Synthesis of Different Nanoparticles for Biological Application

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Abstract. Compared with traditional materials, the application of nanomaterials in biomedical fields will bring many excellent performances. This review summarizes some new developments and applications of nanoparticles in recent years from the perspective of biology and medicine, including magnetic resonance imaging, treatment for Alzheimer's disease, diabetes and plant infection disease, oxygen-releasing scaffolds, engineered water nanostructures (EWNS) based sanitizer, drug loading system and cancer treatment. This article summarized and discussed the synthesis methods, characterization, advantages, and applications based on these aspects. Introducing nanoparticles into biomedical fields can provide useful ideas for applying nanoparticles in biology and pharmacy in the future.

1. Introduction

Nanomaterials as relative novel research areas have been flourishing to a great extent over the past century. Due to the interdisciplinary property of nanomaterials-based technology, there are multitudinous science subjects, such as physics, chemistry, biology, and some engineering sciences, applying or taking advantage of its unique characteristics to achieve theoretical research and practical application goals.

This cutting-edge and novel technology is primarily based on different forms and types of nanomaterials. Nanomaterials are defined as materials with a size that is less than 100 nm at least in one dimension [1]. Based on their dimensions, the common nanomaterials can be divided into different groups, such as zero-dimension (0-D) nanomaterials, one-dimension (1-D) nanomaterials two-dimensions (2-D) nanomaterials, and three-dimensions (3-D) nanomaterials. Among various nanomaterials, nanoparticles (0-D nanomaterials) are representative examples and one of the most widely used nanomaterials. Caron-based and metal-based nanoparticles are the main forms of nanoparticles, such as fullerenes, ZnS, ZnO, CdSe, CdTe nanoparticles [1]. The most intriguing things about the nanoparticles are their critical sizes (less than 100 nm), which show fascinating chemical and physical properties leading to advanced magnetic, optical, electronic, mechanical, and structure uses compared to the original bulk substance [2]. Specifically, small size can result in a large specific surface area, containing a large proportion of the surface atoms can largely increase the chemical reaction reactivity, efficiency, and structural stability. The band structure is also based on particle size. For metal structures, smaller size causes a larger separation of energy levels, which leads to a change from the metallic character of metal changes into a semiconductor nature. Also, increasing the surface area and decreasing the grain size of magnetic materials, like Fe-based materials, can acquire larger coercivity and saturation magnetization, which indicates a better magnetic property. Thus, there is a great potential...
for the nanoparticles to be used in the biological fields for acting as catalysts, indictors, transducers, and carriers.

Nanoparticles or structures combined with other substances can be facilitated by sensors or detected machines in the biomedical field. Lunin et al. carried a research that focused on iron oxide nanoparticles [3]. Because of their special superparamagnetic, antiferromagnetic, and weak ferromagnetic properties can be used in magnetic resonance imaging (MRI). Pumera et al. review platinum and gold nanoparticles on enhancing detection limits of electrochemical DNA biosensors [4]. Khorshidi et al. [5] first used nano-sized calcium peroxide and nano-sized fibres to successfully experiment in building a reliable oxygen releasing scaffold conducive to bone cell formation. In terms of disease treatment, non-toxic, biodegradable, and easily prepared nanoparticles can be used in diagnostic, therapeutic, and theranostic settings [6]. One approach that has great potential is to use ZnO nanoparticles as a treatment for diabetes, as it could act like insulin in lowering blood sugar levels [7]. In addition, some studies in recent years have shown great prospects for iron oxide nanoparticles in treating anaemia, cancer and Alzheimer's disease [6, 8]. Nanomedicine has many features, such as higher solubility, utilization, efficacy and low toxicity. However, the problem of low drug loading, inefficient drug delivery, and high cost of nano drugs limit their wide application [9]. Therefore, several nano drug loading systems have been developed to solve the problem. Also, the Coordination-driven self-assembly nanoparticles (NPs) as effective and problem-solving materials are increasingly used in cancer treatment and diagnosis. Moreover, nanoparticles possess an anti-pathogen characteristic that is extremely beneficial to both agriculture and infectious-related fields. Researchers recently found that engineered water nanostructures (EWNS) produced by electrospaying of water vapor have a possible way to kill hospital pathogens as well as various types of food-borne bacteria [10, 11]. ZnO nanoparticles prepared by bio-assisted methods to be used to treat bacterial leaf blight (BLB) in rice [12] are reviewed in the article.

Thus, this article will mainly review and cover two commonly seen types of nanoparticles (ZnO NPs and Iron oxide NPs) and several NPs based systems’ synthesis processes combined with various applications in biological fields.

2. Applications of zinc oxide nanoparticle

2.1. Preparation method

Because of its unique structure and properties, zinc oxide nanoparticles (ZnO NPs) have been recognized as a safe material for humans and have been widely applied in plenty of fields, especially in the biological field. The synthesis of ZnO NPs can be based on physical and chemical methods, which could consume a large amount of energy and produce toxic substances and wastes that are difficult to dispose of in the preparation process. Therefore, researchers have explored bio-assisted synthetic methods, which could be efficient and environmentally friendly by using chemical substances from plants and microorganisms.

Ogunyemi et al. used a bacterium named *P. polymyxa strain SX3* to prepare ZnO NPs, based on the antibacterial effect of ZnO NPs [12]. After a series of purification, standing, centrifugation and filtration treatments, the extracts were taken to react with ZnO solution. Then the prepared ZnO NPs were purified through centrifugation and other treatments. Bayrami et al. used the extracts of a plant called *Vaccinium arctostaphylos L.* The fruits were dried and ground into powder and were soaked in ethanol solution [7]. After the plant extracts were mixed with zinc nitrate and adjusted the pH value to 10, the prepared ZnO NPs were centrifuged to gain the final products.

2.2. Antibacterial effects on treating plant disease

ZnO NPs has a relatively larger surface area, allowing them to exhibit higher reactivity and efficiency. In terms of biological applications, it has the characteristics of high efficiency, environmental friendliness and lowers adverse reactions when combined with organisms. The two main properties of ZnO nanoparticles in biological applications are antibacterial and anti-diabetes functions. The mechanism of ZnO NPs using in the biological application is that they can produce reactive oxygen...
species (ROS), which can penetrate holes in the cell membrane of bacteria, piercing the cell membrane and thus kill the bacteria. Based on these properties, researchers in this field have conducted a number of application experiments.

Ogunyemi et al. conducted experiments involved ZnO NPs to treat plant diseases [12]. *Xanthomonas oryzae pv. oryzae* (Xoo) is the bacterium that can cause bacterial leaf blight (BLB), one of the most destructive rice diseases occurring in rice-growing areas worldwide, resulting in the loss of about 50% of the economic plant segment.

![Figure 1. TEM images of Xoo strain GZ 0006 cells treated with double distilled water (Control) and ZnO NPs (A) [12]](image)

They tested the antibacterial properties of Xoo strain GZ 0006 by ZnO NPs synthesized by *P. Polymyxa* strain SX3. Rice was infected with Xoo bacteria, and ZnO NPs (16.0 µg/mL) were sprayed on the Xoo-infected leaves. The experimental results show that ZnO NPs have an effective anti-bacterial effect on Xoo (Figure 1), and the average diameter of bacteria removal on infected leaves is 17.0 mm. Another study presented by Nazir et al. showed that ZnO NPs synthesized from *S. marianum* extract and callus extract were highly resistant to both *Klebsiella pneumoniae* and *Bacillus subtilis* [13].

2.3. Anti-diabetic activity

Bayrami et al. evaluated the anti-diabetic activity of bio-synthesized ZnO NPs in diabetic rats and compared it with chemically synthesized ZnO NPs [7]. Alloxan-induced diabetic male Wistar rats were divided into two groups: a healthy experimental group and a diabetic control group. They were given insulin (10 U/kg), chemically synthesized zinc oxide (8 mg/dL), plant extract (150 mg/dL), and bio-synthesized zinc oxide (8 mg/dL). The fasting blood sugar (FBS) level of diabetic rats induced by bio-synthetic ZnO NPs was significantly reduced, followed by chemically synthesized ZnO NPs, insulin and plant extracts, demonstrating that bio-synthetic ZnO NPs have a considerable anti-diabetes effect on alloxan diabetic rats.

3. Applications of iron oxide nanoparticles

3.1. Preparation method

Iron oxide nanoparticles (IONPs) such as magnetite (Fe$_3$O$_4$), magnetite (γ-Fe$_2$O$_3$) and mixed ferrite, have a wide range of applications due to their special magnetic properties and a variety of synthetic methods which can be used to synthesize IONPs in different sizes and shapes. There are four main preparation methods for IONPs: co-precipitation, thermal decomposition, microemulsion, and sol-gel Methods [6]. Co-precipitation is one of the most simple, effective, and widely used synthetic methods that precipitate Fe$^{2+}$ and Fe$^{3+}$ aqueous solutions simultaneously by adding weak or strong bases. However, the nanoparticles produced by co-precipitation are generally dispersed and exhibit low
crystallinity. To overcome these shortcomings, the researchers tried to improve the method by using static magnetic fields and ultrasonic waves in preparation and using alkanolamine as a substrate.

Thermal decomposition is the reaction of organic iron precursor with heat in an organic solvent in the presence of a stable surfactant [14]. IONPs with controllable size, shape and high crystallinity can be prepared by this method. However, this method is not environmentally friendly because toxic chemicals such as chloroform are used in the synthesis process.

The microemulsion method uses isotropic dispersion systems of two immiscible liquids as the nano/micro-reactor to provide the environment for the nucleation and controlled growth of nanoparticles. The main advantage of this method is that the size of the nanoparticles can be controlled by changing the size of the micelles, but the temperature required for the reaction is low, which leads to a decrease in the yield of IONPs.

The sol-gel method uses tetraethyl orthosilicate (TEOS) in ethanol and 30% H2O2 aqueous solution to hydrolyze and condensate with Fe3+ solution to form colloidal sol, and then gelatinize the sol by chemical reaction to obtain IONPs. Since this method is performed at room temperature, further heat treatment is required, and the method also produces contamination by by-products.

3.2. Application of IONPs in magnetic resonance imaging

After surface modification, IONPs can be transformed into superparamagnetic iron oxide nanoparticles (SPION), which can be used in magnetic resonance imaging (MRI) and diagnosis. Contrast agents in MRI can enhance the image contrast of the transported tissue/region by shortening the water protons spin-lattice T1 or spin-spin T2 relaxation time. In contrast, SPION works primarily through the latter because of their superparamagnetic behaviour. SPION can be applied in a wide range of diagnostic processes, such as the location and metastasis of tumours in the liver, spleen, and lymph nodes.

IONPs can be used to treat cancer in two ways: (1) producing ROS or topical heat, and (2) using in chemotherapy drugs [15]. Some IONPs can enhance cellular uptake ability and antiproliferative activity of cancer cells during chemotherapy, reducing the toxicity of some cancer drugs, such as docetaxel, mitoxantrone, cisplatin and carboplatin. One of the IONPs called Ferumoxytol is administered intravenously to treat anemia in patients with chronic kidney disease (CKD) [6]. After injection, iron levels in macrophages in the blood, liver, spleen, and other parts of the body will increase, promoting the production of red blood cells and alleviating anemia.

3.3. Application of IONPs in Alzheimer's disease

Alzheimer's disease is a neurodegenerative disease in which the structure and function of the nervous system of patients deteriorate over time. Elimination of tau-induced microglia activation can delay neurodegenerative sclerosis. According to Luo et al. [8], a fibrin γ̂377-395 peptide that can inhibit microglia activity was combined with γ-Fe3O3 nanoparticles with a diameter of 21±3.5 nm, and the conjugate specifically inhibited microglia activity in rTg4510 Tau mutant mice, which could be more efficient than free peptides.

4. Review of NPs based biomedical engineering systems

4.1. Nanofibers for oxygen-releasing scaffolds

For tissues such as bone and muscle, oxygen is the key to the survival of these tissues. Under the condition of inappropriate oxygen concentration, the viability and function of highly aerobic tissues such as muscle and bone will be reduced. Therefore, compared with a normal scaffold, a scaffold that releases oxygen can overcome these problems, and it can even accelerate bone regeneration [16]. Many studies use calcium peroxide (CPO) as the main component of the scaffold to release oxygen, because CPO can better control the rate of oxygen release than other peroxides. In previous studies, micron sized CPO was added to scaffolds and proved to have good oxygen release properties [17]. Recently, some studies have prepared and tested the addition of nano-sized CPO to scaffolds.
Khorshidi et al. used nano-sized calcium peroxide infiltrated into nanofibers to make scaffolds [18]. As shown in Figure 2, these nanofibers are defect free and uniform. Then the oxygen release capacity of this fiber was detected using a blood gas analyzer. Results show that this fiber can release oxygen for at least 14 days. The study also used rabbit stem cells for cell culture. The results showed that there were more living cells on the fibers loaded with nano CPO particles than the control group. This is because the fiber loaded with nano CPO particles provides a more suitable oxygen concentration for cell survival. The detection shows that the calcium ion concentration produced by this fiber does not exceed 0.36 mM, and hydroxide ion concentration does not exceed $10^{-7.03}$ M, which was lower than the cytotoxicity level.

Abudula et al. prepared poly(glycerol sebacate)/poly(ε-caprolactone) (PGS/PCL) nanofiber scaffolds with nano calcium peroxide [19]. Then they tested the antibacterial ability of the nanofibers. The results showed that a high concentration of CPO had a significant inhibitory effect on *Staphylococcus aureus*. This may be due to the decomposition of CPO to produce hydrogen peroxide and calcium hydroxide, which has a killing effect on bacteria. Moreover, the metabolic rate of cells was higher on nano scaffolds containing a higher concentration of CPO. This is because the oxygen produced by the scaffold can promote cell metabolism, and the hydrogen peroxide and calcium hydroxide produced are not enough to poison cells. Therefore, this oxygen releasing antibacterial scaffold can eliminate bacteria without damaging human cells.

4.2. *Engineered water nanostructures*

Infectious diseases have become an increasing concern for many countries due to their relatively easy transmission ways-direct contact, indirect contact, and airborne routes, as well as their tenacious vitality. Some pathogens are even able to survive on an inanimate surface for weeks. There are about 2 million Americans who develop hospital-acquired infections (HAI) and caused the total financial burden to be 35 to 45 billion dollars annually [20]. To effectively inactivate harmful pathogens, a novel method containing engineered water nanostructures (EWNS) was introduced, and its basic structure is shown in Figure 3. Compared to traditional inactivation methods, including ultraviolet radiation (UV), high efficiency particulate (HEPA) air filters and chemical agents, which are usually companies by problems of health risks, high costs and drug resistance.
Figure 3. The basic structure of EWNS [25]

Figure 4 shows the EWNS producing facilities. When the system works, the pumping area increases the air pressure and pushes the solution in the reservoir to flow into the steel capillary, where a high voltage is applied to generate EWNS [20]. Electrospray will cause the bulk liquid to break into several small water droplets under high electronic fields due to the Rayleigh effect. When the surface charge density increases, a smaller distance of charges will bring larger unstable energy, forcing the breakup of the water droplets. Water droplets will be continual to break up until the Rayleigh diameter, under which the breaking force will be countered by the surface tension of the droplets [10]. Moreover, the operational environment temperature and relative humidity influence the experiment, which needed to be kept within a proper range to reach the best research results. The previous results show that engineered water nanostructure-an aggregation large amount of water droplets with each has a sphere shape with approximately 25 nanometers in diameters [10]. Also, the EWNS are treated as a nano-carrier platform that can be utilized for precise and targeted delivery of active ingredients (antipathogen agent).

Figure 4. The simple EWNS producing facilities [20]

The basic mechanism of the inactivation process is using a strong oxidizing property of reactive oxygen species (ROS) inside the EWNS, like peroxide, to react with the outer membrane of the bacteria, including side chains and nucleic acids of polyunsaturated fatty acids associated with phospholipids, enzymes, and membrane receptors, and form lipid peroxide (LPO). This membrane metamorphic will cause changes in the fluidity and permeability of the cell membrane and eventually lead to changes in cell structure and function to achieve the purpose of inactivation. As shown in Figure 5, the outer membrane of S. marcescens bacteria exposed under the EWNS environment has been almost completely damaged (over 98% compared to the control group) [20].
4.3. Drug loading system

In recent years, with the development of nanotechnology, nanotechnology has had more applications in medicine. Making materials into nano scale will bring many new properties to drugs, such as reducing toxicity, increasing effect and better controlling drug release. However, drug loading and difficult control of drug release limit the further development of nanomedicine. Therefore, some systems and strategies are needed to improve drug loading capacity [9]. New research results bring new ideas and drug loading methods for nano drugs.

Bao et al. designed pH-responsive carbon quantum dots–doxorubicin nanoparticles, which load nanodrugs into cells and kill tumors [21]. The prepared N-CQD particles were evenly distributed with an average size of 4.6 nm. Through spectral characterization, the prepared N-CQD has good fluorescence properties. The results showed that the drug release rate was faster in an acidic environment. This feature can make the drug release in the specific acidic environment of cancer cells and reduce the toxicity to normal cells. This drug loading system can reduce the normal cytotoxicity and increase the inhibitory effect on cancer cells through the cytotoxicity test. And this nano drug loading system can be well absorbed by cancer cells to eliminate cancer cells. These results make this drug loading system have a wide application prospect in the treatment of cancer.

Mesoporous silica nanoparticles (MSN) has been proved to be an effective and harmless drug loading system because the porous structure of MSN can interact with active molecules and metal nanoparticles, and MSN has good biocompatibility and will not poison organisms [22]. In another study on nano drug loading systems, Huang et al. designed a gated mesoporous carbon nanoparticles (MCN) system for drug loading because MCN and MSN have similar properties [23].

In addition, ZnO QD can be dissolved into zinc ions under acidic conditions, which can be used for pH triggered drug release. The results showed that the increase of temperature and the decrease of pH could increase drug release. ZnO-gated MCNs loaded with a small number of drugs have strong cytotoxicity to tumour cells. And the drug was released inside the cell but not outside the cell. These results show that ZnO QD gated MCNs Drug loading system has great development prospects in tumour therapy.

4.4. Cancer treatment

Among all the cancer treatment methods, biological gene therapy for cancer is highly regarded, which has high targeting and remarkable effect and basically does not damage normal tissues when killing cancer cells. It is considered the most promising diagnosis and treatment technology and the most effective method for future cancer diagnosis and treatment. However, DNA that cannot penetrate the membrane is one of the major technical obstacles for accurate gene therapy. As shown in Figure 6, using a hybrid nanomaterial containing genetic materials and nanocarriers [24], such as cationic polymeric
and liposomal systems, can provide a possible and effective way to deliver functional DNA to both in vitro and in vivo of cells.

Li et al. used Fe (II) as the functional nanocarriers integrated with the DNA and formed Fe-DNA nanospheres via the coordination-driven self-assembly method [24]. The Fe–DNA hybrid nanospheres were synthesized by using the one-pot reaction of DNA molecules and Fe(II) ions. After examining through scanning electron microscopy (SEM) and transmission electron microscopy (TEM), spherical Fe-DNA nanoparticles can be observed clearly and obviously. The self-assembly process probably happens because of the strong coordination interactions between the abundance of binding sites, such as the nitrogen, oxygen atoms, and phosphorus residual, on the nucleobases and metal ions. The CpGs, which is "an immunostimulatory DNA with unmethylated cytosine–guanine motifs", and macrophages are the primary targets of CpG for cancer therapy [24], was used as a DNA model in the experiment to assess the system’s delivery efficiency. The experimental group was labeled with Cy5, a fluorescence indicator, to highlight the absorption process. The results showed that Fe–CpG NPs had higher uptake efficiency than free CpG and Fe–CpG NPs treated group largely increase bioactivities levels of macrophages. Also, they found that the formation size of Fe-DNA NPs could be generated in a gradually small range if the reaction was a time within 2 minutes under the observation of a time-dependent transmission electron microscope [24].
5. Conclusion

This paper mainly summarizes the biological applications of nanomaterials and their corresponding synthetical methods in recent years. Two systems are introduced, respectively: (1) zinc oxide nanoparticles and iron oxide nanoparticles. The former synthesized by the bio-assisted method can be used for antibacterial and anti-diabetic treatment, including plant infection disease in rice and diabetes in mouses. At the same time, the latter plays an important role as contrast agents in magnetic resonance imaging and in delaying the development of Alzheimer's disease. (2) nanostructure-based biomedical engineering systems, including four parts: (i) the production of oxygen-releasing scaffolds, which could accelerate the regeneration and offer oxygen for bones and muscles, and PGS/PCL nanofiber scaffolds with antibacterial effect using calcium peroxide nanoparticles; (ii) engineered water nanostructures (EWNS) as a fungicide platform for inactivated pathogens; (iii) nano-based systems for loading drugs, for example, pH-responsive carbon quantum dots-doxorubicin nanoparticles, mesoporous silica nanoparticles and ZnO QD; and (iv) biological gene therapy for cancer by combining DNA with nanomaterials. Compared with ordinary materials, these nanoparticles and nano-structure systems often show higher efficiency and better effects, leading to a wide development prospect of nanomaterials in biological applications.

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