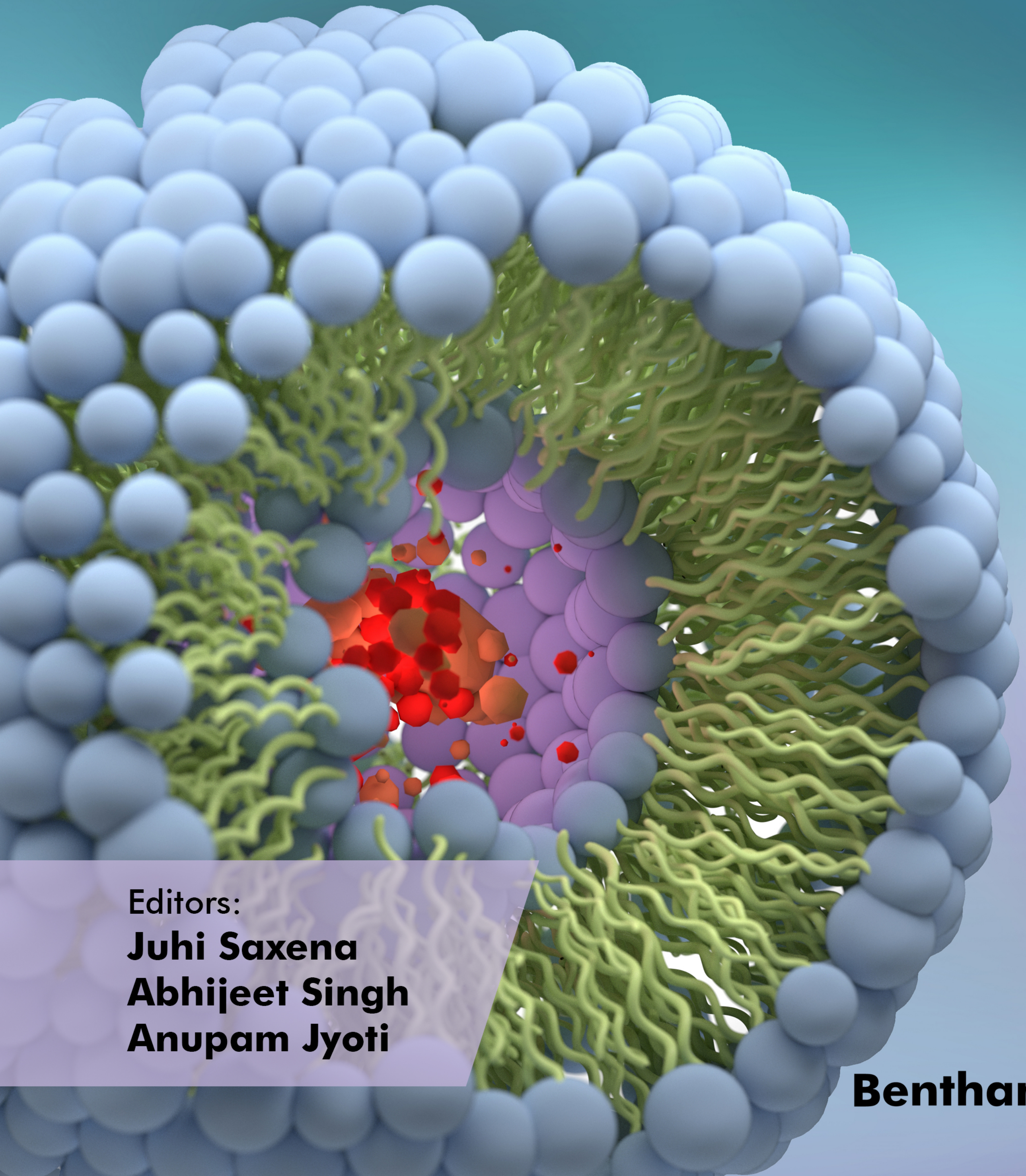
NANOBIOTECHNOLOGY

PRINCIPLES AND APPLICATIONS



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FOREWORD

Research in nanobiotechnology is gaining prime attention as it is directly affecting many socio-economic sectors including medical, agriculture, food, textile, and other industries. Biological, chemical and physical sciences are the backbone of nanobiotechnology. In recent years, nanobiotechnology research has provided solutions for several problems including human health because of its integrated approach involving various disciplines. Lately, it has been integrated rapidly with new emerging branches like molecular biology, pharmaceutical chemistry, animal cell science and drug development and discovery for output-oriented research.

This eBook 'Nanobiotechnology: Principles and Applications' presents a broad overview of the principles and applications of nanotechnology in the diverse areas of biotechnology. The expert group of authors exhibit distinguished expertise and will belong to the academic world, creating a broad perspective. This volume covers the basics and applications of nanotechnology in drug delivery, combating pathogens, nanobiosensors, improving plant health by fertilizers, bioremediation, disease sensing, and diagnosis.

As a biotechnology scientist, I am happy to recommend this eBook to the students of universities as a text and reference book both. The theory, concepts and technique's part will be used as textbook and the application part as a standard reference. This eBook has been written in a way so that it is student-friendly with clean diagrams and protocols of specific techniques. I sincerely hope that the eBook has been prepared with scientific skills and will serve as a useful document for graduate and undergraduate students.

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PREFACE

This eBook titled ‘Nanobiotechnology: Principles and Applications’ will provide insight into the principles and practices of nanotechnology in biological fields. Nanobiotechnology, an amalgamation of nanotechnology and biotechnology has gained attention due to its diverse applications. It utilizes the power of nanotechnology to solve the problems of various aspects of biotechnology like agriculture, medicine, industry and many more. In view of this, there is an unmet need to compile different horizons of Nanobiotechnology. Additionally, the biological toxicity to nanomaterials needs attention.

We strongly believe this book is a reader’s delight and will help in dealing with the fundamental principles, and applications of nanobiotechnology. This will help students to understand the importance of nano techniques in all domains of biotechnology which will set a benchmark for further research. This eBook will cover topics like nano drug delivery, nano fertilizers, nano bioremediation, nanotoxicology, and nano biosensors to be written by authors who have quality publications in their proposed chapter area. We sincerely hope our efforts will be embraced by students with appreciation and enthusiasm for learning.

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CHAPTER 1

The Roles of Nanoparticles in Ovarian Cancer Treatment and Diagnosis

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Abstract: Ovarian cancer, an aggressive epithelial cancer, remains a major cause of cancer mortality worldwide among women, but it can be diagnosed at an early stage also. Surgical removal of ovarian tumour is a good option for the initial treatment, but this is suitable only at the early stage of cancer. Surgery and other therapies like chemotherapy, hormone role therapy and immunotherapy alone are insufficient for the treatment of today's advanced ovarian cancer. The aim of this book chapter is to review the use of nano-particles in the treatment of ovarian cancer, along with surgery. It is believed that nano therapies have lots of advantages like they stabilize drugs in our body, deliver and penetrate the drugs to tumour-specific cells and can profile the toxicity of chemotherapy. This book chapter also covers the development of nanotherapies, types of nanocarriers and their role in ovarian cancer diagnosis and treatment.

Keywords: Apoptosis, Biomarker, Chemotherapy, Detoxification, Drug cargo, DNA repair, Drug resistance, Graft rejection, Gynaecological cancer, Heterogeneous nature, Hydrophilic corona, Intracellular delivery, M alignancy, Metastatic tumour, Nanocarriers, Nanomaterial, Nanotechnology, Nano transmitter, Photodynamic therapy, Prophylactic, Photo thermal therapy, Renal clearance, Silent Killer, Systematic toxicity.

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INTRODUCTION

The most deadliest female reproductive cancer is ovarian cancer [1]. It is the sixth most common malignancy of females worldwide and the second most common malignancy of the female reproductive system. Ovarian cancer is responsible for 4% of all types of malignancies in women and 5% of cancer deaths [2]. Annual incidence rates vary from less than 5 per 1,00,000 in underdeveloped countries like Brazil, India, Thailand *etc.* to greater than 13 per 1,00,000 in developed countries like the United States, Germany, Denmark, Norway *etc.* It is the most common type of gynaecological cancer, ranking the third behind uterine and cervical cancers, and has the greatest incidence of mortality rates. Ovarian tumour pathology is one of the most challenging areas of gynaecology since the ovary produces a wider range and types of tumours than any other organ however; it is a high-grade serous subtype that is frequently misdiagnosed as a systemic disease. Because 75 per cent of Ovarian Cancer is found at an advanced stage, such as stage III or IV, it is also regarded as the “Silent Killer” [3]. The reason of high death rate is due to the fact that tumour grows secretly, and there is lack of appropriate examination to detect the certain stages. It is generally believed that the fatality rate from this type of cancer will surge very high in the following 20 years [4].

Because of the heterogeneous nature of ovarian cancer, prophylactic and early detection strategies have not yet shown effective result. Identifying risk factors and creating protective factors were the main prevention methods of ovarian cancer in the past [5]. But unfortunately these strategies did not greatly reduce the disease's occurrence. Although, surgery is the initial and effective treatment but most of the time, the disease re-occurs due to the aggressive nature of the tumours [6]. And most of the time it is seen that metastatic tumour of the ovary develops a very strong resistance to conventional systemic therapies (like Chemotherapy, targeted therapy and hormone therapy *etc.*). The resistance of cancer cells is caused by a variety of processes, including decreased absorption, increased excretion, drug inactivation and detoxification, and the loss of DNA repair power.

Currently, although many novel ways have been created to increase drug delivery to cancerous cells, nanotechnology has been identified as one of the best therapy methods for overcoming the barriers in advanced cancer treatment [7]. Nanoparticles have the ability to cope up very easily with molecular imaging, carrying drugs to the specific site, treatment, and tumour cell specific destruction. Conventional chemotherapies show very poor systematic toxicity and toxicological effects towards normal and tumour cells. However, nano therapy can be used to manage the cytotoxic effects of healthy cells while also lowering the toxicity of chemotherapeutics [8]. So, there is a hope for an effective treatment of ovarian cancer with the efficient use of nanocarriers as a solution along with multiple chemotherapeutic drugs.

NANOTECHNOLOGY APPLICATION

Through the knowledge and control of matter at nanometre range, mostly 1 to 100 nm, novel functionalities and qualities of matter can be seen. Employed for a broad array of applications, nanotechnology creates Nano composites, sensors, and processes.

In biology, this technology is called nano biotechnology and in the medical field as nano medicine. The primary goal of nanotechnology in medicine is to improve the efficacy of cancer diagnosis and treatment procedures.

Nanocarriers

Nanocarriers are multifunctional nanomaterials and can be used for the treatment and diagnosis of cancer. Their surface can absorb different types of compounds, such as pharmaceuticals, are absorbed by physical absorption and antibodies by chemical conjugation interactions (Fig. **1**) [9]. Nanocarriers can be classified into several types like micelle, dendrimer, carbon nanotube, liposome, *etc*.

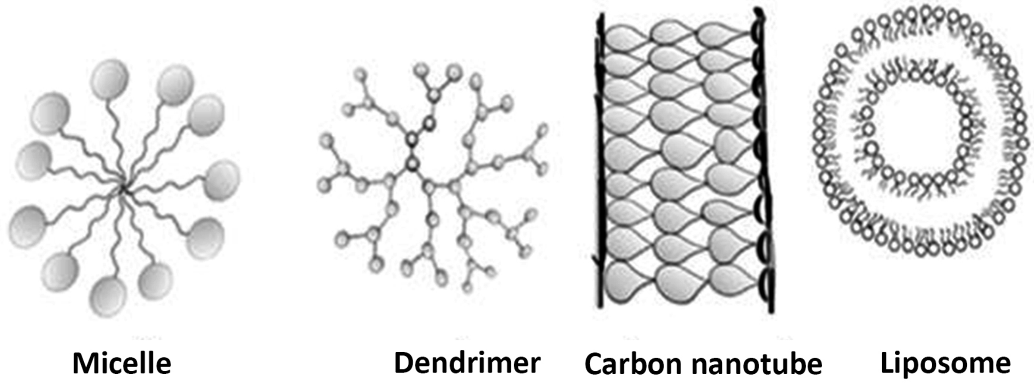

Fig. (1). Examples of some Nanocarriers.

As compared to conventional chemotherapies, Nanocarriers have lots of advantages like delivery of poorly soluble drugs, ability to reduce systematic side effects of chemical treatments, drug stability maintenance by extending their time in bloodstream, and reduced drug resistance by targeting cancer cells [10].

Nanocarriers have the ability to surround the poorly soluble drugs within the hydrophobic interface and can act as carriers for them in blood.

CHAPTER 2

Advances in Nano-remediation of Textile Dyes in Textile Industry Effluents: Current Developments and Future Prospects

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Abstract: Environmental clean-up for the removal of recalcitrant pollutants is a global concern, especially in the terms of industrial waste. Research over the years has led to the development of various conventional physicochemical and biological methods for the decontamination of numerous pollutants. These methods however are reported to be extremely expensive and with limited success. Nano-remediation has been reported as an effective alternative in this regard. The chapter outlines the use of various nanoparticles as an innovative and cutting-edge technology for the clean-up of environmental pollutants. It describes the use of fabricated nanoparticles to remove pollutants. The chapter offers an overview of current research developments in the emerging field of nano-remediation with special emphasis on textile dyes, elucidating the mechanisms involved.

Keywords: Adsorption, Environment, Nano-remediation, Textile dyes.

INTRODUCTION

Human activities have been constantly affecting the quality of air, water and soil. Constant inclusion of heavy metals, pesticides, particulate matter, oil spills, toxic gases, fertilizers, dyes and other organic compounds into the environment has become a major threat to the environment [1, 2] leading to the development of

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nanomaterial based remedial technologies for mitigation of toxic effects of these environmental pollutants through various clean-up mechanism [3 - 5].

Owing to the unique properties of the nano-sized materials, nanotechnologies have achieved immense attention during the last decades. Environmental remediation technologies have utilised the property of higher surface-to-volume ratio for nanomaterials in order to bring efficiency to the remediation processes [4, 6]. Apart from this, nanoremediation has also leveraged the surface chemistry of nanomaterials for trapping target-specific pollutant molecules [7]. Apart from surface chemistry, other tuneable physical parameters of nanomaterials such as size, porosity, morphology along with their unique chemical composition aid the process of remediation confirming additional advantages [8, 9]. The afore-mentioned advantages have therefore popularised the use of nanomaterials for the mitigation of environmental pollutants, especially from aqueous sources.

Furthermore, it is important to note that matrices utilised for the purpose of environmental remediation are not pollutants by themselves. In this connection, different biodegradable materials having desired properties along with nano-sized materials are considered more advantageous then using single nano platforms [10]. Such approaches of using nano-composites have been utilised for scaling up the nano-remediation technology by making it more acceptable amongst the consumers due to its greener and safer nature. Moreover, it also enhances the stability and specificity of the clean-up process by eliminating, off-targeting and promoting target-specific removal of contaminants from the wastewater [11]. Therefore, studies have focused on utilising the core principle of nanotechnology by combining physicochemical surface modifications for nano-composites or functional nano-materials for specific removal of a variety of pollutants from aqueous medium.

NANO REMEDIATION: DEFINITIONS AND AGENTS

Nano-remediation has been defined by various authors in different contexts. For instance, Ganie [9] defines Nanoremediationas "an innovative approach for safe and sustainable remediation of persistent organic compounds such as pesticides, chlorinated solvents, brominated or halogenated chemicals, perfluoroalkyl and polyfluoroalkyl substances (PFAS), and heavy metals". Similarly, Grieger [12] defines it as "nano-remediation is the term used to describe various techniques and methods to clean up contaminated sites using engineered nano-materials". Nanoremediation has also been defined as "Tiny Objects Solving Huge Environmental Problems" in simpler terms [13]. From the perspective of functionality, Zhang [14] simplies nanotechnology as "the use of small size, high specific surface area, reactivity and versatility of engineered nanomaterials to potentiate them for the removal of recalcitrant contaminants and achieve selectivity of target contaminants in complex environmental media". Nano remediation has also been defined as "the practice of using various types of nanoparticles such as TiO_2 based NPs, dendrimers, Fe based NPs, Silica and carbon nanomaterials, Graphene based NPs, nanotubes, polymers, micelles, nanomembranes, *etc.* to diminish environmental hazards" [15]. Thus, leveraging the characteristics of nanoparticles such as high surface area to mass ratio, sensitivity, catalytic behaviour and electronic properties for removal/degradation of pollutants is termed nano-remediation.

Numerous nano-sized materials have been developed using different modes of synthesis for the purpose of environmental remediation. However, there seems no such classification of nanomaterial types utilised for nano-remediation. The chapter, therefore, classifies agents of nanoremediation into three major categories namely, polymer-based nanomaterials, inorganic nanomaterials and carbon-based nanomaterials on the basis of literature review.

Carbon-based Nanomaterial

Carbon-based nanomaterials are known for their unique physicochemical and electronic properties. The mutable hybridization property of carbonaceous materials helps to yield different structural configurations of these nanomaterials such as single-walled nanotubes (SWCNTs), multi-walled nanotubes (MWCNTs), *etc.* Earlier investigations have shown the utility of graphene and carbon nanotubes for environmental remediation applications. It has also been reported that surface treatment of these carbon materials helps in improving the efficacy of these materials as they are otherwise ineffective for remediation purposes. The literature demarcates the dominance of single-walled carbon nanotubes (SWCNT) and multi-walled carbon nanotubes (MWCNT) owing to their absorption properties for the removal of a variety of pollutants from air as well as large-scale aqueous medium [16 - 19]. In order to enhance the adsorption properties further, researchers have been working on opening the close ends of pristine carbon nanotubes (CNTs) [16, 20]. It has been stated that the open-ended CNTs can typically absorb pollutants in four different available sites based on their adsorption energy. Apart from this, other modifications that have been proposed for improving absorption efficacy is by oxidation of CNT. For instance, post oxidation nitric acid treated CNTs proved to improve their heavy metal adsorption capabilities [21]. Furthermore, physical properties such as temperature, molecular weight, pH, and electric dipole moment also have a huge impact on the adsorption phenomenon by CNTs [20]. Thus, tuning physical parameters has also been employed as a strategy to activate carbonaceous nano-materials.

CHAPTER 3

Interaction between Metal Oxide Nanoparticles and Terrestrial Plants: An Overview of the Mode of Action and Future Perspectives

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Abstract: Nanotechnological interventions have extensively been used as an efficient non-invasive approach in agriculture for disease protection, to improve yield and many more. The use of engineered nanomaterials (like metal-oxide nanoparticles) as fertilizers,pesticides, carriers for genetic material/RNA/protein, sensors for detection of contaminants and toxic compounds *etc.* have been extensively studied and reported. Interaction between plants and nanomaterials plays an important role in their applications for various purposes in agriculture and otherwise. In this chapter, mechanisms of uptake and mode of action of three commonly used metal oxide (TiO_2, CuO, ZnO) nanomaterials in plants have been reviewed. The chapter also summarises the various studies conducted on the effect of these nanomaterials on different agricultural food crops in the last 2 decades. The thorough review of existing literature on the aforementioned areas indicates that although the published data on terrestrial phytotoxicity of metal oxide NPs is increasing continuously but surprisingly the range of selected plants is still narrow (mostly agricultural crops and seed plants), thus random selection of plants (outside this narrow range) should be made to gain better insights into the various impacts of nanomaterials on plants.

Keywords: CuO, Mode of action, Phytotoxicity, TiO_2, Uptake mechanisms, ZnO.

INTRODUCTION

Nanotechnology has been identified as one of the most promising and revolutionary technologies, which is going to affect people's life. Now developing

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countries have started investing more in nanotechnology by considering the potential of NPs to overcome the challenges associated with development in key areas such as energy, water, agriculture, health and environment [1]. Therefore, the production of nanomaterials (NMs) has escalated in recent years due to their multifaceted utilities. The estimated global production of engineered nanomaterials (ENMs) in the year 2010 was 260,000 - 309,000 metric tons; out of which approx. 63-91%, 8 - 28%, 0.4 - 7%, and 0.1 - 1.5% were estimated to end up into landfills, soils, water bodies and atmosphere respectively [2]. The most common contaminating ENPs of the environment are carbonaceous nanoparticles (NPs), quantum dots, zero-valent metals, metal oxides and nanopolymers [3]. Certain exceptional properties of NPs such as high specific surface area, abundant surface reactive sites and mobility are greatly affecting the environment and health as well [4, 5]. Organisms especially algae, fungi and plants have direct interaction not only with NMs but also with their existing environment thus may be considered as the first target life forms to be exposed which are indirectly affecting higher species through the food chain [6]. Although, the evolution of plants took place in the presence of natural NPs, but because of expanded production of ENPs and their use in diverse processes and goods; the possibility of plant exposure has increased incalculably [7].

With these points of view, in this chapter, the interaction of NPs and plants has been discussed. As the most encountered group of NPs is metal oxides that are being produced largely for enormous applications, this chapter focuses on 3 leading candidates in line *viz*. TiO_2 CuO and ZnO for the study of interaction with plants.

Titanium Dioxide NPs (TiO_2 NPs)

Certain properties of TiO_2 NPs such as high stability, anticorrosion and photo-catalyst activity make them an excellent candidate to be used in cosmetic and skin care products, antibacterial and air cleaning articles; paints and pigments; and in organic matter decomposition in wastewater. In the year 2010, 64,000 - 81,000 metric tons of ENMs were used in coatings, paints, pigments and cosmetics, with approx. 34,000 and 10,000 tons/year for TiO_2 and SiO_2 NPs respectively. The above-mentioned applications contribute around 42% of the total global ENM flow, 82 - 87% of total ENM emissions to soil and 89 - 97% to water [2].

Copper Oxide NPs (CuO NPs)

Due to manifold uses of CuO NPs, they serve as potent NPs to enter the most important and sustaining environmental compartment *i.e.* soil [8, 9] and, therefore, catching the attention for numerous bio-toxicity studies [10 - 12]. These NPs are owing antimicrobial nature thus predominantly being used in antimicrobial formulations [13]. Plentiful literature is available on the protection of wood products from fungi and insect-induced biodegradation using nano-CuO and nano-$CuCO_3$-based biocides [14]. 50% of the global wood preservation is occupied by the wood preservation market of North America with 79000 tons consumption of Cu salts annually [14, 15]. In the year 2010, the predicted world-wide production of Cu-based NPs was ~ 200 tons/year, and it is increasing continuously [2].

Zinc Oxide NPs (ZnO NPs)

In 2010, the estimated global annual production of ZnO NPs was 30,000 metric tons, which was used primarily in paints, medicine, cosmetics, optics, electronics, coatings and pigment products. Emission at the time of manufacturing was estimated to be around 32–680 tons/year, from which the highest amount is being contributed by emissions from the use of ZnO ENMs in cosmetics. Overall predicted emissions are 90–578 tons/year to atmosphere, 3,100–9,283 tons/year to soils and 170–2,985 tons/year to receiving water bodies [2].

Thus, the inevitable rapid use of metallic NPs in multiple areas have raised the demand for assessment of their impact on different biotic and abiotic components [16 - 18]. Scanty reports are available about the impact of NMs on food crops and the food chain [19, 20]. As plants are in direct contact with the environment and are first targets to face NPs, thus increasing the curiosity to know the way NPs affect plants, the method of their uptake and the way they act in plant systems (Fig. **1**). The focal point of this chapter is to discuss all these aspects of interactions of MONPs and plant systems.

UPTAKE AND TRANSLOCATION OF MO NPS IN PLANT SYSTEM

TiO_2 NPs

The worldwide production of titanium dioxide (TiO_2) NPs is up to 2 million tons/year [21], which eventually contaminate soils and plants on its release in the environment. In Arabidopsis thaliana, the uptake and translocation of nano-conjugate of an ultra-small TiO_2 (<5nm) complexed with Alizarin red were studied by Kurepa *et al.* (2010) [22]. They demonstrated that the inhibition or facilitation of entry of nano-conjugate depends on the pectin hydrogel capsule formed by the mucilage that was released from the surrounding roots. Numerous other studies show that depending on the plant species, toxic heavy metals in the rhizosphere could either be accumulated or inactivated by the polysaccharides present in the mucilage [23]. Asli and Neumann (2009) [24] investigated that in maize (Zea mays), TiO_2 NPs were not taken up by the root cells of excised roots with intact apices, the probable reason for this might be their large size compared to the size of pore diameter (6.6 nm). Servin *et al.* (2012) [25], evaluated the

CHAPTER 4

Role of Nanofertilizers in Agriculture-Futuristic Approach

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Abstract: Chemical fertilizers are crucial in the production of cost-effective agricultural crops. However, long-term usage of chemical fertilizers will deteriorate the soil quality and it is hazardous to human health. Scientists and researchers across the globe are seeking the help of nanotechnology as a possible solution to combat the hazardous effect of chemical fertilizers. Nanotechnology is a branch of science and engineering concerned with the matter at the nanoscale or one billionth of a meter. Nanofertilizers are modified fertilizers that are synthesized using techniques of nanotechnology involving various physicochemical and biological methods. These methods aid in enhancing their attributes and composition, which leads to a positive effect on crop productivity. Nanofertilizers are far more beneficial when compared to chemical fertilizers as the former are cost-effective, less toxic and show controlled and regulated release of nutrients to plants. This chapter is primarily concerned with the various methods employed in nanofertilizer synthesis, the economic importance of nanofertilizers and their advantage over conventional chemical fertilizers.

Keywords: Chemical fertilizers, Cost-effective, Nanofertilizers, Nanotechnology.

INTRODUCTION

Soil is a storehouse of nutrients that serve as the medium in which plants grow. Nutrients are lost from the soil in several ways such as crop harvest, weeds,

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leaching, volatilization, and erosion thereby affecting the fertility status of the soil, thus affecting productivity. Therefore, these losses when combined altogether, a significant amount of nutrients are lost from the soil such that the crop requirement exceeds the soil supplying power thus nutrients are applied from external sources [1].

The bush-fallow strategy, which allows arable land to switch back to fallow after 3-4 years of intensive cropping, was once the conventional technique of sustaining soil fertility and production when the human population was low. With the increasing growth of the human population and other socioeconomic demands, an attempt was made to substitute the fallow system with the use of manures, mainly where significant numbers of animals were present. This highlights the agricultural benefits of organic manures such as farmyard manure, compost, green manure, poultry droppings, cow dung, and household refuse, among others. Nowadays, agriculture became more demanding with the usage of crops giving high yields. But such crops require more nutrition to grow than the natural nutrients present in the soil. It became evident that manures could not fulfill the nutrient requirements of these crops for increased productivity, and could not be procured in adequate quantities to meet farmers' needs. Even when manure is readily accessible, transportation and labor costs (inevitably) restrict its frequent use. In this case, mineral fertilizers were considered a viable alternative [2].

Fertilizers, also referred to as inorganic fertilizers are the mineral source of plant nutrients that are industrially manufactured and their nutritional content is higher than that of farmyard manures and are almost released instantly, thus meeting the nutrient demand of the crop. Fertilizers supply macronutrients such as Nitrogen (N), Phosphorus (P) and Potassium (K) which are necessary for plant growth and development. Fertilizers also supply micronutrients such as Zinc (Zn), Sulphur (S), and Iron (Fe) for plant uptake and utilization in various metabolic processes. Fertilizers can be straight fertilizers (such as Urea, SSP and MoP) containing only one type of primary macronutrients or complex containing two or more primary macronutrients that are chemically bound together [3].

Commercial chemical fertilizers are expensive and include substances that are harmful to the skin or respiratory system. Because of their huge particle size and low solubility, they are less bioavailable to plants. Furthermore, they cause toxicity and disrupt the soil's ecological equilibrium. Implementing nanoparticles in sustainable agricultural practice might be defined as using modern and advanced agro-nanofertilizers over conventional fertilizers in a sequence of environmentally and farmer-friendly inputs [4].

CONVENTIONAL FERTILIZERS

Today's agriculture is growing increasingly intensive, requiring higher dosages of chemicals such as fertilizers, herbicides, and pesticides to achieve maximum productivity per unit area to fulfill the demands of an ever-increasing human population. These chemicals have with no doubt increased crop productivity but simultaneously usage of these chemicals is more than optimum, severely affecting natural resources and ecosystem services.

Challenges of Fertilizers In Present-Day Agriculture Practices

Fertilizers play a significant role in obtaining higher crop yields as they contribute up to 40-60% of agricultural productivity [5]. However, applying higher doses of fertilizer than optimum does not guarantee an increase in crop productivity rather it results in several problems such as soil health degradation, environmental pollution, multi-nutrient deficiency (especially micro-nutrients), element toxicity, rise in the cost of production, among others [6]. The use of inorganic fertilizer helps in increasing the yield of the crop but it increases the cost of production (cost of fertilizer plus cost of transportation) and applying higher doses leads to environmental pollution. In addition, the application of higher rates of chemical fertilizer leads to significant land problems as a result of over-exploitation of land and land pollution [7]. Furthermore, applying higher doses of fertilizer more than crop requirement leads to losses of nutrients through various sources such as leaching (especially for nitrate), volatilization, immobilization, *etc.* The nutrients lost will not be utilized by the plant as such will increase the cost of crop production. Nutrients lost through leaching cause groundwater pollution while those lost through volatilization such as NO_2 (especially in rice field) is among the greenhouse gases that cause climate change. Pandey and Awasthi [8] concluded that using too many chemical fertilizers reduces soil health quality attributes (physical, chemical, and biological qualities) as well as crop productivity.

Solutions to the Use of Fertilizer

Despite all these challenges regarding the use of a fertilizer, it plays a significant role in obtaining higher productivity (contributes 40-60% of crop yield) as its nutrient concentration is high and is released immediately to the soil for plant uptake. Therefore, the use of fertilizer cannot be eliminated and this paved the way for several nutrient management practices to be employed in present-day agriculture to minimize many problems linked with the usage of fertilizer. The concept of integrated nutrition management is one of these nutrient management strategies. To improve crop and soil productivity as well as the sustainability of

CHAPTER 5

Nanobiotics for the Treatment of MDR Infections

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Abstract: Nanoparticles are those agents that are made-up of single or a combination of single or multiple materials which are very small in size ranging from 1 to 100 nanometers. Several studies reveal that nanoparticles have features that interact effectively with microorganisms and can help in treating multidrug-resistant organisms. These have intrinsic antimicrobial activity and are of various types broadly divided into organic and inorganic nanoparticles. Nanoparticles can engage with bacteria and travel across the bacterial cells and host cell membranes, and help treat ESKAPE pathogens which are among the most notorious multidrug resistant superbugs. These pathogens have MDR features and have multiple types of MDR mechanisms including drug inactivation/alteration, modification of drug binding sites/targets, reduced intracellular drug accumulation and biofilm formation. For targeting different types of MDR, there are multiple types of nanoparticles such as metal nanoparticles, nanostructures, leukocyte membrane-coated nanoparticles, red blood cell membrane-coated nanoparticles, cancer cell membrane-coated nanoparticles, and platelet membrane-coated nanoparticles among others. Antimicrobial nanobiotics identified and synthesized to date harbor a vast diversity of intrinsic and modified physicochemical properties and have applications in diagnostics. No technology is without its challenges and the same is true for nanobiotics. The major challenges in this field of nanobiotic-based therapeutics are their allergic responses, assembly and pharmacokinetics. This chapter will elaborate on the mechanisms of action of various types of nanobiotics present as cost-effective solutions useful in a variety of applications in the treatment of MDR pathogens with a special focus on ESKAPE pathogens.

Keywords: Antimicrobial resistance, ESKAPE, Multi drug resistance, Nano biotics, Nanoparticles.

INTRODUCTION

The first antibiotic Penicillin was discovered and commercially produced in 1928. From the 1920s to the present, we have taken for granted that every infection can be cured completely by antibiotics. Because of the generous unchecked use of antibiotics in human therapy; it has resulted in the birth of pathogenic bacteria resistant to multiple drugs [1]. MDR is a serious threat to public health. Efforts for

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controlling MDR in "ESKAPE" pathogen (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter spp.)* have been hampered by their ability to escape drugs. They can cause life-threatening nosocomial infections. These pathogens also known as "Superbugs" [2] carry MDR genes on the bacterial chromosome, plasmid, or transposons. Drug resistance mechanisms fall into several categories, some bacteria produce enzymes that can irreversibly modify and inactivate the drug; these are β-lactamases, aminoglycoside-modifying enzymes, or chloramphenicol acetyltransferases. Some bacteria perform modifications of drug binding sites/targets to avoid recognition. The balance of antibiotic uptake and elimination determines the susceptibility of bacteria to a particular drug. Bacterial cells often reduce intracellular drug accumulation to develop antibiotic resistance. Biofilm formation [3] contributes to 65% to 80% of microbial infections and is advantageous in the survival of bacteria. Biofilm can be produced in recurrent tonsillitis, cystic fibrosis lung infection, urinary tract infections and chronic wounds. Clinical illnesses attributed to bacterial adhesion to implants and medical device-related infections are among the most challenging issues to be addressed in MDR infections [4]. Owing to the multidrug resistance nature of pathogens and the failure of the treatments, we must find a better option for treating these microorganisms. Nanobiotics, a revolutionary concept can be seen as a future of drugs for MDR [4].

Nanobiotics are small materials 1- 100 nanometers in size. Nanobiotics also known as Nano particles are categorized into numerous classes; these classes are based on their size, forms and qualities [5]. These categories include numerous subcategories which are elaborated further in the chapter. A brief glimpse into nanobiotics reveals that inorganic nanoparticles are introduced as nanobiotics and used for drug carriage [6]. Nanoparticles that are covered by metal oxide shell are known as metallic nanoparticles. After chemical modification, metal nanoparticles can be used in diagnostic imaging, and targeted drug delivery [7]. Organic nanoparticles focus on the utilization of nanoparticle-based materials having an organic structure [8]. Leukocyte membrane-coated nanoparticles, red blood cell membrane-coated nanoparticles [9], and cancer cell membrane-coated nanoparticles [10] are additional nanoparticles' categories that have been recently designed. In this chapter, we will be discussing about the roles and use of nanoparticles in MDR treatment and diagnostics.

Nanobiotics

Nanobiotics, also known as nanoparticles, are small materials that range in size from 1 to 100 nanometers. They are categorized into numerous classes based on their qualities, forms, and sizes. Nanoparticles may consist of a single material or a combination of different materials. They are used in research and technology because of their small size and unique features. Nanoparticles show various physical as well as chemical properties that include the optical, mechanical, and magnetic properties [5]. Optical properties like absorption, transmission, reflection, and light emission of nanoparticles are dynamic. The optical property of nanoparticles is of great importance in several ways. It was discovered that their optical qualities are influenced by their internal electronic structure, providing a thorough understanding of the structure. They can use their electrical properties to develop quantum effects which may lead to variations in size, shape, and color they produce. The optical properties of nanoparticles can be recognized by using various spectroscopic techniques [11]. Nanoparticles' magnetic characteristics have a wide range of applications, including drug administration, therapeutic treatment, MRI imaging, and in-vitro diagnostics. According to one study, nanoparticles perform best when their size is less than the critical value, which is 10–20 nm. Nanoparticles' magnetic characteristics can dominate more effectively at this low scale, making them cost-effective and useful in a variety of applications. Nanoparticles have a magnetic property due to their unequal electrical distribution [12]. The unique mechanical properties of nanoparticles have numerous applications in the field of surface engineering, nanofabrication, and nanomanufacturing. Different mechanical parameters such as elastic modulus, hardness, stress and strain, adhesion, and friction can be examined to better understand the mechanical nature of nanoparticles. Surface coating, coagulation, and lubrication, in addition to these characteristics, play a role in the mechanical properties of nanoparticles. Controlling the mechanical properties of nanoparticles and their interaction with any type of surface, on the other hand, is critical for highlighting surface quality [13].

The Amalgamation of Nanoparticles with Antimicrobials

Antimicrobial resistance to hazardous bacteria is on the rise across the world, posing a serious threat to human health. This has led the researchers to look for new therapeutic options. One of the approaches that have been explored currently includes drug-associated nano systems. Several studies have revealed the intrinsic antimicrobial activity of various types of organic and inorganic nanoparticles. These nanoparticles have many unique properties, including small size and a high surface-area-to-volume ratio in comparison to bulk material, both of which are important for antimicrobial activity. Nanoparticles' special features allow them to engage with bacteria and rapidly traverse the bacterial and host cell membranes, obstructing the main microbial metabolic pathways and allowing the eradication of intracellular infections where antibiotics typically fail [5]. Nanoparticles can functionalize the surface, especially when it comes to linking chemical functional

CHAPTER 6

Metallic Nanoparticles as Antibacterial Agents

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Abstract: Metallic nanoparticles against bacteria have increased recently due to their unique properties. Many metals like silver, gold, copper, aluminum, zinc and their oxides have been shown to have antibacterial properties. The activity of the nanoparticles is affected by their physico-chemical properties. Different types of mechanisms are proposed for the antibacterial actions against various types of bacteria. The metal-based nanoparticles are synthesized by the top-down methods and bottom-up methods. However, the latter methods are used effectively against many types of bacteria including antibiotic-resistant bacteria.

Keywords: Antibacterial activity, Antibiotic resistant, Metallic nanoparticle, Physico-chemical properties, Synthesis.

INTRODUCTION

Nanoparticles are exceptionally tiny particles that vary from 1-100 nanometer. These particles possess different chemical and physical properties in contrast to their larger counterparts. Since the particle size is extremely small, thus they follow the Brownian movement and do not sediment. Moreover, these are not seen by the naked eyes and with an ordinary microscope. Nanotechnology is one of the several techniques, which is employed in biology, chemistry, environment, food industry, agriculture, engineering, therapeutic application, sensor and medicines [1, 2]. The pharmaceutical field in medicine is mainly used for the

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improvement of drug solubility, bioavailability and delivery to various sites of action and real-time monitoring of drugs [3].

Nanoparticles are classified into different categories based on their physical and chemical properties. These may be metal nanoparticles, non-metal nanoparticles, ceramic nanoparticles, semiconductor nanoparticles, ceramic based nanoparticles, carbon-based nanoparticles, lipid-based nanoparticles, polymeric nanoparticles, organic based nanoparticles, *etc.* Among different types, metal-based particles are important in pharmacy. They are used in drug and gene delivery for their effectiveness against micro-organisms, in diagnostic assay, in thermal ablation and anticancer properties [4, 5].

Since ancient times, metals are used to cure several types of diseases and to combat infections against many micro-organisms like bacteria, fungi, viruses *etc.* Many types of metals are used among which silver, copper, gold, aluminum oxide, copper oxide, and titanium oxide have found their wide application against various diseases. In this chapter, we have discussed the properties, mode of action and the preparation of metallic nanoparticles.

PHYSICO-CHEMICAL PROPERTIES OF METAL NANOPARTICLES

Metal nanoparticles have quite distinctive features in comparison to their larger counterparts. Such properties provide them the mechanism of toxicity to different types of bacteria. Different types of physico-chemical properties affect their toxicity which are detailed as under:

Size of Nanoparticles

The size as well as the surface area of metal nanoparticles is crucial for the antimicrobial activity. The small size of nanoparticles has the larger surface area relative to volume that makes the nanoparticles more active by facilitating their entry in the bacterial cell membrane in comparison to larger nanoparticles [6]. The nano shape of the nanoparticles facilitates better contact with the plasma membrane of the bacteria mostly because of their larger surface area showing preferable interaction with the membrane than the larger nanoparticles [7]. The size of the nanoparticles thus greatly affects the various types of biological mechanisms [8, 9]. It clearly indicates that size and surface area of the particle govern the systems [10]. Due to the smaller size, nanoparticles are able to enter the biological system [11] due to which modification of various biomolecules takes place [12] which ultimately interferes the biological functioning of the cell. Although various mechanisms are attributed to the toxicity of nanoparticles, yet the production of ROS (reactive oxygen species) that is because of the creation of free radicals since the liberated electrons try to make a stable bond is prime mechanism. The size of nanoparticles is essential in the generation of ROS which imparts hazardous effects on DNA in comparison to their larger counterparts [13, 14]. Besides, the size less than 100nm causes adverse respiratory effects in human beings in comparison to the larger nanoparticles. However, it is not necessary that the size of nanoparticles alone is responsible for antibacterial activity, other physico-chemcial properties of NPs should also be considered for antibacterial mechanism [15].

Shape of Nanoparticles

The shape of metal nanoparticles is important for their antibacterial activity. Shapes of nanoparticles interact with periplasmic enzymes of the bacterial cell thereby causing bacterial cell damage [16]. In nanoparticles, the most common shape is spherical however, other shapes like triangular, cubical, hexagonal, oval, helical, prism, tubes and rod-shaped are also found which impart toxicity and influence the wrapping process in the membrane during endocytosis and phagocytosis [17]. It has been shown that triangular and truncated (cut-off corners) sized nanoparticles show better inhibition [18] whereas nanotubes and rod shapes have been reported to be more effective due to their exposed planes. The exposed planes having higher density facets help in increasing reactivity because of the large surface area to volume proportion thus facilitating to increase the adsorption and binding of nanoparticles [8].

Charge of Nanoparticles

Charge on nanoparticles is a pivotal factor for the antibacterial property. Positively charged nanoparticles are attracted to the anionic cell wall of bacteria electrostatically thus alter the functioning of electron transport chain in bacteria which results in the creation of ROS [8]. Whereas, the negatively charged nanoparticles do not stick to the bacterial cell wall, however, the higher concentration of the negatively charged bacteria leads to interaction between bacteria and the nanoparticles due to molecular overcrowding [19]. The potential of nanoparticles increases vascular permeability [20].

Acidic Conditions

Acidic conditions favour bindings of the nanoparticles to the bacterial cell wall through electrostatic interaction [21]. Acidic conditions have been shown to increase the dissolution and release of Zn^+ [22]. In acid medium, the silver

CHAPTER 7

Promises of Nanobiosensors in Pathogen Detection

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Abstract: Rapid and accurate identification of pathogens has always been challenging. There are a number of methods for the detection of pathogens, but still they face critical challenges. In general, rapidity, sensitivity, and accuracy are the important criteria that limit the applicability of classical methods. Nanomaterials-based biosensors have been proven to be effective for the early and accurate quantification of pathogens. Interactions between target pathogen and nanomaterials are very important, as they provide a measurable signal in biosensors. Nanobiosensors are effective in detecting pathogenic bacteria in various samples, including food, water, blood, and other matrices. In this chapter, we intend to discuss the existence and importance of electrochemical-based biosensors for quantification.

Keywords: Bacterial Sensing, Electrochemical, Nanobiosensors, Pathogenic bacteria.

INTRODUCTION

Bacteria, fungi, and protozoans are infectious agents that cause disease. Viruses and prions are molecular scale infectious agents, enter the body causing infection and lead to millions of deaths annually worldwide [1, 2]. The most prominent pathogens include bacteria such as *S. aureus* and *E. coli*, and viruses like influenza virus which bring exotoxins, mycotoxins and enterotoxins. They vary in several regards, like in contagiousness, virulence, transmittable dose and mode of spread. For example, the world is at present facing a global pandemic linked with the COVID-19 virus, for which infectious dose and virulence data are still promising.

Food products are the actual provision for a healthy life and are the strong transmitting media of more than 200 known diseases [3]. Drinking water is also a major source of contamination by microorganisms that have increased fast in

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recent years [4, 5]. Community concern has significantly increased regarding water and food safety in the past decades.

Therefore, it is of primary importance to observe these microorganisms for the avoidance of infections, the maintenance of human health at large and to comply with quality standards.

Different pathogen recognition techniques have been developed in recent times. Traditional methods containing immune-diffusion, latex agglutination, immune-precipitation, *etc.* were multi-step and time-consuming, taking several days to confirm the presumptive results [6, 7]. This has made them less appropriate for the fast and direct study of pathogens.

Some other techniques such as Enzyme-linked immunosorbent assays (ELISA), and Polymerase Chain Reaction (PCR) were frequently opted to detect pathogens but they could be time-consuming, sensitive and expensive to the given qualitative and quantitative information [8 - 10]. PCR technique not only requires expensive reagents for routine analysis but also bears the problem of false positives by amplifying nonviable cells [11]. Hence, it is required to develop suitable detection methods that authorize an accurate, rapid, and sensitive investigation to monitor pathogens.

Biosensors are integrated devices, developed to quantify and measure biomarkers particularly for infectious pathogens since they exhibit the advantages of selectivity, simplicity, rapidity, and high sensitivity [12, 13]. A biosensor has two elements, a transducer and a bioreceptor, which have great importance. A transducer converts the chemical recognition information into an assessable signal, and the bioreceptor can recognize and combine the target biomolecules. On the basis of signals, biosensors, and transducers are divided into colorimetric, fluorescent, electrochemical, SERS biosensors, and so on.

Electrochemical-based Biosensors

Electrochemical biosensors can perform chemical or biological analysis with simplified sample preparation and facilitate high sensitivity in lesser time. Electrochemical biosensors are made up of three elements, with signal transduction element, target recognition element, and electrochemical signal output elements. Combined with electrocatalytic activity, electronic properties and nanoparticles with a huge surface area [14], nanoparticles-based electrochemical biosensors have achieved significant attention for pathogen detection [15]. Nanoparticles offered an appropriate microenvironment for the immobilization of biomolecules to support the transfer of electrons between electrodes and immobilized biomolecules and enlarge the surface area of electrodes for target identification. Therefore, electrochemical-based biosensors have a unique advantage of fast response, high sensitivity and ease of operation in thick media compared with conventional methods. Based on monitored electric parameters, electrochemical biosensors could be divided into voltammetric biosensors, amperometric biosensors, and impedimetric biosensors, and potentiometric biosensors.

Voltametric Biosensors

Voltammetric biosensors supervise the current changes caused by the reduction or oxidation reaction of the electrochemically energetic analytes, which can be studied by the unstable potential in the electrochemical system [16].

Shoaie&Omidfar (2018) prepared a voltammetric biosensor for rapid detection of *E. coli* based on AuNPs and a polyaniline customized screen-printed carbon electrode with a LOD down to 4 CFU/ml. The low LOD biosensors have great applications of AuNPs and polyaniline, which may significantly increase the surface area and conductivity for immobilized biomolecules [17].

Likewise, Zhu *et al.* (2014) developed a unique amperometric biosensor based on the rolling circle amplification (RCA) approach for *Salmonella* detection in milk samples. The *Salmonella* DNA was first arrested on the electrode surface by a DNA-AuNPs probe. After a chain of amplification processes, the DNA-AuNPs identified the RCA product and formed an enzymatic amperometric signal. The range for target DNA detection by proposed biosensors was from 10aM to 10pM and the LOD down to 6.76 aM [18].

Nze *et al.* (2019) also developed a technique for separating and electrochemically identifying *E. coli* in ground meat also developed by. In this technique, antibody-coated magnetic beads and hydrodynamic cavitation are used for the separation of immunomagnetic samples, which significantly amplified the detection potential of the biosensors [19].

In 2017, Chen *et al.* developed a voltammetric biosensor for the detection of *Mycobacterium tuberculosis* DNA. If nanoparticles are incorporated with DNA amplification approach, this can improve the detection limit of biosensors.

Metal-based nanoparticles, such as MWCNTs and GO carbon-based NPs, have also been commonly applied in electrochemical biosensors because of their excellent electron transfer properties and high surface area.

Amperometric-based Biosensors

Amperometric biosensors are working on the principle that the number of

CHAPTER 8

Breaking the Barriers of Nanotoxicological Assessments: The Importance of Available Models and Future Perspectives

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Abstract: Nanoparticles (NPs) and nanotechnology have penetrated every walk of life. The nanotechnology-based products include pharmaceuticals, cosmetics, electronic goods, food, food packaging, and household products of daily use. The unique physicochemical properties of nanoparticles also make them a potent toxicant. The evidence suggests that nanoparticles are used in humans' neurological disorders, pulmonary disorders, and other ailments. The situation is alarming as NPs may make their way to the human fetus. The regulations for checking the use of NPs are still in their early stages. The NP toxicity has not only affected the human race but the entire Biosphere. The chapter discusses the different assays and models to study nanotoxicity. The models used in deciphering the molecular mechanism are primarily *in vitro* models, particularly 2D and 3D cell cultures of primary, cancerous and normal cell lines. 2D cultures are monolayers, while 3D cultures can be spheroids and organoids derived from stem cells. Cell culture models serve to be a good assessment model but due to lack of systemic complexity, results may not be explicitly extrapolated to humans. In order to fill the gap, *in vivo* models are available. *In vivo* models are helpful in assessing the systemic toxicity in organisms. The *in vivo* models are further categorized as models to study human nanotoxicity and the models to study nanoecotoxicity. Out of the plethora of models, certain specific models are briefly discussed here. The ethical regulations for the usage of animal models are stringent which sometimes make it challenging to acquire animal models. Such challenges can be overcome by developing futuristic models like a lab or animal on a chip, and other computation models which may make nanotoxicological assessments easy and accurate, thereby helping in making efficient regulatory policies for NPs usage in various consumer products safeguarding the mankind and the biosphere.

Keywords: Cell viability, Cytotoxicity, Ecotoxicity model, *In vitro* models, *In vivo* models, Nanoparticles (NPs), Nanotoxicity.

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INTRODUCTION

The advances in nanotechnology have been unprecedented in the last two decades. The technology is penetrating every aspect of human life in the form of nanomedicines, disease diagnostics, food preservatives, cosmetics (sunscreen), detergents, personal wears, vehicles, paints, surface coatings and electronic goods. Nanoparticles (NP) are everywhere. "Nanotechnology product Database," a website that maintains the data of nanotechnology based products worldwide, shows currently there are 9422 products that are being manufactured by 2797 companies set in 64 countries across the globe [1]. The number of such products has increased more than seven times in the last ten years as it was just 1317 in 2011.

The database shows that the electronics, cosmetics, medicine and textile industries are amongst the top industries having maximum numbers of nanotechnology enabled-products [1]. This ever increasing trend shows how fast we are dumping our environment with such nanotech enabled products containing various NPs whose fate and effects on the human and the biosphere is drastically unknown. NPs are more reactive as compared to their larger counterparts with similar particle mass due to their smaller size, higher surface area, and high tensile strength. The characteristics that make them such an advanced technology, also make them a threat to us and our Biosphere. The term 'nanoparticle' was coined in 1970s but its potential to be a toxicant was acknowledged in the year 2004 when the term 'Nanotoxicity was used for the first time [2]. The immense increase in the uses of NPs in consumer products has enhanced the chances of human exposure due to which the instances of toxic outcomes have also increased. There are studies which highlight the neurological, pulmonary, vascular, and genetic toxicities in humans caused by NPs' exposure. For example, in a clinical study performed over 22 human subjects, chronic exposure to Fe_3O_4 NP of size less than 20nm is reported to be neurotoxic. Age-associated bio-mineralization of Fe_3O_4 in the brain is manifested as Alzheimer's disease [3]. In another study, 37 human subjects showed symptoms of neurodegenerative disorders due to an enhancement in ROS levels upon exposure to Fe_3O_4 with a size ranging up to 150 nm [4]. To investigate the pulmonary toxicity of nanomaterials, Khatri *et al.* studied the effects of acute exposure to NPs (30-40 nm) from photocopiers in 9 healthy volunteers. These nanoparticles can trigger immune responses in the upper airways, resulting in systemic oxidative stress with the generation of pro-inflammatory cytokines [5]. In a study dealing with vascular dysfunction, an incidental exposure of diesel fumes consisting of NP (<100 nm) showed increased systolic blood pressure in 16 human subjects, which might be due to vasodilation induced by oxidative stress [6]. Genotoxicity associated with exposure to silver NPs is also reported in mononuclear leukocytes in 76 subjects employed in the silver jewellery industry. The genotoxicity was attributed to oxidative stress induced by silver NPs [7].

The situation is alarming as NPs are not only affecting human health, but also being accumulated in our ecosystem. The release of NPs in water bodies and landfills is 69000 and 189200 metric tons per year [8], respectively. The biological magnification of NPs is still in its early stages of research. In the early 1960s, pesticides and chemical fertilizers came up with a lot of hope and promise in solving hunger issues by increasing plant yield. However, after decades of prolonged usage, we now know that these toxic chemicals enroute to our biological system and cause various diseases ranging from mild allergies and hormonal disorders to severe genotoxicity and cancers [9 - 11].

The human race would certainly not want to be caught off guard in the case of NPs, and that is why it is important to study the behaviour of NPs in terms of toxicity for which we need to device a vast setup of model systems along with the robust test batteries. The fate of NPs in the environment depends upon the aggregation, disaggregation, chemical interaction, and change in their surface properties. There is very little research available regarding these aspects of various NPs in both biological and ecological systems. Due to a lack of robust knowledge about the prospective harms of using these NPs in various consumer products like cosmetics and other routinely used stuffs, developing countries like India fail to make stringent policies for regulating the usage of NPs in consumer products. Thus, the area needs appropriate model systems for nanotoxicity evaluation in order to decipher the potential threats as well as to form stringent regulations.

Here in this chapter, we would discuss the present advances in nanotoxicology research in terms of various assays and various available models for the assessment of toxicity of such NPs along with the future perspectives just for the ease of the young researchers because very few such articles are available that illustrate all these assays and models in one write-up.

NANOTOXICITY CAUSES AND MECHANISM

Nanoparticles enter the human body *via* oral ingestion, inhalation, occular exposure, deposition on the skin, and intravenous administration. NPs then translocate *via* the bloodstream to distant organs and tissues. While translocating NPs may interact with serum proteins, thus resulting in the structural changes of interacting proteins, causing them to accumulate around the NP (protein corona formation) and may change the protein's functionality. Subsequently, NP may also trigger certain pathways that lead to immunotoxicity, loss-of-function in proteins, new antigenic site formation, and may hinder gene expression [12, 13]. Some of

SUBJECT INDEX

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