, Suresh D, Rajanaike H and Sharma S C 2016 Green, nonchemical route for the synthesis of ZnO superstructures, evaluation of its applications toward photocatalysis, photoluminescence, and biosensing Cryst. Growth Des. 16 6828–40

Umar H, Kavaz D and Rizaner N 2018 Biosynthesis of zinc oxide nanoparticles using Alliazia lebeck stem bark, and evaluation of its antimicrobial, antioxidant, and cytotoxic actions on human breast cancer cell lines Int. J. Nanomed. 14 87–100

(US), National Institutes of Health, and Biological Sciences Curriculum Study 2007 Understanding cancer NIH Curriculum Supplement Series (available at: www.ncbi.nlm.nih.gov/books/NBK20362/)

Vahed Z, Sepidne N F, Samiei M, Dizaj S M and Sharifi S 2019 Targeted cancer drug delivery with aptamer-functionalized polymeric nanoparticles J. Drug Target. 27 292–9

Vijayakumar S and Vaseeharan B 2018 Antibiofilm, anti cancer and ecotoxicity properties of collagen based ZnO nanoparticles Adv. Powder Technol. 29 2331–45

Vijayakumar S, Vaseeharan B, Malaikozhundan B and Shobhiya M 2016 Laurus nobilis leaf extract mediated green synthesis of ZnO nanoparticles: characterization and biomedical applications Biomed. Pharmacother. 84 1213–22

Vimala K, Shanthi K, Sundarraj S and Kannan S 2017 Synergistic effect of chemo-phothermal for breast cancer therapy using folinic acid (FA) modified zinc oxide nanosheet J. Colloid Interface Sci. 488 92–108

Wang B, Wei Q and Qu S 2013 Synthesis and characterization of uniform and crystalline magnetite nanoparticles via oxidation-precipitation and modified co-precipitation methods Int. J. Electrochem. Sci. 8 3786–93

Wang J, Gao S, Wang S, Zhaonan X and Wei L 2018 Zinc oxide nanoparticles induce toxicity in CAL 27 oral cancer cell lines by activating PINK1/Parkin-mediated mitophagy Int. J. Nanomed. 13 3441–50

Wang X 2012 Piezoelectric nanogenerators-harvesting ambient mechanical energy at the nanometer scale Nano Energy

Wang Y, Zhang X, Wang A, Xiang L, Wang G and Zhao L 2014 Synthesis of ZnO nanoparticles from microemulsions in a flow type microreactor Chem. Eng. J. 235 191–7

Wang Y D, Ma C L, Sun X D and Li H D 2002 Preparation of nanocrystalline metal oxide powders with the surfactant-mediated method Inorg. Chem. Commun. 5 751–5

Wang Z L and Song J 2006 Piezoelectric nanogenerators based on zinc oxide nanowire arrays Science 312 242–6

Winter P M, Lanza G M, Wickline S A, Madou M, Wang C, Deotare P B Loncar M and Wang Z L 2006 Piezoelectric nanogenerators based on zinc oxide nanowire arrays Semicond. Process. 9 2331–45

Wu H and Zhang S 2018 Chitosan-based zinc oxide nanoparticles for enhanced anticancer effect in cervical cancer: a physicochemical and biological perspective Saudi Pharm. J. 26 205–10

Yadav E, Singh D, Yadav P and Verma A 2018 Ameliorative effect of biofunctionalized ZnO nanoparticles of: TriantHEMA portulacastrum Linn. on dermal wounds via removal of oxidative stress and inflammation RSC Adv. 8 21621–35

Yadav M et al 2018 Current developments in green synthesis of metallic nanoparticles using plant extracts: a review Artif. Cells, Nanomed., Biotechnol. 46 5336–543

Yan Z and Jiang L Y 2011 The vibrational and buckling behaviors of piezoelectric nanobeams with surface effects Nanotechnology 22 245703

Yang G and Park S J 2019 Conventional and microwave hydrothermal synthesis and application of functional materials: a review Materials (Basel) 12 1177

Yang L 2015 2—Nanotechnology-enhanced metals and alloys for orthopedic implants Nanotechnology-Enhanced Orthopedic Materials, ed L. Yang (Oxford: Woodhead Publishing) pp 27–47

Yuvakumar R, Suresh J, Saravanakumar B, Joseph Nathanael A, Hong S I and Rajendran V 2015 Rambutan peels promoted biomimetic synthesis of biospired zinc oxide nanochains for biomedical applications Spectrochim. Acta A 137 230–8

Zhang J and Meguid S A 2017 Piezoelectricity of 2D nanomaterials: characterization, properties, and applications Semicond. Sci. Technol. 32

Zhang J, Wang C and Bowen C 2014 piezoelectric effects and electromechanical theories at the nanoscale Nanoscale

Zhang L, Jiang Y, Ding Y, Daskalakis N, Jeucken L, Povey M, O’Neill A J and York D W 2010 Mechanistic investigation into antibacterial behaviour of suspensions of ZnO nanoparticles against E. coli J. Nanopart. Res. 12 1623–36

Zheng Y, Wang Z, Peng F and Li F 2016 Application of biosynthesized ZnO nanoparticles on an electrochemical H2O2 biosensor Braz. J. Pharm. Sci. 52 781–6

Zhu X, Pathakoti K and Hwang H-M 2019 Green synthesis of titanium dioxide and zinc oxide nanoparticles and their usage for antimicrobial applications and environmental remediation Green Synthesis, Characterization and Applications of Nanoparticles (Elsevier) pp 223–63