

PATHWAYS TO GREEN NANOMATERIALS:

**PLANTS AS RAW MATERIALS,
REDUCING AGENTS AND
HOSTS**

Li Fu

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Pathways to Green Nanomaterials: Plants as Raw Materials, Reducing Agents and Hosts

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**Pathways to Green Nanomaterials:
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FOREWORD

I was honored to receive an invitation from Dr. Li Fu to write a foreword for his new book.

The development of nanomaterials within the last few decades has been a very exciting chapter in the history of science. Not only material scientists but also a large number of physicists and chemists are involved in this field. This gives the illusion that nanomaterials are man-made materials and that there is no connection between them and nature. In fact, the development of nanomaterials is inextricably linked to the natural world. Nanotechnology is neither a mystery, nor it is exclusive to humans. Nanomaterials and nanotechnology have existed since the dawn of the universe. In the long evolution of the earth, the natural world, from the pavilion of the lotus, ugly spiders, weird sea snake tail, flying bees, water striders on the surface, shells in the sea, gorgeous butterflies, palm-sized geckos, to bacteria, each of them is a master of nanotechnology. These plants and animals are used to make a living or to defend against enemies through their exquisite nanotechnology skills. Their tenacious survival in nature not only enriches the world around us, but also brings countless inspirations and insights to modern nanotechnologists.

In the 1970s, Barthelot, a botanist at the University of Bonn in Germany, was studying plant foliage and found that smooth leaf surfaces were dusty and had to be cleaned before they could be viewed under a microscope, while leaf surfaces such as lotus leaves were always dry and clean. They used artificial dust particles to contaminate the foliage of eight types of plants, including magnolia, forest beech, lotus, taro, and kale. The condition of the dust particles remaining on the foliage was observed after washing with artificial rain for 2 min. Experiments have found that some plants have up to 40% or more contaminants left on their foliage. The percentage of contaminant residues on the foliage of plants such as lotus is less than 5%. The presence of very complex multiple nano- and micron-scale ultrastructures on the surface of lotus leaves can be observed by electron microscopy. The surface of the lotus leaf has some tiny waxy particles and is covered with numerous protrusions of about 10 microns in size. The surface of each protrusion is covered with even finer villi of only a few hundred nanometers in diameter. The part between the protrusions is filled with air. This forms a layer of air that is only a nanometer thick immediately adjacent to the leaf surface. This makes it possible for dust and rain, which are much larger in size than this structure, to fall on the leaf surface without coming into direct contact with it on a large scale, but with a very thin layer of air. This also makes it possible for them to contact only a number of raised points on the leaf surface. This is the result of the long-term evolution of living organisms in nature. It is this special nanostructure that keeps the surface of lotus leaves clean from water droplets.

However, nanomaterials also pose some challenges for us. The amount of nanoparticles entering the ecosystem and human living environment is increasing, and the number of species is gradually increasing, and all these nanoparticles will have more or less effects on living organisms. In studies on the effects on animals, scientists have found that nanomaterials have certain neurotoxic effects, with different forms of toxicity on different organs, and determined by the dose and duration of action. In addition to the safety risks to animals or humans, nanomaterials also have an impact on plants. Although research on the effects of nanomaterials on plant growth is just beginning, it can be expected that nanoparticles will have varying degrees of impact on plants and even ecosystems. Nanomaterials and plants have a delicate relationship. Sometimes you will find them "mutually beneficial", while at other times, they seem "incompatible".

Initially, people wanted to know how nanomaterials would affect the growth of plants if they entered water bodies and ecological environments. The first concern was the effect on the seed germination rate. For example, moderate concentrations of TiO₂ nanoparticles were found to promote the germination of some seeds, while having no effect on others. The effect of nanomaterials on seed germination is related to the nature of the nanoparticles. Some scientists have confirmed that Zn nanoparticles and ZnO nanoparticles can significantly inhibit seed germination in rye and rape, and root growth in corn and cucumber. Meanwhile, other scientists have found that TiO₂ nanoparticles can enhance photosynthesis in spinach, and this has been shown to be closely related to its photocatalytic activity. For example, spraying 0.35% TiO₂ nanosol on cucumber foliage can significantly reduce the leaf spot area, and promote the synthesis of chlorophyll and carotene.

There are many possibilities for the relationship between nanomaterials and plants. In this book, Dr. Li Fu focuses on the relationship between nanomaterials and plants. In his book, he tells more about the relationship between plants and nanomaterials. For example, plants can be used as effective reducing agents in the synthesis of nanomaterials. Plants can also be carbonized at high temperatures into very useful nanomaterials. Plants can even synthesize nanomaterials *in vivo*. This is not only an academic book that combines the scientific research results between botany and nanomaterials; it is also an interesting work of science and popularization. I believe that every reader can also feel the fun brought by nature from the book

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PREFACE

As an important strategic emerging technology for mankind in the 21st century, nanotechnology has become an important driving force for innovation and economic and social development in many fields. Nanomaterials are widely used in sensors, medical imaging, diagnostics and therapy, antimicrobial agents and drugs, catalysts, optoelectronics, environmental remediation and in other fields for their unique and excellent properties and show good prospects for application. The most prominent properties of nanoparticles are small size, high specific surface area and high reactivity, which open the way for the design and application of new materials, new systems and new devices. Nanomaterials are inevitably released into the environment during production, use and disposal, thus interacting with plants. The presence of nanomaterials may affect plant growth, and plant metabolic processes and uptake and accumulation may in turn affect the environmental behavior and fate of nanoparticles, even amplifying them in the food chain. Several studies have also shown that plant or algae extracts can be important raw materials for the synthesis of nanomaterials. Compared with other chemical raw materials, plant extracts have the advantages of being mild and environmentally friendly. This book focuses on the connection between nanomaterials and plants, and discusses this topic in the following aspects.

There are two main types of strategies for nanoparticles' synthesis: "top-down" and "bottom-up". "Top-down" refers to the mechanical and physical reduction in the size of the bulk material to the nanometer scale. "Bottom-up" refers to the self-assembly of atoms into nuclei and their growth into nanoparticles, including chemical and biological synthesis methods. In recent years, bio-mediated green nanosynthesis technology (biosynthesis technology) is gaining more and more attention in the field of nanoparticle synthesis and preparation, which has outstanding advantages such as mild reaction conditions, safety, no need for special expensive equipment and harmful chemicals, and good biocompatibility of the synthesized products. Among them, the synthesis of nanomaterials using plants usually requires only the addition of ionic precursors of the material and the reduction or chemical transformation of the ions by biomolecules in the plant to synthesize nanoparticles. The synthesized nanoparticles can also be modified by biomolecules in the system to obtain high biocompatibility and new properties, which are beneficial to broaden the application of nanoparticles. The first chapter of this book summarizes the research progress in the synthesis methods and applications of different plant-mediated nanomaterials. The content also analyzes the mechanism of nanomaterial biosynthesis. Plant synthesis regulation and application prospects are also explored.

Cellulose is a polysaccharide, a highly crystalline natural polymer linked by β -(1-4) glycosidic bonds to glucose units. It comes from a wide range of sources, including wood, cotton, ramie, crop straw, bamboo, *etc.* Amorphous cellulose and semi-crystalline cellulose can be removed from biomass resources by top-down methods of physical cutting and stripping, chemical acid digestion or oxidation treatment to obtain nanocellulose. Chapter 2 describes the structural properties and classification of nanocellulose, the sources of raw materials, and their structural characterization methods. Progress in the preparation process, methods, and applications of nanocellulose were also reviewed. Meanwhile, the future development direction of nanocellulose was prospected in order to provide a reference for its development and utilization.

Plant-derived biochar is a material derived from plant-derived biomass as a carbon source. It has a large specific surface area, high pore capacity, adjustable surface functional groups, and good environmental compatibility. Its raw material plant is inexpensive, widely available and

can be regenerated, making it a cheap and efficient adsorbent. Biochar was initially used only in agriculture. Nowadays, biochar has been applied in many different fields, allowing this plant-based raw material to take full advantage of its positive effects. Chapter 3 describes the preparation and modification of biochar materials. The application of plant-derived biochar in different fields is also presented.

Exosomes are small nanoscale vesicles secreted from most cells. It has a phospholipid bilayer structure and contains DNA, small RNA (sRNA), proteins and other substances that carry proteins and nucleic acids and participate in intercellular communication. The diameter of plant exosomes is about 40-150 nm, which is similar to the morphological structure of animal exosomes. In plant cells, exosomal nanoparticles containing miRNAs, bioactive lipids, and proteins act as extracellular messengers to mediate intercellular communication in a manner similar to that of secreted exosomes in mammalian cells. These exosomal nanoparticles are edible and can be used for the effective delivery of specific drugs that can be used as natural therapies against various diseases. Chapter 4 describes the research progress of plant exosomes and their potential applications

Plants, as an important part of the ecosystem, are the first step for nanoparticles to enter the food chain. Therefore, it is significant to study the production and transport of metal nanoparticles by plants. Based on the nanoparticles observed in plants, some studies conclude that nanoparticles can be directly absorbed by plants in the form of particles. Other studies concluded that plants would synthesize the absorbed metal ions *in vivo*. Chapter 5 mainly summarizes the reports on the synthesis of nanomaterials by plants *in vivo*. How nanoparticles are transported in the plant body is also discussed.

Plant polyphenols have strong antioxidant properties, along with anti-cancer, anti-aging, anti-cardiovascular and other effects, so they are widely used in many fields such as food and medicine. The application of polyphenols is limited by their physicochemical properties, such as their high susceptibility to oxidative degradation and sensitivity to light and alkaline solutions. The expression of the pharmacological activity of polyphenols can be enhanced by the nano-embedding technique. Nanotechnology-treated natural product nanoparticles are also more versatile. Chapter 6 describes the research related to polyphenol nanoparticle preparation technology. The effects of different preparation techniques and the functionality of polyphenol nanoparticles were summarized and analyzed

Nanomaterials are inevitably released into the environment during production, use and disposal, thus interacting with plants. The presence of nanomaterials may affect plant growth, and plant metabolic processes and uptake and accumulation may in turn affect the environmental behavior and fate of nanoparticles, even amplifying them in the food chain. In addition, under conditions of high metal ion contamination, plants themselves can synthesize nanoparticles on the root surface or even *in vivo*, which is considered a self-detoxification mechanism for plants to resist metal toxicity. Chapter 7 provides a discussion on the phytotoxicity of nanomaterials and the uptake, transport and accumulation of nanomaterials by plants

Through long-term selection and evolution in nature, many plants have developed multidimensional, hierarchical fine structures that assist them in achieving one or several functional purposes. This provides a good reference for the design and development of new functional materials, so the study and mimicry of biological structures have become one of the major hot spots in the research of new materials. However, since most biological structures are very fine and complex, it is difficult to prepare similar structures directly using traditional artificial methods. The morphology genetic method can prepare novel functional

materials with bio-fine graded structures by directly using the biological structure as a template and selecting a suitable physicochemical method to transform the framework components into the target material while maintaining the fine-graded structure of the template. Chapter 8 gives several common preparation methods from the ideas and principles of the morphology genetic method. This chapter also introduces the progress of research on morphology genetic materials based on several typical biological structures in recent years.

Plant virus particles are an ideal natural nanomaterial with the advantages of high accumulation level in plant cells, low regeneration cost, simple purification process, and safety to humans. With the development of bio-nanotechnology, plant virus nanoparticles show increasing potential for application in the medical field. Chapter 9 introduces the research progress and applications of plant virus nanoparticles in the medical field from drug delivery, molecular imaging and vaccine preparation.

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CHAPTER 1**Biosynthesis of Nanomaterials *via* Plant Extracts**

Abstract: Nanoparticles (NPs) have become a hot research material in many fields, such as catalysis, sensing, clinical diagnosis, medical treatment, antimicrobial agents, and environmental remediation, due to their small size, high surface area, high reactivity, and unique optical, electrical, and thermodynamic properties. The type, morphology, size, and surface function modification of NPs determine their performance and application scope. The development of green, simple, and controllable NP synthesis methods is an important research direction at present. The biosynthesis of NPs is a kind of green synthesis method that uses organisms or biomolecules to reduce NP precursors. The reaction conditions are mild, the energy consumption is low, and there is no need for expensive equipment or harmful chemicals. It has been developed into an important branch of nanobiotic technology. This chapter summarizes the latest progress in the synthesis of NPs from different plant tissue extracts. It also summarizes the biosynthesis mechanism and application of NPs, analyzes the main problems faced by the biosynthesis method, and prospects its future research direction.

Keywords: Au nanoparticles, Biosynthesis, Fe-based nanoparticles, Plant selection, Silver nanoparticles, Se nanoparticles, ZnO nanoparticles.

INTRODUCTION

Nanoscience is a field that combines various disciplines to design and create functional systems at the molecular level. It focuses on the production, characterization, and usage of materials and devices on a nanoscale. The objective of nanoscience is to manipulate matter at the nanoscale to create unique materials. Nanoscaled materials are defined as having components with at least one dimension less than 100 nm. The field has had a significant impact on various branches of science and all aspects of life. There are two methods for creating nanostructures: top-down and bottom-up. The top-down method involves reducing the size of bulk material through techniques such as grinding, milling, sputtering, and thermal or laser ablation. The bottom-up method, on the other hand, involves creating nanoparticles from smaller entities like atoms, molecules, and particles through mostly chemical and biological processes. This method has the advantage of producing metallic nanoparticles with fewer defects and a more uniform chemical composition. Typically, nanoparticles are produced through three distinct methods: physical, chemical, and biological. Physical synthesis

methods include thermal decomposition, laser irradiation, and electrolysis. For instance, thermal decomposition occurs at high temperatures. The primary drawbacks of physical methods are that they are energy-intensive and often require expensive vacuum systems or equipment. Chemical reduction using agents like sodium borohydride or sodium citrate is the most commonly used method for chemical synthesis. However, the use of toxic chemicals and non-polar solvents in the synthesis process can limit their applications in the clinical field. In an effort to find cleaner, biocompatible, non-toxic, and eco-friendly methods for nanoparticle synthesis, researchers have been exploring biological synthesis routes. This has led to the use of various biomaterials, such as bacteria, fungi, yeast, viruses, microalgae, macroalgae, and plant biomass/extract, for nanoparticle production. The use of biological organisms in this field is growing rapidly due to their success and ease of forming nanoparticles. These biologically synthesized nanomaterials have potential applications in treatment, diagnosis, surgical nanodevice development, and commercial product manufacturing. Among the various biological materials, plant biomass/extract has several advantages over other microscopic organisms in nanoparticle synthesis.

BIOSYNTHESIS OF SELENIUM NANOPARTICLES

Plant Selection

Selenium (Se) is an essential trace element in the human body, closely related to daily life. Se has the functions in beauty, anti-cancer, anti-radiation, and immune regulation. Insufficient intake of Se can lead to Creutzfeldt-Jakob disease. Excessive intake of Se can lead to Se poisoning, which can harm human health. Therefore, the problem of finding a low-toxicity alternative to Se to meet the needs of people needs to be addressed urgently. It was found that low toxicity and high bioactivity of Se NPs could meet the needs of people. Therefore, how to prepare Se NPs has attracted much attention. The particle size of Se is significant in studying its properties, and the properties of organic Se, inorganic Se, and Se NP are entirely different. Organic Se is involved in protein synthesis and can be easily absorbed and utilized by the human body. Inorganic Se is toxic and unsuitable for the human body to absorb and utilize. With a small particle size, Nano Se can be easily absorbed by the human body. At the same time, research shows that Se NPs have high dispersion, high biological activity, and low toxicity, so it is highly tolerable to the human body.

Biological methods for preparing Se NPs are used to synthesize red Se NPs by reducing Se salts (sodium selenite, selenite, *etc.*) using effective substances (metabolites [1], enzymes, *etc.*) produced during the growth and development of microorganisms. Another approach is the reduction of sodium selenite to red Se NPs using the active substances from plant extracts. In the case of plants, the reaction of plant components such as leaf extracts, fruit extracts, and root extracts

with Se salts (selenite, sodium selenite, *etc.*) is the primary way to prepare Se NPs. Se NPs from plant extracts generally use the active ingredients (polysaccharides, proteins, vitamin C, *etc.*) in plant extracts as reducing agents, dispersants, stabilizers, *etc.* The Se NP synthesized from plant extracts has a small particle size, high stability, no cytotoxicity, and is environmentally friendly.

Yilmaz *et al.* [2] synthesized spherical Se NPs in the particle size range of 20-50 nm using the reaction of tarragon extract and sodium selenite. Kumar *et al.* [3] prepared spherical Se NPs from *Azadirachta indica* extract. The plant extract species reported so far that can be used to prepare Se NPs include Lemon (Citrus) [4], *Emblica officinalis* [5], fenugreek seed [6], *Psidium guajava* [7], *Diospyros montana* [8], *Vitis vinifera* [9], *Theobroma cacao* [10], green tea [11], *Aster tataricus* [12], hawthorn fruit [13], *etc.* The synthesized Se NPs were in the size range of 15~500 nm, with high bioactivity and stability, which are beneficial for later development and utilization. There are differences in Se NPs prepared from different plant extracts. They differ in particle size, optimal reaction time, optimal reaction concentration, and optimal reaction temperature. However, they all have milder reaction conditions, short reaction times, high biological activity, and no cytotoxicity. The synthesis of Se NPs from plant extracts is summarized in detail in Table 1.

Table 1. Source, precursor, reductant, size of biosynthesized Se NP from plants.

Plant Source	Precursor	Reductant	Size of Se	Ref
Tarragon	Sodium selenite	Plant extract	20-50 nm	[2]
Neem	Se	Plant extract	-	[3]
Lemon	Sodium selenite	Plant extract	60-80 nm	[4]
<i>Emblica officinalis</i>	Sodium selenite	Fruit extract	15-40 nm	[5]
Fenugreek	Seleninic acid	Seed extract	50-150 nm	[6]
<i>Psidium guajava</i>	Sodium selenite	Leaf extract	8-20 nm	[7]
<i>Diospyros Montana</i>	Seleninic acid	Leaf extract	4-16 nm	[8]
<i>Vitis vinifera</i>	Seleninic acid	Fruit extract	3-18 nm	[9]
<i>Theobroma cacao</i>	Seleninic acid	Seed extract	50-500 nm	[10]
Green tea	Sodium selenite	Plant extract	334.7 nm	[11]
Hawthorn	Sodium selenite	Plant extract	113 nm	[13]

Characterization of Biosynthetic Se Nanoparticles

In the study of the preparation of Se NPs, TEM is often used to observe the particle size and surface morphology of synthetic Se NPs. Yilmaz *et al.* [2]

CHAPTER 2**Preparation of Nanocellulose From Plants**

Abstract: Plants contain the most abundant natural polymer cellulose in nature. Plants have a multilayered structure, made up of cells at the microscopic level. The main material of cells exists in the cell wall, mainly in the form of cellulose nanofilament-matrix composite structure, which plays a crucial role in the performance and function of plants. Nanocellulose, as a kind of environmentally friendly renewable polymer material, has great application potential and ecological benefits. In this chapter, the structural properties and preparation methods of nanocellulose are introduced, and the characterization methods and modification methods of nanocellulose are summarized. Finally, the design and construction of new nanocellulose materials in the future are prospected.

Keywords: Characterization, Cell wall, Functional material, Material construction strategy, Mechanical defibrillation, Nanocellulose, Preparation, Structure.

INTRODUCTION

Plants are one of the main forms of life, including trees, shrubs, vines, grasses, *etc.* After a long evolution on earth, plant materials such as wood have become the most abundant natural organic polymer material in nature. Compared with synthetic polymers, plant materials have significant advantages such as renewable raw materials, abundant accumulation, and relatively low cost. In addition, plants are living organisms, and their unique structural characteristics give them the biological function to adapt to the laws of nature. As a natural polymer material, all plant materials have a multilayered structure, constructed at the microscopic level from a stack of cells. In most of the plants, cellulose nanofibrils (microfibrils, elemental fibrils) - matrix (hemicellulose, lignin, *etc.*) complexes are formed in the cell wall during cell synthesis and growth. This nanocomposite structure plays a crucial role in the performance and function of plants. Therefore, the study of the nanostructures of plant cell walls is particularly important to help us further understand the biological nature of plants and also to support the development of new materials using the structural advantages of plants.

The cellulose nanofilaments inside plant cell walls are also known as nanocellulose. Nanocellulose within plant materials exhibits excellent mechanical

and thermal properties due to the parallel aggregation of cellulose molecular chains and the formation of hydrogen bonds between hydroxyl groups on the cellulose surface as well as van der Waals forces. Combined with its unique one-dimensional nanofiber structure and flexibility, this makes it a highly promising new polymeric nanomaterial. For this purpose, effective strategies are needed to release nanocellulose from plant cell walls. The structure, surface chemistry and properties of the obtained nanocellulose vary by using different preparation strategies and selecting different plant cellulose as raw material. Based on the preparation methods and structural characteristics of the resulting nanocellulose, nanocellulose is mainly classified into three categories: cellulose nanocrystal (CNC), nanofibrillated cellulose (NFC) and bacterial cellulose (BC). CNC can be produced by removing the amorphous zone from cellulose by strong acid hydrolysis (concentrated hydrochloric acid, concentrated sulfuric acid, *etc.*). It is short in length, but has high crystallinity and chiral characteristics when present in water at certain concentrations. BC is mainly synthesized by microorganisms in specific genera under specific conditions and has a very high cellulose purity. NFC is mainly made from vegetable cellulose pulp with the help of high strength mechanical defibrillation method, which has a very high aspect ratio. Chemical modification of the fibrillated pulp (TEMPO catalytic oxidation treatment, *etc.*) before the mechanical defibrillation process can result in NFC with higher nanofibrillation degree. In contrast, cellulose pulp is produced from the plant material using only chemical purification and then separated using mechanical defibrillation to maximize the retention of its native structure in the plant cell wall. The resulting high aspect ratio nanocellulose has a reticulated entangled structure, as well as high crystallinity, mechanical strength and thermal stability, and shows great potential for applications in the development of new materials, energy storage, and environmental purification [1 - 3].

All plants have multilayered structural features, exhibiting different structural characteristics ranging from macroscopic to microscopic. The multi-level structure and the “perfect” span between them have given life to plants and allowed them to survive the long evolution of the earth. Although there are some differences in the multilevel structural characteristics of different plants, there are many commonalities in the overall. Take trees for example, growing trees show a large tree trunk with a well-developed root system underground. The General Sherman tree is the largest tree in the world, 83.8 m tall with a maximum diameter of 11.1 m at its base, and can be 2300 to 2700 years old. Trees are felled and processed in series to obtain logs, veneers and related profiles. Further fine processing results in small pieces of wood at the centimeter/mm level, which can be observed under the electron microscope in cross-section, diameter and chordal sections. Further magnification reveals that the tree is composed of an orderly stack of cells with lengths of millimeters and widths of tens of micrometers. The solid part of the cells in the xylem is mainly gathered in the cell wall. The cell walls are multilayered and composed of microfibrils with high aspect ratios and diameters at the nanometer scale. Inside the microfibrils, there are finer sized primitive fibrils with diameters of about 2 ~ 5 nm. Both microfibrils and primitive

fibrils are nanoscale fibers with cellulose as the main component. These nanocelluloses, together with polymeric polysaccharides such as hemicellulose and lignin, constitute the multilayered structure of the tree cell wall. The elementary filaments are composed of cellulose molecular chains arranged in parallel. Inside the elementary filaments, crystalline regions can be formed due to the oriented and ordered arrangement of cellulose molecular chains, while amorphous regions are formed due to the undirected and irregular arrangement of cellulose molecular chains. The crystalline region of cellulose coexists with the amorphous region inside the primitive filament. Cellulose molecular chains are several hundred nanometers to a few micrometers in length and a few Ångstrom in width, and are composed of D-glucose groups linked by β -1,4 glycosidic bonds. The glucose unit is mainly composed of carbon, hydrogen and oxygen atoms connected by chemical bonds. The structural characteristics of trees are representative among plants, and other types of plants also have multi-layered structural characteristics similar to those of trees.

Every plant is made up of a stack of cells. At the microscopic level, the cell is the basic structural unit of the plant. It is estimated that 1 m^3 of spruce wood contains about $3.5 \times 10^{11} \sim 5.0 \times 10^{11}$ cells. The structure of the cell largely determines the structure, properties and functions of the plant. Usually, the cells of plants go through stages of growth and development such as meristem, enlargement and cell wall thickening to reach maturity. The types, structures and sizes of the cells that make up a plant are diverse due to various influences such as growth needs and environmental factors. Taking trees as an example, their growth mainly consists of height growth and diameter growth. Their constituent cells also mainly consist of two main types of cells growing along the longitudinal direction and lateral growth, which are obtained by the division of spindle primitive cells and ray primitive cells, respectively. The cell composition and arrangement vary in different tree species. Coniferous trees mainly contain axial tubular cells, wood rays, axial thin-walled tissues and resin tracts. Broad-leaved wood mainly consists of ducts, wood fibers, axial thin-walled tissues and wood rays. Only a few broad-leaved trees contain a certain amount of tubular cells. The axial tubular cells in coniferous wood are the longest cells in trees, closed at both ends and hollow inside, with an average length of $3000 \sim 5000 \mu\text{m}$ and width of $15 \sim 80 \mu\text{m}$. The size of the ducts in broadleaf timber varies greatly depending on the species and location, and the ends can be fully or partially open. Short ones can be less than $175 \mu\text{m}$ in length and long ones can be more than $1900 \mu\text{m}$. Axial thin-walled tissue is formed by many axial thin-walled cells aggregated in a brick-like pattern with small size. The wood rays of coniferous trees are all composed of transverse cells, while those of broad-leaved trees are mainly composed of ray-thin-walled cells, including transverse cells, erect cells and square cells. Therefore, the size, structure, arrangement, and number of cells that make up plant resources are

CHAPTER 3**Preparation and Application of Plant-derived Biochar**

Abstract: Plant-derived biochar is derived from biomass as a carbon source. It has a large specific surface area, high pore capacity, adjustable surface functional groups, and good environmental compatibility. Its raw material, plant-derived biomass, is widely available and renewable. It is a cheap and efficient adsorbent. Most biochar contains more than 70 percent carbon. Biochar can adsorb heavy metal ions, hormones, and organic pollutants. It can also be used for soil improvement, carbon sequestration, and the development of new materials with BC as the main component. In this chapter, biochar preparation and plants' selection are introduced. How to characterize biochar is also discussed. In addition, biochar adsorption applications in different fields are also introduced.

Keywords: Adsorption, Preparation method, Plant biomass derived biochar, Soil remediation, Waterbody.

INTRODUCTION

Plant-derived BC (BC) materials are materials derived from plant-derived biomass as a carbon source and can be prepared using a variety of agricultural wastes and by-products. The biomass commonly used as carbon sources are wheat straw, corn cobs, and rice husks. Due to the diversity of carbon sources, there are many types of plant-derived BC materials, and the raw materials for preparation are abundant and widely distributed. At the same time, the composition of plant-derived BC materials makes them biodegradable compared to synthetic polymers and other materials, and they can be quickly degraded by microorganisms into water, carbon dioxide and small molecules in the natural environment, making them very friendly to the environment. At present, the main methods for preparing BC materials are pyrolysis, hydrothermal carbonization, and microwave-assisted carbonization, among which pyrolysis is the most widely used and mature method for the preparation of BC materials. At the same time, the total pore volume, specific surface area and surface functional groups of BC materials made by pyrolysis and other methods are small, which makes the adsorption energy of

the materials weak. Modification of BC materials by some physical or chemical activation methods is essential and can greatly enhance the performance of BC materials.

PREPARATION OF BC MATERIALS

Pyrolysis of biomass is a thermochemical process that converts most of the biomass into gas, bio-oil and BC under limited oxygen supply or anaerobic conditions at 400 to 700 °C. For the composition of plant-derived biomass, hemicellulose decomposes first at temperatures between 200 and 250 °C. Cellulose decomposes in the temperature range of 240 to 350 °C, and lignin decomposes last at 280 to 500 °C [1]. Considering the complete carbonization of biomass, the pyrolysis temperature is generally higher than 500 °C.

Pyrolysis temperature affects the yield, pore size distribution and surface chemistry of BC materials. On the one hand, high pyrolysis temperature will reduce the yield of BC materials [2]. On the other hand, the increase in pyrolysis temperature can reduce the average pore size of the product, increase the total pore volume and increase the specific surface area [3]. The pyrolysis temperature can also affect the surface chemistry of BC materials by influencing the formation of functional groups on their surfaces. For example, oxygen-containing functional groups are more abundant at low pyrolysis temperatures, when most of the BC materials obtained are hydrophilic [4].

Hydrothermal carbonization is a method for producing BC materials in an aqueous solution. Compared with pyrolysis, the temperature of hydrothermal carbonization is generally lower than 350 °C, which is an alternative to pyrolysis requiring high-temperature heat treatment. Hydrothermal carbonization involves a series of chemical reactions, such as hydrolysis, dehydration, decarboxylation, and recondensation in the presence of water [5]. The surface of biomass materials prepared by hydrothermal carbonization has more oxygen-containing functional groups [6].

Hydrothermal carbonization is dependent on temperature, reaction time and the ratio of reactants. As with pyrolysis, high temperature decreases the yield of BC material, but increases the total pore volume and specific surface area of the material. Compared to the products obtained by pyrolysis, the products obtained by hydrothermal charring have the lower specific surface area and are less stable. Therefore, most researchers prefer the method of pyrolysis to obtain BC materials.

Microwave-assisted technology is an effective method for reducing the reaction time of pyrolysis and improving the quality of products from different raw materials. The principle of microwave-assisted carbonization is that when a high-frequency voltage is applied to the sample material, the molecules with permanent

dipole moments are aligned in the opposite direction of the applied electric field, thus generating heat through dipole-dipole rotation [7]. Therefore, microwave-assisted pyrolysis can heat biomass more uniformly, and energy can be saved by using the characteristics of microwave propagation within the sample. In microwave-assisted carbonization, the properties and yield of BC materials are mainly affected by the heating rate and radiation power. It has been demonstrated that the addition of acids, bases, or other chemical reagents in microwave-assisted carbonization can improve the performance of the products [8].

The physical activation method usually uses some gases such as air, CO₂, or water vapor to modify the BC material [9]. Physical activation using water vapor is cheap and simple and can increase the specific surface area of BC materials. Also, the oxygen in water vapor molecules can react with carbon on the surface of BC materials, which helps to produce the content of functional groups such as carboxyl groups and hydroxyl groups [10]. CO₂ is also often used for physical activation to obtain BC materials with higher carbon content. In addition, CO₂ activation results in BC materials with more micropores and higher specific surface area than those obtained by air and water vapor activation [11]. The use of nitrogen allows the formation of nitrogen-containing functional groups such as amino groups on the surface of BC materials. The effect of physical activation on the product properties depends mainly on the time and temperature of activation.

Chemical activation is an activation method in which the BC material is impregnated with chemical reagents for reaction. Chemical activation can use acids, bases or salts as activators. These activators can improve the surface structure of the BC material and create hydrophilic or hydrophobic functional groups on its surface to enhance its adsorption properties. In addition to the type of activation reagent, the performance of BC materials may be degraded by excessive use of activators, high activation temperature or long activation time. Masoumi *et al.* [12] used KOH for the activation of algal biomass and found that good yields and total porosity were achieved at a mass ratio of KOH to algae of 1.5. Excessive amount of activator would instead reduce the specific surface area of the product by destroying the pores. For the activation temperature, Zhao *et al.* [13] used KOH to activate walnut shell char materials. The specific surface area of the product increased to 1101.4 m²/g when the temperature was increased to 800 °C. When the temperature was increased to 900 °C, the specific surface area decreased to 1033.0 m²/g. The variation in activation time also has a large effect on the biomass material. Too long activation time may reduce the total pore volume and average pore size, which may be due to the collapse of pores. Therefore, when chemical activation is performed, the ratio of activator to biomass, temperature and time should be controlled and mixed uniformly before pyrolysis.

Extraction and Application of Plant Exosomes

Abstract: Plant extracellular vesicles (EVs) are membranous vesicles secreted by plant cells, with a lipid bilayer as the basic skeleton, which encapsulates various active substances such as proteins and nucleic acids. Plant exosomes are nanoscale vesicles secreted by plant cells, containing DNA, small RNA, sRNA, microRNA, miRNA and proteins, which mediate cell-to-cell communication. Plant exosomes play anti-inflammatory, antiviral, anti-fibrosis, anti-tumor and other roles through the substances contained in them, and participate in the defense response to pathogen invasion. Plant exosome nanoparticles are mostly edible and can be used as delivery vehicles for specific drugs without toxicity or side effects. In this chapter, the recent literature reports on plant exosomes are reviewed, and the sources and functions of plant exosomes are summarized and analyzed.

Keywords: Carrier, Extracellular vesicles, Exosomes, Fruits and vegetables, miRNA, Pharmacological application, sRNA.

INTRODUCTION

Vesicles released into the extracellular space by eukaryotic cells are called extracellular vesicles (EV). EV plays an important role in intercellular and interspecies communication and immune response. So far, three types of EVs have been identified in eukaryotic cells, including exosomes produced by the multivesicular body (MVB)-plasma membrane (PM) fusion, micro-vesicles budding directly from PM, and apoptotic vesicles produced during cell death [1 - 3]. Among them, exosomes are extracellular vesicles of 40-100 nm in diameter secreted by eukaryotic cells.

Many mammalian cells have the ability to release exosomes, including reticulocytes, dendritic cells, B cells, T cells, mast cells, epithelial cells, and tumor cells. After the discovery of exosomes in animal cells, there is increasing evidence that MVB and exosome-like vesicles occur in plants. These findings were mainly focused on studies of cell growth and differentiation (including processes related to the cell wall) or on the response of plants to various stresses [4]. In addition to animal body fluids, exosome-like NPs containing proteins and small RNAs have been found in many plants, such as ginger, carrots, watermelon

grapes and olives. These plant exosomes have the potential for mass production due to their lack of toxicity. Their intrinsic properties and the possibility of carrying other compounds such as drugs or small RNA molecules have attracted a lot of attention in medical applications (Fig. 1) [5]. Regente *et al.* [6] first identified the presence of exosome-like vesicles in plants in 2009, which were 50-200 nm in diameter in sunflower seeds. Many subsequent studies have focused on the deeper characterization of vesicles and their bioactive components to better understand their intrinsic properties and possible biotechnological applications. Some studies have isolated extracellular vesicles with specific proteomic, lipidomic and transcriptomic profiles from plant species such as grape, grapefruit, ginger and broccoli by ultracentrifugation [7 - 10]. They also found that these vesicles of edible plant origin have anti-inflammatory properties. Grape- and grapefruit-derived exosome-like NPs have a role in targeting intestinal cells and in protecting mice from sodium dextran sulfate-induced colitis.

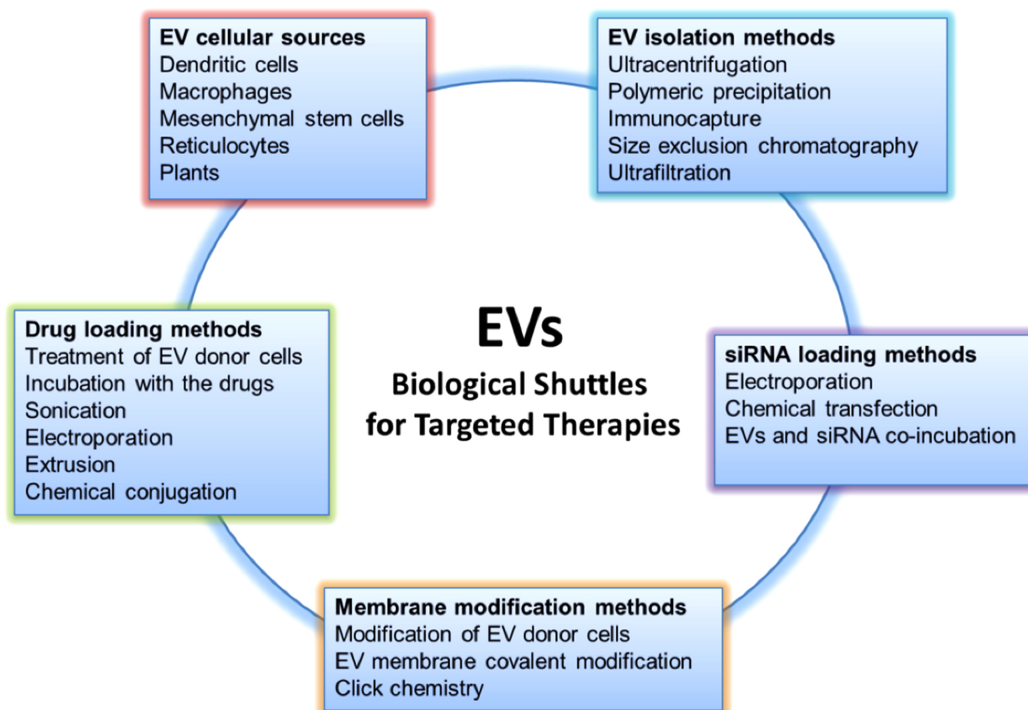


Fig. (1). Aspects to be considered for the use of EV as drug delivery systems. Reproduced with permission from [5]. Copyright 2023, MDPI.

Plant exosomes are similar to animal exosomes in morphology and structure, with a phospholipid bilayer structure, containing proteins and micro RNAs in the shape of a teat or cup. Extraction of the total exosomal lipids allowed a more detailed analysis. Protein analysis revealed the inclusion of proteins involved in signaling,

many of which are highly induced in stress and immune responses. It also contains proteins involved in reactive oxygen species signaling and oxidative stress responses, and various membrane transport proteins and intra-vesicular transport proteins [11]. Plant exosomes are rich in miRNAs, a class of endogenous single-stranded non-coding RNAs of approximately 22 nucleotides in length, which play an important role in organisms and are efficient regulators of gene expression [12]. A growing number of reports show that miRNAs not only perform biological functions within their native systems but also regulate cross-border gene expression [13].

ISOLATION AND PURIFICATION OF PLANT EXOSOMES

Differential centrifugation, which uses different centrifugal forces to separate plant exosomes from other substances, is currently the most common method for the separation of plant exosomes [14]. The pure ginger plant exosomes were obtained by centrifuging the extracted ginger juice at $3\,000 \times g$ for 20 min, $10\,000 \times g$ for 40 min and $150\,000 \times g$ for 2 h [15]. However, this method is less efficient for the separation of plant samples that are more viscous or contain more impurity particles, such as *Pueraria lobata* and yam.

Density gradient centrifugation is a method in which each component with different sedimentation coefficients settles at a certain speed under the action of centrifugal force, forming strips in different density gradient regions. The commonly used gradient building blocks are sucrose and iodixanol. The plant exosomes were purified by density gradient centrifugation after differential centrifugation to obtain high purity plant exosomes [15], but this method is limited in terms of scale up preparation.

Ultrafiltration is a method that uses the microporous structure of ultrafiltration membranes to separate samples at different scales under a certain pressure [16]. Since ultrafiltration membranes are commercially available and easy to choose the size, this method can be considered as an alternative to ultra-high speed centrifugation. Filtration is a method of separating plant exosomes based on molecular mass and size, usually using fiber bundle filters, mostly in combination with ultrafiltration. Compared with differential centrifugation, the filtration method is less stressful and provides better purification, but the ultrafiltration/filtration process may change the structure of plant exosomes due to the squeezing effect, and the membrane may have problems such as clogging and contamination [17].

The immunomagnetic bead method uses the functional groups on the surface of magnetic beads to specifically recognize the surface proteins of plant exosomes, and achieves the separation of plant exosomes under the action of magnetic field.

CHAPTER 5***In vivo* Synthesis of Metal Nanoparticles Using Plants as Hosts**

Abstract: The precursors of nanomaterials can be transformed into nanomaterials in plants. This chapter introduces plants as hosts for nanomaterial synthesis. Although the synthesis of nanomaterials by this method cannot be obtained in large quantities, the existence of nanomaterials in plants will have a certain impact on the growth of plants. This technique may not be useful in the synthesis of nanomaterials, but it has potential applications in agriculture.

Keywords: A plant organ, Cells, *In vivo* synthesis, Nanomaterials transport, Toxicity.

INTRODUCTION

The field of nanotechnology has grown from being a multidisciplinary concept to a primary area of scientific study in recent years. The growth in new technologies has resulted in the development of nanoscale devices, advanced sensors, and innovative biomimetic materials. A new, simple, and cost-effective way of synthesizing metal nanoparticles using biological tools such as bacteria, yeast, fungi, and plants have been introduced in addition to chemical and physical methods. Most research has focused on the *ex vivo* synthesis of nanoparticles in plants, which has proven to be an economical and valuable alternative for the large-scale production of metal nanoparticles. However, very little research has been done on the potential of living plants for synthesizing metal nanoparticles. This review discusses the synthesis of metal nanoparticles using living plants. So far, metal nanoparticles' formation in living plants has been observed for gold, silver, copper, and zinc oxide. Although the results achieved to date demonstrate the feasibility of this process, there are still aspects of plant physiology that need to be better understood to have better control and modulation of the formation of these new materials. Plant science can play a significant role in fully exploring the potential of phyto-synthesis of metal nanoparticles.

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UPTAKE AND TRANSPORT OF METAL ELEMENTS BY PLANTS

In the natural environment, metal elements exist in different forms in the atmosphere, water and soil, and are more easily enriched in water and soil. Therefore, most of the pathways of metal elements entering plants in nature to participate in migration and transformation are through the root system, and a small portion is absorbed through foliar contact. Various metals undergo translocation and morphological transformation when they enter the plant through absorption. For example, Koontz *et al.* [1] labeled AgNO_3 by isotopic tracer method using $0.5 \mu\text{M } ^{110}\text{AgNO}_3$ in root exposure of soybean. They detected radioactive signals in the stem and leaves and were increasing in the upper stem and the first three nodes of the leaves even when the supply of new extrinsic sources of AgNO_3 ceased, demonstrating that AgNO_3 undergoes bottom-up transport in the plant. At the same time, there is experimental evidence that plants can absorb different forms of metal substances, even NPs can be directly absorbed by plants. Dang *et al.* [2] controlled root secretion and root surface reactive oxygen species (ROS)-induced dissolution of Ag NPs by creating anoxic conditions, removing root secretions, and flushing plant roots with superoxide dismutase and catalase to avoid Ag^+ production from Ag NPs that undergoes leaching before being taken up by plants. They exposed wheat to 10 mg/L Ag NPs and detected 29.1 ± 0.03 nm and 34.3 ± 1.7 nm of Ag NPs in roots and stems, respectively, using single-particle inductively coupled plasma mass spectrometry (spICP-MS), demonstrating that plants can directly absorb Ag NPs.

The concentration, size, shape, and biological interactions of NPs with the plant can affect plant growth. In particular, the particle size of NPs, the size of which directly affects their migration and accumulation to the plant. Marusenko *et al.* [3] showed that *Arabidopsis thaliana* did not absorb $\alpha\text{-Fe}_2\text{O}_3$ NPs with a mean particle size of 40.9 nm (range 22.3-67.0 nm). However, other studies have shown that $\alpha\text{-Fe}_2\text{O}_3$ NPs with an average particle size of 14 nm seem to have entered barley and reached the above-ground parts [4]. The size (10, 14 and 18 nm in diameter) and concentration (1, 10 and 100 mg/L) of AgNPs significantly affected the growth of *Arabidopsis thaliana* roots [5]. Larue *et al.* [6] conducted exposure tests on wheat using TiO_2 NPs of different particle sizes (14- 655 nm). The results showed that TiO_2 NPs with particle size greater than 140 nm were intercepted by the root epidermis and did not migrate to the wheat roots. Particle sizes larger than 36 nm are prevented from entering the cell wall of wheat by the Casparian band and do not reach the vascular tissues and transfer into the stems and leaves of wheat. The TiO_2 NPs shift to the leaf part when the particle size is below 36 nm. Insoluble Pd particle agglomerate solutions may be too large in particle size to be directly absorbed by plant roots [7]. However, the rate of accumulation of Pd NPs in plants exposed to Pd NPs (5- 10 nm in diameter) in solution was higher than

that in plants exposed to PdCl solution [8]. This indicates that particle size has a significant effect on plant uptake. The pore size of maize primary roots (6.6 nm) selectively allows the uptake of smaller sized CeO₂ NPs in the root cells and subsequent propagation to the aboveground parts [9]. On the other hand, CeO₂ NPs with diameters greater than 7 nm can be taken up by other crops such as chicory, tomato, cucumber and corn [10]. CeO₂ NPs with diameters ranging from 7 nm to 25 nm were absorbed by cucumber roots and migrated to their shoots. These studies have shown that the uptake of NPs differs between cereal and vegetable crops, depending on the NP and root pore size. Similarly, NPs with sizes larger than those mentioned above have been found to cross the cortical and vascular systems in epidermal cells [11]. All these results indicated that NPs of different particle sizes had different effects on the same plant. The same particle size also has different effects on different plants. NPs with smaller particle size are more likely to enter and migrate in the plant.

Several research groups have shown that the accumulation and translocation of metal-based NPs in plants is also species-specific. ZnO NPs (44.4 ± 6.7 nm) produced significant germination toxicity to *Arabidopsis thaliana*, while SiO₂ NPs (42.8 ± 3.9 nm) with similar particle size did not show significant inhibition of *Arabidopsis thaliana* at the same concentration [12]. Differences in Au bioavailability of tobacco and wheat grown in modified nutrient solution were reported by XRF images. After exposure to three different sizes of Au NPs coated with citrate or tannate exposure, Au was not found to penetrate the surface of wheat roots, but elemental Au was observed in tobacco leaves after treatment with 30 nm citrate-coated Au NPs [13]. There is now conclusive evidence of changes in vitamin composition and content in edible tissues of cucumber and soybean exposed to TiO₂ NPs and CeO₂ NPs [14]. 10 nm of Al₂O₃ NPs on lettuce which is root elongation showed a promotional effect in the concentration range of ≤2 mg/mL [15]. Lee *et al.* [12] observed a positive effect of 150 nm Al₂O₃ NPs on root elongation of *Arabidopsis thaliana* seeds exposed to 4 mg/mL. Lin and Xing [16] reported that treatment with 60 nm Al₂O₃ NPs at 2 mg/mL had no effect on seed germination or root elongation of lettuce. In contrast, Al₂O₃ NPs at around 13 nm at the same concentration showed significant inhibition of cabbage, carrot, maize, cucumber and soybean [17]. This deleterious effect was attributed to the cellular damage of epidermal and cortical cells by Al₂O₃ NPs, which inhibited cell expansion in the elongation zone. These studies clearly show that the same NPs exert different effects on different plants and that different NPs of similar particle size bring significant differences in the effects on the same plant. However, these differences do not affect the accumulation and migration of metal-based NPs in plant tissues (especially edible tissues). Once NPs enter the food chain, their transfer and accumulation within humans become possible.

Plant Polyphenol Nanoparticle's Preparation and Application

Abstract: Plant polyphenols are important secondary metabolites in plants. They have strong antioxidant activity, which has a certain effect on the prevention of cardiovascular diseases and other chronic diseases, and can be widely used as antioxidants in food, drugs, and many other fields. The use of nanotechnology to make polyphenolic compounds into nanoparticles can effectively protect polyphenols from destruction, and improve the antioxidant and stability of polyphenols. In this chapter, the preparation technology and functional characteristics of polyphenol nanoparticles are discussed in detail, and the preparation and application of polyphenol nanoparticles are provided with references.

Keywords: Anti-aging, Coalescence precipitation embedding, Flavonols, Freeze drying, Plant polyphenols.

INTRODUCTION

Plant polyphenols, also known as plant tannins, are a general term for substances with a benzene ring combined with multiple hydroxyl chemical structures and are important secondary metabolites of phenols in plants. Plant polyphenols have strong antioxidant properties with anti-cancer, anti-aging and anti-cardiovascular disease effects, and are therefore widely used in many fields such as food and pharmaceuticals [1]. The application of polyphenols is limited by their physicochemical properties, such as their high susceptibility to oxidative degradation and sensitivity to light and alkaline solutions. Therefore, scholars at home and abroad have studied various methods to enhance the expression of the pharmacological activity of polyphenols, among which nano-embedding technology has the most promising development. NPs are natural products made by nanotechnology with bioactive functions. Plant polyphenols are mainly classified into anthocyanins, catechins, flavanones, flavonols, *etc.* Most polyphenols have a complex composition, unstable structure, and easy oxidation and decomposition. Most of the polyphenolic compounds also have a complex composition, unstable structure, and easy oxidation and decomposition, thus limiting their applications in food and pharmaceuticals. The loading of poly-

-phenolic compounds onto nanocarriers by different preparation techniques can enhance the stability and functional properties of polyphenolic compounds.

POLYPHENOL NANOPARTICLE PREPARATION TECHNOLOGY

Nanocarriers can transport and deliver drugs that are unstable in biological fluids and do not readily diffuse through mucosal barriers, thus effectively protecting the drug of interest from degradation. Oral NPs have good bioavailability, targeting, bioadhesion, and controllability of the drug in the gastrointestinal tract. Polyphenol NPs are nanotechnology-embedded or adsorbed polyphenols on the surface of NPs. They are more active in terms of solubility, antioxidant activity and stability, and have a wider range of applications. At present, there are several techniques for the preparation of polyphenol NPs: coalescence precipitation, spray drying, inclusions encapsulation, freeze-drying, microencapsulation, yeast embedding, *etc.* The use of NP preparation techniques has received a lot of attention from researchers as they can effectively protect polyphenolic compounds for better applications.

Coalescence precipitation embedding is a technique that separates one or more gels from the initial solution, thereby allowing the active material in a suspension or emulsion to coalesce and embed. Cohesive embedding can be formed either by simple gel solutions or by more complex coalescence, which often forms amorphous structures. Currently, there is a method to prepare NPs by NP precipitation using pH drift in a mixed micellar system. Its process is mainly to mix micellar solution, surfactant, acid, *etc.* through a mixer and then circulate ultrafiltration through an ultra-filter to obtain the precipitated NPs. Coalescence is currently a very important technology in food preparation, with the advantages of mild conditions, high production efficiency, better environmental tolerance and controlled release of the prepared microcapsules. The particle size, distribution, microsphere morphology and system stability of the microspheres produced by coalescence precipitation are excellent.

Spray-drying embedding is an embedding technique in which the core and wall materials used for embedding are homogeneously mixed and then the mixture is passed through a spray dryer for atomization to obtain micro-particle capsules [2]. It was found that the content of black carrot anthocyanins after maltodextrin encapsulation obtained by spray drying method was dependent on the inlet air temperature. Higher inlet air temperatures (>160 °C) can lead to higher anthocyanin losses. The 20-21DE maltodextrin with glucose 210 as wall material had the highest anthocyanin content at the end of drying [3]. Chiou *et al.* [4] used natural fruit fibers as an embedding medium to extract the bioactive substance *Hibiscus sabdariffa* L. by spray drying and obtained particles with particle sizes

ranging from 16 μm to 23 μm , thus demonstrating that natural fruit fibers can be used as an alternative carrier for spray-dried viscous materials. They have also developed a new nutritional product that can be used in a variety of applications in functional food manufacturing.

Spray drying is commonly used for the encapsulation of fragrances, lipids, carotenoids, and other ingredients. Spray drying is the most widely used microencapsulation technology in food processing because of its speed, low cost, simplicity, and the quality of the products obtained. After spray drying, spherical particles with diameters of 10 μm to 100 μm are generally obtained. However, because the wall material used must have a certain solubility in water, there is a limit to the wall material that can be utilized in spray drying. Some microorganisms, biological components or volatile oils are microencapsulated to reduce their original efficacy, so the process should be optimized to meet the actual needs.

The molecular inclusions are generally produced by using CD as a coating material. CD are non-reducing cyclic oligosaccharides with a slightly tapered hollow cylindrical stereospecific ring structure, obtained by the action of CD glycosyltransferase on precipitated powder and linked by α -1,4-glycosidic bonds. The common CD include α , β and γ , among which β -CD is more widely used. Ficarra *et al.* [5] complexed hesperidin, hesperidin, naringenin and naringenin with β -CD using a co-precipitation method and studied their solubility in solutions and solids. The results showed that β -CD formed 1:1 inclusion complexes with hesperidin, naringenin and naringenin in solid and aqueous solutions, respectively, which can be used to improve the dissolution and absorption properties of drugs in therapeutic formulations. It was found that mixing olive leaf extract (rich in olive bitter glycosides) with β -CD in an aqueous medium and freeze-drying resulted in the formation of an inclusion complex. The inclusion complex has the advantage of high water solubility. The complex of olive leaf extract and β -CD solids can be used either as a leaf extract alone to fortify foods or as a food supplement [6]. The complexation of flavonols, kaempferol, quercetin, and prunetin with hydroxypropyl- β -CD was found to enhance their antioxidant capacity to a great extent. The antioxidant activity of each flavonol was maximized when they were complexed in the hydrophobic cavity of CD [7]. The water solubility of polyphenols is not only improved by CD encapsulation, but also the antioxidant activity of some chemical substances with low water solubility is enhanced. On the other hand, the encapsulation technique is expensive and the embedding capacity inside the CD is influenced by the core material. Different wall materials also affect the embedding capacity of CDs.

CHAPTER 7**Good Guy vs. Bad Guy: The Opposing Roles of Nanoparticles in Plant**

Abstract: The increasingly wide application of artificial nanomaterials is bound to lead to a large number of nanomaterials in the ecological environment, so the possible environmental pollution and ecological effects of nanomaterials have also attracted great attention. Plants are an important part of the ecosystem. On the one hand, nanomaterials may affect the development and growth of plants. On the other hand, the metabolic activities of plants can affect the migration and transformation behavior of nanomaterials in the environment and their transmission in the food web. This chapter reviews recent studies on the interaction between nanomaterials and plants, and discusses the toxic effects of different nanomaterials on plants from the plant to the plant cell level, as well as the process of plant uptake and transport of nanomaterials.

Keywords: Absorption, Nanomaterials, Plants, Plant toxicity, Transmission.

INTRODUCTION

The use of NPs has seen a significant increase in recent years in commercial products and industrial applications, but there is a lack of understanding of the interaction mechanisms between NPs and biological systems at a molecular level. Some NP properties, such as their high specific surface area and increased reactivity, have raised concerns about their potential adverse effects on human and environmental health. To promote the sustainable development of nanotechnology, it is important to evaluate the risks associated with the use of NPs. However, there is limited information about their fate in water and soil. The idea that NPs might be bioaccumulated through the food chain has been suggested, and plants as producers in the food chain are important components in the ecological system and could potentially be a pathway for NP transport and bioaccumulation into the food chain. Studies on the impact of NPs on plants have produced mixed results, with some indicating positive effects and others negative effects on germination and growth. Despite this, the potential for NPs to enhance agricultural applications has garnered interest among researchers. However, the toxicity of NPs to plants is still not fully understood and more research is needed in this area. While some studies have shown that NPs can harm seed germination and growth, their unique properties also hold the potential for improving seed

germination and crop performance. The field of NPs and plant toxicity is still in its early stages, and further studies are needed to reach a conclusion.

TRANSPORT AND TRANSFORMATION OF METAL NANOPARTICLES IN SOIL-PLANT SYSTEMS

Metal NPs can not only release homogeneous metal ions to produce toxicity, but the particles themselves may also have a higher toxic effect. For example, the toxicity of the more stable titanium dioxide NPs to wheat stems mainly from the toxic effects of the material itself [1]. The less stable ZnO NPs, on the other hand, can leach zinc ions that are toxic to wheat [1] or algae [2]. The dissociated Fe^{3+} in $\alpha\text{-Fe}_2\text{O}_3$ affects the growth of algae. However, it has also been found that the biotoxicity of Zn NPs to ryegrass is mainly caused by the particles themselves [3]. The entry of metal NPs into plants can produce ROS, which causes damage and death of plant cells and may also affect nutrient transport and uptake, thus affecting plant growth [4]. For metal NPs such as Ag NPs, ZnO NPs, CeO_2 NPs and TiO_2 NPs, whether they can release metal ions or not, their own toxicity cannot be ignored. At present, the mechanism of toxic effects of metal NPs is still controversial, and some key issues need to be studied in depth.

Soil is an important aggregation medium for metal NPs in the environment and plays a key role in their biogeochemical cycling. Metal NPs in soil can infiltrate into groundwater and may be absorbed by plants and animals and then enriched through the food chain. There is a biomagnification effect on the transfer of metal NPs along the food chain with an amplification factor of 5.32. The plant is the primary producer of the ecosystem. As the starting trophic level of the food chain, the uptake and transformation of metal NPs by plants not only affect the safety of plants themselves, but also may affect consumers at all levels of the food chain. Therefore, it is of great theoretical and practical significance to study the migration transformation of metal NPs in soil-plant systems, transport processes and mechanisms in plants, biotransformation in plants and their biological effects on plants for ecological risk evaluation of metal NPs.

Soils have complex structural systems and chemical heterogeneity. The movement of water in the soil is also dominated by non-stationary (transient) processes of infiltration, drainage and evaporation. Soil heterogeneity can influence the interaction between nanocolloids and soil. For example, transient pulses of water (simultaneous changes in water flux and velocity) during the alternation of wet and dry soils are similar to “hydraulic activators”. It can change the geometric layout of the pore water and the energy gradient and state of the liquid-gas interface in the pore. At the same time, it allows the nanocolloids, which were kept at the liquid-solid and liquid-gas interfaces, to gain momentum

and overcome the “energy barriers” that prevent them from moving, and thus to remobilize and diffuse further [5]. Thus, in the case of unsteady unsaturated flow, the extent of nanomaterial transport may be much greater than that predicted by current steady-state flow models. In addition, the response of nanomaterials to soil heterogeneity, pore water saturation and water flow stability may vary significantly due to significant differences in their size and surface properties. Therefore, the transport of nanomaterials in the natural environment under non-steady-state inhomogeneous flow patterns (*e.g.*, rainfall) and spatially heterogeneous adsorption is an important scientific issue to be investigated.

Metal NPs in the environment are subject to a variety of environmental factors and have a complex distribution pattern. NPs with a wide range of structural properties (surface functionalization, size, crystalline structure) designed and produced to meet specific application requirements are discharged into the environment during production and application. These NPs are subject to morphological transformations in response to environmental conditions, which include but are not limited to the following processes:

(1) Adsorption/desorption: Metal NPs can adsorb with dissolved organic matter (DOM) in the environment to form organically bound nanometals/metal oxides. The main factors affecting DOM adsorption on metal NPs include adsorbent properties (specific surface area, surface charge, crystalline form, surface functional groups and agglomeration), DOM structure and properties (hydrophobicity, surface charge, molecular weight, hydroxyl and carboxyl group content and spatial structure) and environmental conditions (pH, ionic strength, ionic valence, temperature) [6]. The adsorption mechanism of metal NPs with DOM mainly includes electrostatic interaction, coordination, hydrophobic interaction, entropic change, hydrogen bonding and bridging of cations [7]. Among these effects, ligand interaction, *i.e.*, ligand exchange with the hydroxyl-carboxyl functional groups of DOM on the surface of metal NPs, is considered to be the most important one.

(2) Dispersion/sedimentation: Free or organically bound nanometal/nanometal oxide particles can agglomerate with themselves or with minerals in the environment to form agglomerates of different water and ionic radii. These agglomerates may either be suspended in solution due to spatial potential resistance, electrostatic repulsion, or may settle, depending mainly on the relative magnitude of the gravitational and repulsive forces between the agglomerate particles. Multivalent cations can significantly reduce the stability of agglomerates, *e.g.*, the critical flocculation concentration (CCC) of Ag NPs can vary by two orders of magnitude depending on the monovalent and multivalent cations [8]. DOM can also significantly affect the stability of agglomerates. In the

Plant Tissues as Templates for Morphology Genetic Material Synthesis

Abstract: In order to ensure the needs of survival and reproduction, plants have formed various, diverse, multi-dimensional, and multi-scale fine and subtle configurations for millions of years, which provides rich inspiration for scientific research in many fields today. Research on morphology genetic material converts natural biological components into target materials by directly using biological structures as templates and selecting appropriate physicochemical methods while maintaining the fine-graded structure of the template. It can be used to prepare new functional materials with a biological finely-graded structure. This section describes methods for preparing functional materials with biological structures using morphology genetic material research ideas. In this chapter, we briefly introduce the structure of residual materials prepared by using several typical plant structures as templates, and discuss the related functional performance of materials with different structural characteristics.

Keywords: Biological structure, Composite, Functional materials, Morphology genetic materials.

INTRODUCTION

In 1859, Darwin's book "On the Origin of Species" was published, and human understanding of natural organisms entered a new stage. Darwin considered human reproduction to be "unnatural" selection. Humans themselves undergo systematic changes, so wildlife is also a process of selection. The process of natural selection can be equated to "natural warfare", which includes other animals, parasites, food supply, and external environmental stresses such as temperature and water. The evolutionary view is that the complex, fine structure and functional organization of animals and plants that have evolved to date have been reproduced by mutation through brutal natural selection and constant resistance. This evolution has resulted in endless, beautiful and perfect forms of life and natural substances.

The structure and function, formed by evolution and natural selection over time, are far superior and more effective than human-designed materials, for which the later development of bionics was spawned. The common recognition of bionics

was the first American Bionics Symposium in 1960. Jack Ellwood Steele used the Latin word “bion” (meaning way of life) and the ending “ic” to represent bionics. There are 2 models for the fabrication of bionic materials in a simple overview. One is to make materials by imitating biological structures or forms, such as biomimetic bones, biomimetic hollow materials, biomimetic ion channels, *etc.* Another type of bionic material is made to imitate the special functions of living things to meet the needs of people; for example, super-tough fibers made by imitating spider silk, superhydrophobic bionic materials made by imitating lotus leaves, high-strength bionic materials made by imitating animal shells, and bionic materials made by imitating the high adhesion of geckos' feet. However, simple imitation is not the best way to solve the problems in the field of material application, and it is difficult to obtain a true replication of the fine structure and organization of the organism, especially in the field of modern nanotechnology. In this context, morphology genetic material emerged.

As a new concept in the field of materials research, the morphology genetic material has been receiving international attention for more than 20 years since its birth. The term morphology genetic ” is a combination of both “genetic” and “morphological”, which represent the “inheritance” of the “morphology” of an organism. Morphology genetic materials mainly refer to the borrowing of the multi-level and multi-dimensional intrinsic structures and morphological appearance of organisms themselves optimized in nature for billions of years as templates, and using artificial coupling processing technology to prepare new structure-functional integrated materials with finely-graded morphological structure and appearance of natural organisms through a biological structure and morphological inheritance combined with a variety of chemical components. Such materials, with the help of optimized structures formed as a result of biological evolution, can obtain material properties that are more functional than artificial biomimetic materials and can obtain more complete functional properties than the original organism [1].

PLANT TEMPLATES

Leaves are the sites where plants use solar energy for photosynthesis, and their structure plays an important role in sunlight absorption, energy conversion and transmission. It is a hierarchical microstructure that evolves well from the macroscopic scale to the nanoscale, facilitating solar energy capture and conversion to chemical energy through photosynthesis. We can construct functional metal carbides or oxides by replicating the layered structure of green leaves from the macroscopic scale to the nanoscale. Tree leaves have been widely studied for their three-dimensional finely graded porous structure that facilitates light absorption and material transport, and have become one of the typical representatives of plant morphology genetic material.

By using leaves as templates, Li *et al.* [2 - 4] prepared nitrogen-doped titanium dioxide, platinum-deposited nitrogen-doped titanium dioxide, cadmium sulfide and gold-deposited nitrogen-doped titanium dioxide composites for photocatalytic hydrogen production. The three-dimensional finely graded porous structure of the leaves improves the absorption of incident light by the catalyst and also provides more reaction sites for the hydrolysis reaction, resulting in several times better photocatalytic hydrogen production performance of the composites than the semiconductor materials without the leaf template configuration. Tong *et al.* [5] further prepared graphitized carbon nitride (g-C₃N₄) with different number of layers by hard template method inspired by the nano-ordered shell lamellar stacking of cystoid in tree leaves. The results show that when the number of layers increases, the absorption of incident light by the catalyst increases significantly due to the increase of surface area and the effect of scattering and reflection of multiple layers. Therefore, the light absorption performance and photocatalytic performance of 3-layer g-C₃N₄ are significantly stronger than those of the monolayer and bilayer.

The layered porous structure of plants plays an important role in mass transport, which facilitates the preparation of functional materials. The structural hierarchy of cell wall components and the arrangement of vascular bundles can be used as templates [6, 7]. Thus, a series of works have explored the practical applications of porous carbon materials with various plant structures [8 - 12]. The high performance of a porous carbon is mainly caused by a reasonable combination of its specific surface area and pore structure [13]. Therefore, controlling the pore size and channel structure is the key to enhance the kinetic performance of porous carbon. Its graded channels are essentially optimized for mass transport, making the plant structure an ideal platform for scalable production.

Unfortunately, the plant tissue does not have enough micro- and mesopores, which limits the specific surface area of the product [14]. The activation process is commonly carried out. This structure can meet the needs of these applications, and these new properties of naturally-derived carbon are necessary for the rapid development of energy science. Porous graphitic carbon with high electrical conductivity is commonly used as an electrode material. Transition metal NPs (*e.g.* Co, Fe and Ni) can catalyze graphitization of solid carbon skeletons as catalysts. Ultrathin graphite nanostructures can be formed with the catalysis of metal NPs [15]. They consist of 3 to 7 graphite layers that produce a conductive coating. The specific surface area of the resulting carbon is as high as 1622 m²/g. When used as an electrode material for super-capacitors (1 M H₂SO₄ as electrolyte), this material with graphite nanolayer can provide advanced capacitive performance than the control sample without catalytic activation. In theory, all natural biomass based on lignocellulose, including leaves, wood and bamboo

Plant Virus Nanoparticles and Their Applications

Abstract: Plant virions, as nano-sized particles, have the advantages of high accumulation levels in plant cells, low regeneration cost, simple purification process and safety for the human body. They are ideal natural nanomaterials. With the development of bio-nanotechnology, plant virus nanoparticles show more and more applicable potential in the field of medicine. This chapter reviews the research progress and application of plant virus nanoparticles in the field of medicine, focusing on targeted drug delivery, molecular imaging and vaccine preparation.

Keywords: Drug delivery, Extraction, Nanoparticles, Plant virus.

INTRODUCTION

In recent years, various nanocarriers capable of delivering drugs that can be used for cancer treatment have attracted much attention in medical applications. Compared to conventional drug delivery platforms, nanocarriers have a larger surface area to accommodate more drugs or markers and can be modified with specific ligands to enhance their targeting. There are several synthetic nanocarriers available, such as synthetic polymers, lipids and carbon nanotubes. They have their own advantages and disadvantages in terms of drug loading capacity, target delivery capability, stability, *etc.* Considering the toxicity and environmental impact, researchers are currently focusing more on some natural nanocarrier materials, such as phage MS₂, small heat shock proteins, ferritin and viral nanoparticles (VNPs) [1]. Viruses in nature have great potential for medical applications because of their nanoscale size, highly symmetric three-dimensional protein structure, good biocompatibility and water solubility, and the ability to undergo various genetic/chemical modifications. Viruses originating from plants do not infect humans and animals and are safer compared to animal viruses.

VIRUSES ORIGINATING FROM PLANTS

Viruses originating from plants do not infect humans and animals and are safer compared to animal viruses. Plant virus particles are used as nanomaterials to attach or encapsulate drugs for the treatment of diseases. Shriver *et al.* [2] successfully delivered therapeutic drugs to the damaged central nervous system using cowpea mosaic virus (CPMV) as a vector. When delivering drugs, the circulation time of the carrier in the body is directly proportional to the amount of drug accumulated in the target tissue [3], while the retention time of the carrier in

the body is related to the charge on the carrier surface and whether the surface is modified [4]. The carriers for transporting drugs are mostly small molecule nanomaterials, but negatively charged nanocarriers have a short half-life *in vivo* [5]. Polyethylene glycol modification not only increases the stability and solubility of nanocarriers, but also reduces the interaction of nanocarriers with proteins or cells *in vivo*, resulting in improved half-life, increased stability and reduced immunogenicity in the organism [6]. Virus-like particles based on potato virus X (PVX) and CPMV were modified with polyethylene glycol to reduce immunogenicity. Animal experiments have confirmed that the modified viral particles penetrate more strongly and accumulate more in the tumor sites of mice, which is more conducive to the delivery and imaging of anticancer drugs.

Tumor cell surface proteins can be used as markers to distinguish tumors from normal tissue [7]. Plant VNPs attached to tumor markers and then transported can directly target tumors without affecting other normal tissues, *e.g.*, F56 peptide specifically binds to the vascular epithelial growth factor receptor 1 (VEGFR-1). In a mouse model transplanted with human colon cancer cells, CPMV nanoparticles expressing F56 peptide were able to target tumor cells with high expression of VEGFR-1 [8], reducing the side effects of the drug to some extent.

The outer shell protein (coatprotein, CP) of phytopathogenic nanoparticles contains many naked amino acid residues such as lysine, cysteine, aspartate and glutamate. They can be linked to antibodies, oligonucleotides, drugs or other small molecules by chemical modification methods [9], thus functionalizing viral particles. Wang *et al.* [10] used N-hydroxysuccinimide ester to bind fluorescein or biotin to the CPMV surface, allowing the CPMV to be used for imaging or localization. Among the various forms of plant viruses, icosahedral plant virus particles have a large internal space and are the most studied carriers used to contain small molecular substances [11]. By changing the concentration of metal ions and pH of the solution, the gap of icosahedral plant pathogenic CP gradually increases [12]. The drug diffuses through the pores to the inside of the virus, reverses the conditions, and the gap in the CP shrinks again, eventually encapsulating the drug inside. Ren *et al.* [11] used Hibiscus chlorotic ringspot virus (HCRSV) as a carrier to encapsulate the anticancer drug azithromycin and prepared a protein cage with a diameter of about 30 nm. They attach folic acid to the surface of the viral particles, giving them the ability to target tumors. The modified HCRSV delivers more drugs to the tumor site and enhances the effectiveness of disease treatment. Cucumber mosaic virus (CMV) [13], CPMV [14], and red clover necrotic mosaic virus (RCNMV) [15] have also been shown to change the conformation of virus particles in response to pH changes, thereby encapsulating the relevant drugs internally. In addition to altering the conformation of plant VNPs by changing the pH, some drugs can be encapsulated

in the lumen of plant VNPs through electrostatic interactions with charged amino acid residues on the inner surface of plant VNPs CP or negatively charged nucleic acids in the lumen [16]. Some of the charged amino acid residues in the viral CP have a strong affinity for nucleic acids and can stably encapsulate the legacy material, which has the potential to deliver gene drugs [17]. Cowpea chlorotic mottle virus (CCMV) has been shown to efficiently and spontaneously encapsulate many long single-stranded RNAs and release them at the appropriate sites [18]. Azizgolshani *et al.* [18] found that the CP of CCMV self-assembled *in vitro* with a heterologous RNA to form heterologous viral particles that were able to release heterologous RNA in the cytoplasm of mammalian cells. The CP of plant VNPs serves as a robust external scaffold that protects the encapsulated gene drug from nuclease degradation, while it has the potential for further functionalization and cellular targeting, providing a viable option for gene therapy of related diseases.

The versatility of modification methods and the advantages of precise assembly allow the use of plant VNPs for the preparation of imaging probes. Currently, plant VNPs have been used for optical imaging, MRI, and positron emission tomography [19]. During the imaging process, it is critical to remove toxicity caused by the retention of tissue in the carrier carrying the imaging reagents. Compared to some synthetic nanomaterials (carbon nanotubes, gold particles and silica) that require months for clearance, VNPs are easily cleared from the body and have a short half-life, which greatly reduces the toxicity produced by the nanocarrier's retention on the organism [3]. Fluorescent imaging is currently an important modality for tumor evaluation, and fluorescent agents can be integrated into VNPs by biocoupling, genetic engineering, and self-assembly [19]. Fluorescence plays a crucial role in the study of cell-specific particles' uptake using flow cytometry, the localization of particles by confocal microscopy, and the determination of biodistribution by *in vivo* imaging [20]. A viral fluorescent probe prepared by connecting CPMV to the fluorescent molecule Oregon Green-488 can be used to detect the circulating pathway of CPMV in living mice. CPMV can be detected in the kidney, lung, stomach, jejunum, ileum and brain of mice after several days of oral administration. The nano-particle structure of the virus recovered from mouse tissues was not damaged [21]. Tobacco mosaic virus (TMV) NPs with NIR fluorescent dye and dysprosium (Dy^{3+}) attached to the inner surface and aspartate-glycine-glutamine-alanine attached to the outer surface were produced with good biocompatibility and low cytotoxicity. The ability to target prostate cancer cells PC-3 both *in vivo* and *in vitro* provides the feasibility of the use of plant viruses for cancer cell detection [22].

In vivo angiography is a powerful tool for non-invasive detection and visualization of disease. Commonly used imaging reagent carriers include nanospheres [23], iron oxide particles [24], and liposomes [25], but there are

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