# NANOPHARMACOLOGY AND NANOTOXICOLOGY:

## CLINICAL IMPLICATIONS AND METHODS

Editors:

**Elham Ahmadian**
**Magali Cucchiarini**
**Aziz Eftekhari**

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# Nanopharmacology and Nanotoxicology: Clinical Implications and Methods

Edited by

**Elham Ahmadian**
*Kidney Research Center*
*Tabriz University of Medical Sciences*
*Tabriz*
*Iran*

**Magali Cucchiarini**
*Molecular Biology*
*Vice-Director, Center of Experimental Orthopaedics*
*Saarland University Medical Center*
*Kirrbergerstr. Bldg 37*
*D-66421 Homburg/Saar*
*Germany*

*Affiliated Researcher*
*Université Paris 13, Sorbonne Paris Cité*
*Unité CSPBAT - UMR CNRS 7244*
*Équipe LBPS*
*F-93430 Villetaneuse*
*France*

&

**Aziz Eftekhari**
*Department of biochemistry*
*Faculty of Science*
*Ege University*
*Izmir*
*Turkey*

**Nanopharmacology and Nanotoxicology:**
**Clinical Implications and Methods**

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# FOREWORD

Nanomedicine is one of the 21st century's most promising technologies and there is rising attention in the world toward the development of new highly specific approaches in the diagnostic and therapeutic applications of nanobiotechnology. This field is a multidisciplinary science and carries the science of incomprehensibly small and portable devices closer to reality. Among the different applications of nanobiotechnology in health sciences, nanopharmaceuticals, gene therapy, regenerative medicine, targeted drug delivery, and disease diagnostics are the most important. It explores the application of nanotechnology in the development of safe, effective, and reliable tools to combat various infections. In the field of pharmacology and toxicology, the future of nanobiotechnology is very bright and facilitates precise and selective occlusion within minutes, and will ensure improved health. Nano pharmacology could also prove an outstanding milestone.

The main focus of the book is dedicated to concepts, applications, and perspectives that combine contributions from analytical, bioorganic, and bioinorganic chemistry, molecular and cell biology, and materials science in an attempt to give the reader a feel for the full scope of current and potential future developments. The chapters in this volume clearly emphasize the high degree of interdisciplinary research that forms the backbone of this joint venture of pharmacology/toxicology and nanoscience. Readers will benefit from all this information if they decide to read this book.

**Prof. Rovshan I. Khalilov**
Baku State University
Head of Department of Biophysics and Biochemistry
Baku
Azerbaijan

# PREFACE

Nanomedicine is one of the 21st century's most promising fields and there is rising attention in the world toward the development of new highly specific approaches in the diagnostic and therapeutic applications of nanobiotechnology. This field is a multidisciplinary science and carries the science of incomprehensibly small and portable devices closer to reality.

Among the different applications of nanobiotechnology in health sciences, nanopharmaceuticals, gene therapy, regenerative medicine, targeted drug delivery, and disease diagnostics are the most important. It explores the application of nanotechnology in the development of safe, effective and reliable tools to combat various infections.

Recently, numerous efforts have been made to improve assays for the diagnosis and treatment of diseases including cancer in terms of selectivity and sensitivity based on nanobiotechnology. These developments will increase the survival rate of cancer patients by enabling early detection and treatment. Although only a few nanobotechnology-based assays have been introduced to clinical trials, these methods of cancer diagnosis are poised to move into the clinic in the near future.

The main focus of this book is dedicated to concepts, applications and perspectives regarding combined contributions from pharmaceutical, pharmacological, toxicological, cell biology, and materials science in an attempt to give the reader a feel for the full scope of current and potential future developments. The articles in this volume clearly emphasize the high degree of interdisciplinary research that forms the backbone of this joint venture of Pharmacology/Toxicology and nanoscience.

The book is divided into 2 main sections. The first section concerns nanobiotechnology for human health including gastrointestinal disease, kidney diseases, pulmonary disorders, reproductive system, COVID-19, and cancer.

The second section is devoted to toxicological aspects of nanomaterials which involve toxicological assessments of Nan- therapeutics and potential solutions for nanotoxicology.

We hope that the results of theoretical, methodological, and practical studies presented in the proposed collective work of the authors will be interesting both for specialists and for the general public.

**Elham Ahmadian**
Kidney Research Center
Tabriz University of Medical Sciences
Tabriz
Iran

**Aziz Eftekhari**
Department of biochemistry
Faculty of Science
Ege University
Izmir
Turkey

**Magali Cucchiarini**
Molecular Biology
Vice-Director, Center of Experimental Orthopaedics
Saarland University Medical Center
Kirrbergerstr. Bldg 37
D-66421 Homburg/Saar
Germany
Affiliated Researcher
Université Paris 13, Sorbonne Paris Cité
Unité CSPBAT - UMR CNRS 7244
Équipe LBPS
F-93430 Villetaneuse
France

# List of Contributors


| | |
|---|---|
| **Ali Jahanban-Esfahlan** | Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran<br>Kidney Research Center, Tabriz University of Medical Sciences, Tabriz, Iran |
| **Ahad Mokhtarzadeh** | Immunology Research Center, Tabriz University of Medical Science, Tabriz, Iran |
| **Ali Mandegary** | Department of Toxicology and Pharmacology, Faculty of Pharmacy Kerman University of Medical Science, Kerman, Iran |
| **Afsaneh Farjami** | Food and Drug Safety Research Center, Tabriz University of Medical Science, Tabriz, Iran<br>Pharmaceutical Analysis Research Center, Tabriz University of Medical Science, Tabriz, Iran |
| **Asma Najibi** | Department of Pharmacology and Toxicology, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran |
| **Atefeh Abedi** | Department of Endodontics, Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran |
| **Aygun Nasibova** | Institute of Radiation Problems, Ministry of Science and Education Republic of Azerbaijan, AZ1143 Baku, Azerbaijan<br>Department of Biophysics and Biochemistry, Baku State University, AZ1148 Baku, Azerbaijan |
| **Asif Selimoğlu** | Department of Otolaryngology, Hacettepe, University Faculty of Medicine, Sihhiye, Ankara, Turkey |
| **Babak Sokouti** | Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran |
| **Cumali Keskin** | Department of Biology, Mardin Artuklu University Graduate Education Institute, Mardin 47200, Turkey |
| **Khadijeh Khezri** | Deputy of Food and Drug Administration, Urmia University of Medical Sciences, Urmia, Iran |
| **Maleki Dizaj Solmaz** | Dental and Periodontal Research Cente, Tabriz University of Medical Sciences, Tabriz, Iran |
| **Mohammadreza Ardalan** | Kidney Research Center, Tabriz University of Medical Sciences, Tabriz, Iran |
| **Mohammad Mehdi Ommati** | College of Life Sciences, Shanxi Agricultural University, aigu, Shanxi , Peoples'Republic of China<br>Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran |
| **Mahdieh Alipour** | Dental and Periodontal Research Center, Tabriz University of Medical Sciences, Tabriz, Iran |
| **Mehmet Fırat Baran** | Department of Food Technology, Vocational School of Technical Sciences, Batman University, Batman, Turkey |

CHAPTER 1

# Advances in Pulmonary Nanopharmacology

**Khadijeh Khezri[1], Solmaz Maleki Dizaj[2,*]** and **Shahriar Shahi[2]**

[1] *Deputy of Food and Drug Administration, Urmia University of Medical Sciences, Urmia, Iran*

[2] *Dental and Periodontal Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

**Abstract:** The field of nanotechnology has revealed unique aptitudes in the manufacture *of* novel and effective drugs/delivery systems for pulmonary diseases. This knowledge bargains numerous profits in the treatment of chronic human pulmonary diseases with targeted drugs/delivery systems. In recent years, numerous approaches have been reported to transport drugs to the lungs. Delivery of the drugs/delivery systems over the pulmonary way can be prescribed in two ways: oral inhalation and intranasal administration. In nanomaterial-based aerosol inhalation systems, drug delivery to the lungs can be accomplished by repeated high-dose inhalation. New tools deal with major clinical profits to increase the efficiency of pulmonary drug delivery and target specific areas of the lung. Factors such as size distribution, surface charge, quantitative analysis of lipid composition, drug loading rate, and formulation stability are vital in nanomaterials-based nanopharmacology. The alteration from *in vitro* phase to the clinical stage and production step for nanomaterials is a multipart action with requirements to overcome various limitations. In the present chapter, we focus on new progress in pulmonary nanopharmacology and the supporting approaches for designing new nanomaterials for this arena. Some patents have been gathered about this topic as well. The future viewpoints have also been discoursed.

**Keywords:** Lung, Pulmonary, Nanotechnology, Drug delivery.

## INTRODUCTION

Nanotechnology-based medicine and drug delivery systems are relatively new knowledge that is constantly evolving. In these sciences, nanoscale materials are used as a tool to diagnose diseases or targeted therapeutic agents to treat diseases. This technology offers several benefits in the treatment of chronic human diseases with precision site-specific drugs. In recent years, a number of prominent functions of nanomedicine (chemotherapeutic agents, biological agents, immunotherapeutic agents, *etc.*) have been reported in the treatment of incurable diseases [1].

* **Corresponding author Solmaz Maleki Dizaj:** Dental and Periodontal Research Center, Tabriz University of Medical Sciences, Tabriz, Iran; E-mail: maleki.s.89@gmail.com

**Elham Ahmadian, Magali Cucchiarini and Aziz Eftekhari (Eds.)**

Knowledge of nanotechnology has shown special capabilities in the production of new and effective drugs for lung diseases. To date, many methods have been used to deliver drugs to the lungs, including lipid drug systems, polymer matrices, production of polysaccharide particles, biocompatible metal mineral particles (iron, gold, zinc) [2]. The respiratory system, as one of the most important and extensive organs of the human body, is known as an organ for gas exchange [3]. Functionally, this organ can be divided into two parts: a conductive airway (nasal cavity, oral cavity and associated sinuses, nasopharynx, pharynx, larynx, trachea, bronchi, and bronchioles) and a respiratory area. (Respiratory bronchi, alveolar ducts), alveolar sacs and alveoli) [4]. We know that particle size is of particular importance in sedimentation in the lungs (Fig. **1**). Many studies have been performed to model particle deposition in the human lung.

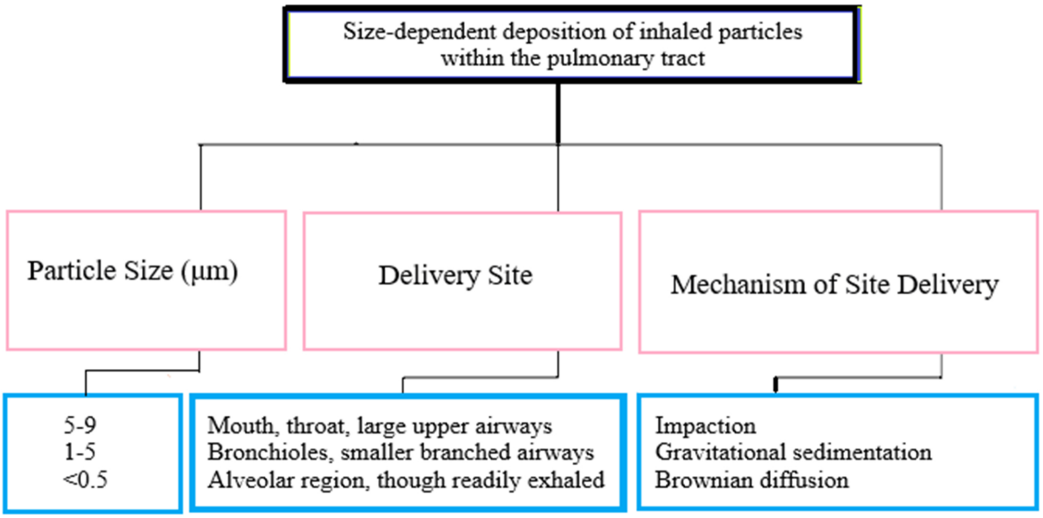


**Fig. (1).** The particle size is of particular importance in sedimentation in the lungs.

However, over successive respiratory cycles, accurate estimates of regional particle dosimetry for long-term lung exposure cannot be specified without considering the transport and deposition of preserved particles, especially those in the size range of 0.1 μm [5]. Then, awareness to control the drug deposition and absorption in the lungs is the main factor to attain better therapeutic results in the clinic.

Nanopharmacology is a new division of pharmacy and nanotechnology that studies the interaction of nanoparticles with living systems at the nanoscale level. Studies have shown that targeting specificity, the type of formulation and its design method, selective localization of formulation to the target site, and site-

specific activation of drug can play a key role in nanopharmacological success, overcoming physiological barriers and in drug delivery [6, 7]. In this chapter, we concentrate on new advances in pulmonary nanopharmacology and the assistance strategies for developing new nanomaterials for this field. Besides some patents have been summarized about this subject. The future outlooks have also been discussed.

## THE RESPIRATORY TRACT

The respiratory tract with a surface area of about 150 $m^2$ has been identified as an organ for gas exchange [3]. Functionally, this organ is divided into two parts, including a conducting airway (the nasal cavity, oral cavity, and the associated sinuses, nasopharynx, oropharynx, larynx, trachea, bronchi, and bronchioles) and a respiratory region (respiratory bronchioles, alveolar ducts, alveolar sacs, and alveoli) [4]. Also, human lungs have five lobes including three lobes for the right lung and two lobes for the left lung [8]. There are three main barriers in the lungs that protect the inhalation tract from foreign particles, including mechanical barriers (from nose to alveoli), biochemical barriers (the mucus, complement and complement cleavage products, pulmonary surfactant, antimicrobial peptides, secreted immunoglobulins (mainly IgA), and mucins), and cellular barriers (the epithelial and immunological cells) [9].

The presence of more than 300 million alveoli and 280 billion capillaries in the lungs provides a large gas-blood barrier in the pulmonary system. Alveolar gas exchange is mediated by the alveolar epithelium, endothelium, and interstitial cell layers. The pulmonary alveolar epithelium cells are formed of two types of cells including type 1 and type 2 pneumocytes. The capillaries are connected to the alveolar epithelium by an endothelial layer (about 0.5 μm thick) and gas exchange takes place in this part. To reduce surface tension, the alveoli are coated with a layer of surfactant-containing phospholipids and surface proteins. This improves gas exchange function in the lungs. The alveolar surface is covered with various cells, such as lymph vessels, nerves, fibroblasts, and macrophages [8]. Studies have shown that the lungs (as a non-invasive and attractive route) have a high capacity for drug delivery because of a large surface area for drug absorption, the avoidance of first-pass metabolism, access to an extensive vasculature, a relatively low enzymatic activity in the alveolar space compared with the GIT/liver, high permeability and low thickness of the epithelial barrier [10]. Furthermore, adequate knowledge of the anatomy and physiology of the lungs is essential for the treatment of various diseases associated with the lungs including cystic fibrosis, asthma, lung cancer, pulmonary hypertension, bacterial, viral, fungal and parasitic infections, chronic obstructive pulmonary disorders, acute respiratory distress syndrome in infants, pneumonia, and tuberculosis. These

CHAPTER 2

# Advances in Cardiovascular Nanopharmacology

**Solmaz Maleki Dizaj[1,*], Shahriar Shahi[1], Khadijeh Khezri[2]** and **Simin Sharifi[1]**

[1] *Dental and Periodontal Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

[2] *Deputy of Food and Drug Administration, Urmia University of Medical Sciences, Urmia, Iran*

**Abstract:** Nanotechnology has caused the most noteworthy influence on oncology, recently. Many nano-based delivery systems for specific medicines and a diversity of other diseases are being advanced nowadays. Nanomedicine is preferably adapted to resolving the main issues of numerous diseases, as it offers the special opportunity to create specific nanoparticles as a carrier for the targeted and controlled transferal of several therapeutic agents to the targeted location. Moreover, ligand-targeting or receptor-mediated targeting methods relate to an extra degree of complexity that may be implemented in the nanoparticles-based product in cardiovascular diseases. Despite the noteworthy increase in studies on the use of nanoparticles in cardiovascular disease, some reports have shown that different types of nanoparticles have cytotoxic action. Future studies are desired to fully investigate toxicity, especially cytotoxicity and inflammatory responses for nanomaterials. The outline of new plans to reduce toxicity should be the aim of future studies. In the present chapter, we emphasize new developments in cardiovascular nanopharmacology and the assistant methods for scheming new nanomaterials for this field. The future lookouts have also been discussed.

**Keywords:** Cardiovascular diseases, Drug delivery system, Nanotechnology.

## INTRODUCTION

Heart failure, coronary heart disease, inflammatory heart disease, and myocardial infarction, besides other cardiovascular diseases (CVDs), are among the world's most serious health issues. Regrettably, the statistics are anticipated to rise throughout the next decade because of the rise of CVDs risk factors including obesity, diabetes, and also an increase in the senior population. As a result, despite the potential advantages of recent increases in treatment choices and pharmacological breakthroughs (*e.g.*, valsartan /sacubitril), the development of novel and far effective therapeutic methods remains important. Nanomedicine,

* **Corresponding author Solmaz Maleki Dizaj:** Dental and Periodontal Research Center, Tabriz University of Medical Sciences, Tabriz, Iran; E-mail: maleki.s.89@gmail.com

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one of the most rapidly developing scientific topics, is projected to address this need by transforming the CVDs treatment system [1, 2].

The requirement to determine the manners of nanoparticles (NPs) such as carbon nanotubes (CNTs), iron oxide magnetic nanoparticles (IOMNPs), quantum dots (QDs), gold nanoparticles (AuNPs) and various others within the conventional pharmacological parameters have complicated nanopharmacology [3, 4]. In many circumstances, nanoconstructs have restricted metabolism in biological organisms, which is especially important when hazardous elements like cadmium are present [5].

The first deterrent to overcome, depending on the mode of delivery, is absorption into the host system. According to some research, many nanomaterials can pass in an organism by skin absorption, oral delivery, inhalation, and parenteral routs [6].

For QDs, systemic distribution by parenteral delivery appears to be the most relevant delivery path at the moment, however, environmental and occupational exposures through cutaneous and inhalation paths are also feasible. The limited reports that exist on QD absorption at the organism level generally use parenteral IV administration. According to QD reports, QDs with functional groups for targeting aims can be gathered in specified tissues after IV delivery. Though, distribution to other tissues of an organism has not been studied, and more research is urgently needed in this area. The deposition process for a particle should be easily quantifiable owing to the strong fluorescence of QDs and the metallic cores [4 - 6].

Over 26 FDA-approved anticancer medications have been confirmed for clinical use in the last decade, in addition to other pharmaceutical materials for illnesses ranging from cardiovascular disease to inflammation. Although many conventional medications have therapeutic prospective, clinical translation and success are hampered by several obstacles. These constraints consist of the drugs' physico-chemical characteristics, which preclude them from being delivered effectively in a molecular form [7]. The common medications are polycyclic, which means they are water-insoluble [8]. For example, dexamethasone and paclitaxel have low water solubility of 0.0015 mg/mL5 and 0.1 mg/mL6, respectively, which make them unsuitable for intravenous injection in aqueous solution [7]. The drug's very unspecific dispersion, with barely 1 in 10,000 to 1 in 100,000 molecules reaching their designated site of action, is a severe impediment to it reaching its target [8]. As a result, a considerably greater dose is required to achieve the intended therapeutic effect, perhaps bringing the dose closer to the dangerous dose [8], as observed with doxorubicin, which has significant cardiotoxicity [9]. Given these considerations, it would be preferable to change

the drug with properties that would pharmacologically ensure enhanced stability, solubility, and selective targeting of the action site [7]. Nanotechnology has the potential to change the field of pharmacology in this regard. NMs have the potential to improve the curative capacities of several conventional medications due to their unique size and characteristics.

During the previous fifteen years, nanotechnology has made the most significant contribution to oncology. Liposomes were the first commercially available drug nanocarrier for injectable therapies, and nanocarriers played an essential role in cancer treatment [9]. Liposomal doxorubicin was accepted by the FDA in 1990 to be used for Kaposi's sarcoma. Then, it authorized for the treatment of recurrent ovarian cancer and metastatic breast cancer. So far, several drug-delivery systems with nanocarrier have been advanced with varying physicochemical properties, shape, compositions, and surface functionalizations, and are in various steps of progress [8].

Many nanocarriers for specific medications and a variety of other illnesses are now being developed. Liposomes, in cancer, use the neovasculature's increased permeability to localize within the disease site *via* an enhanced permeation and retention (EPR) mechanism including those used in therapeutic applications. Liposomes use the improved permeability of the neovasculature as a mechanism to localize into the illness site in many cancer cases, a process known as the EPR mechanism [10].

The existence of large (several hundred nanometers) vascular fenestrations on freshly created angiogenic arteries favors NM extravasation. Surface modifications with compounds like polyethylene glycol (PEG) can render the nanovectors “stealthy,” averting them from being taken up by the reticuloendothelial system (RES). Several alternative NMs for drug delivery have emerged as a result of significant progress in chemistry and materials science, such as polymer–drug conjugates [11], polymer micelles [12], and dendrimers [13, 14]. Therefore, whereas the first generation of NMs-delivery structures for medicines had no active mechanisms of illness site localization and therapy at the start of the investigations, the second generation now includes targeted nano-based delivery structure [15]. Particular molecular recognition sections on the nanocarrier to receptors overexpressed on cancerous cells or nearby blood vessels (such as Ab conjugated NMs) or an opportunity for active/triggered release of the cargo at the diseased location (*e.g.* magnetic nanoparticles (MNPs) may be attributed to the targeting functionality [16]. As a result of the employment of targeting sections, remote activation, and environmentally sensitive components, the NMs outperform their predecessors, introducing additional levels of sophistication in design that promise enhanced success in achieving the goals.

CHAPTER 3

# Advances in Nanopharmacology: Cancer Treatment

**Soheila Montazersaheb[1], Raheleh Farahzadi[2,*]** and **Afsaneh Farjami[3,4,*]**

[1] *Molecular Medicine Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

[2] *Hematology and Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

[3] *Food and Drug Safety Research Center, Tabriz University of Medical Science, Tabriz, Iran*

[4] *Pharmaceutical Analysis Research Center, Tabriz University of Medical Science, Tabriz, Iran*

**Abstract:** Nanotechnology has attracted considerable attention in the biomedical field, especially in cancer therapy. Nanomedicines are superior to current approaches in cancer treatment due to their unique properties and advantages. Along this line, nanotechnology-based therapeutics can offer greater effectiveness with minimal or no side effects. In other words, the inherent limitations of conventional cancer therapies have led to the development of more effective and safer treatments. In this regard, a variety of nanocarriers have been developed for cancer treatment with high specificity, selectivity, biocompatibility, multi-functionality, and precise sustained-release properties. The focus of this book chapter is therefore on several advancements in nano-based approaches and the potential applications of nanomedicines for hematological malignancies and solid tumors with the hope of developing a robust and efficient nanotherapeutic modality.

**Keywords:** Cancer, Nano-drugs, Nanotechnology, Oxidative stress.

## INTRODUCTION

Cancer is one of the most complex and traumatic diseases that cause enormous health and economic burden. According to the World Health Organization (WHO), cancer is a threatening disease and about 9.6 million people died due to cancer in 2018 worldwide. It is the leading cause of mortality in one-third of deaths in the world. However, the data from the National Center for Health Statistics (NCHS) reported a 27% reduction in cancer rates from 1991 to 2016

* **Corresponding authors Raheleh Farahzadi and Afsaneh Farjami:** Hematology and Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, Postal Code: 5166614731, Iran; Tel: +98-41-33343626; Fax: +98-41-33343844; E-mail: farahzadir@tbzmed.ac.ir and Food and Drug Safety Research Center, Tabriz University of Medical Sciences, Tabriz, Postal Code: 5166614731, Iran; Tel: +98-41-33372250; E-mail: afsanehfarjami92@gmail.com;
*These authors contributed equally to this work

**Elham Ahmadian, Magali Cucchiarini and Aziz Eftekhari (Eds.)**

[1, 2]. Despite differences in terms of phenotypic traits, genetic and molecular profiles, cancer shares six common hallmarks, including self-sustained proliferative abilities, sustained angiogenesis, drastic metabolic alterations, and the capability to invade surrounding tissues and subsequent metastasis [3]. All cancers arise as a consequence of the accumulation of somatic mutations, epigenetic modifications, and copy number alterations [4]. With this background, there is a need to develop novel therapeutic strategies.

A variety of methods can be applied for cancer therapy, including surgery, radiotherapy, and the administration of anticancer agents. In this regard, a growing number of attempts are being made to develop a new intervention with the highest efficacy and low adverse impacts on healthy tissues.

## Nanomedicine: A Review on Nanotechnology for Cancer Therapy

The application of nanotechnology has received considerable attention in the biomedical field. In case of cancer treatment, nano-based therapies offer significant advantages over traditional ones. The nanotechnology-based approach can effectively improve drug internalization, decrease degradation and excretion of drugs, and can create a sustained-release system. All of the mentioned parameters are valuable for prolonging the half-life of drugs in biomedical applications [5, 6]. Nanoparticles (NPs) are highly capable to specifically target and deliver drugs to cancer cells through different modifications, hence it can reduce off-targeting toxicity. On the whole, nanomedicines provide a broad platform for overcoming the shortcoming of traditional treatment in cancer treatment. Based on the source of material, NPs can be primarily classified into organic NPs, inorganic NPs, and hybrid NPs (combining organic and inorganic NPs). Organic NPs such as lipid-based NPs, polymeric NPs, lipoproteins, and dendritic molecules exhibit high biocompatibility drug delivery [7, 8]. Lipid NPs improve drug absorption, drug release, and reduce toxic side effects. Liposomes and micelles are the most typical lipid-based nanocarriers, which are broadly used for the delivery of anti-tumor agents, genetic drugs, peptides and proteins [9]. Polymer-based NPs were synthesized *via* the polymerization of monomers. Easy manipulation of particle size, controlled/sustained release formulation, and high loading capacity of polymeric NPs lead to their use in cancer therapy [10]. Inorganic NPs such as carbon-based NPs, metal NPs, and quantum dots (QDs) provide some advantages in terms of performance and activity compared with other NPs [11]. Collectively, NPs are vehicles for the delivery of small molecule drugs to specific sites and for reducing the side effects of the drugs as observed with traditional treatments.

## Nanomedicine for Angiogenesis in Cancer

Similar to normal cells, tumor cells require nourishments such as food and oxygen which are accompanied by the removal of metabolic excretes and carbon dioxide. To deal with this demand, various patterns of tumor-related neovascularization are mediated by angiogenesis [12]. In fact, angiogenesis has a vital role in the development of tumor growth and tumor-associated metastasis. Without angiogenesis, a primary tumor is able to grow only 1-2 $mm^3$. Accumulating evidence indicates that tumor growth can be triggered by a series of events, including the downregulation of angiogenesis inhibitors, overexpression of angiogenic stimulators, hypoxic status, and so on. Along this line, cancer cells overexpress proangiogenic factors such as vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), angiopoietins (Ang), and interleukin 8 (IL-8) to stimulate the formation of blood vessels. Besides, the activation of hypoxia-inducing factor (Hif1) is found to have a regulatory role in angiogenesis, thereby Hif1 can be used as the target in cancer therapy [13, 14].

In the last few decades, growing attempts aimed at the development of plausible approaches to cure cancers. Blocking of tumor-associated antiangiogenesis can prevent the aberrant capillary formation and tumor growth. In this regard, anti-angiogenic drugs, namely, monoclonal antibody inhibitors in combination with chemotherapy are used to combat tissue invasion and metastasis in cancer [15]. In fact, angiogenic inhibitors can target both existing infiltrating blood vessels and newly formed blood vessels, thus, hampering the tumor metabolism and expansion [16]. Despite the beneficial effects of angiogenic inhibitors in cancer therapy, some limitations need to be addressed for achieving therapeutic efficacy such as toxicity, drug resistance, hypoxia resistance, upregulation of proangiogenic signals, and delayed response to radiotherapy [17].

In this context, nano-based therapies offer an appealing platform to circumvent the existing limitations. This is due to the attractive physicochemical properties of NPs such as small size and high surface area, particularly at the nanoscale level. Therefore, nanomedicines are considered an alternative modality for antiangiogenic cancer therapy [18]. There are two types of angiogenic inhibitors, the first type is based on direct blocking of angiogenesis inducers such as VEGF, bFGF, and PDGF. The indirect inhibitors can target the tumor/stromal cells and modulate angiogenic regulators [19]. Keeping this concept in mind, NPs can conjugate to a variety of targeting ligands for active targeting in antiangiogenic therapy. Growing evidence has reported the application of various inorganic NPs including, gold NPs (AuNPs), silver NPs (AgNPs), copper NPs (CuNPs), carbon nanotubes (CNT), graphene oxides (GO), and so on [20]. In addition to this, other

CHAPTER 4

# Nanomedicine in Nephrology and Urinary Tract Infection

**Sepideh Zununi Vahed[1], Mohammadreza Ardalan[1]** and **Yalda Rahbar Saadat[1,*]**

[1] *Kidney Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

**Abstract:** Nanomedicine is an evolving trend in the biomedical field that can be used for the diagnosis, molecular targeting, imaging, and therapy of a wide range of diseases. The kidneys are essential organs that regulate blood pressure, filtrate blood and remove metabolic waste, produce hormones, and balance electrolytes. The kidney has gained great attention in nanomedicine due to its roles in the clearance of the nanodrugs and affecting the pharmacokinetics of these drugs. Nanoparticles can be used for the diagnosis and treatment of kidney diseases including acute kidney injury (AKI), chronic kidney disease (CKD), and glomerular diseases. Different approved nanodurgs have been developed for the treatment of kidney diseases. In this chapter, we summarize the available nanodrugs for the treatment of kidney diseases and urinary tract infections.

**Keywords:** Rarefied flow, Nanoparticle, Nanomedicine, Drug delivery, Nephrology, Kidney.

## INTRODUCTION

Nanomedicine is an evolving trend in the biomedical field that can be used for the diagnosis, molecular targeting, imaging, and therapy of a wide range of ailments, particularly kidney diseases [1]. An important goal of nanomedicine is developing efficient drugs with enhanced safety, solubility, pharmacokinetics (PK), tissue selectivity, and decreased toxicological issues [2]. Various nanoparticles (NPs) including liposomes, polymers, micelles, nanocrystals, metal/metal oxides, inorganic materials, proteins, and carbon nanotubes are employed in nano drug formulation [2]. Pre-clinical, clinical validation and pre-market authorization are necessary for nanodrugs approval [3]. Moreover, a complete understanding of the physicochemical characteristics of the nanomaterial, reproducibility and scalability of the manufacturing procedures are required for nanomedicines [3].

* **Corresponding author Yalda Rahbar Saadat:** Kidney Research Center, Tabriz University of Medical Sciences, Tabriz, Iran Email: yalda.saadat@gmail.com

**Elham Ahmadian, Magali Cucchiarini and Aziz Eftekhari (Eds.)**

The urinary system is considered a vital organ system, responsible for the maintenance of homeostasis *via* regulation of the blood volume, pressure, and pH of metabolites and electrolytes [4].

The kidneys are essential organs that control blood pressure, filtrate blood and remove metabolic waste, produce hormones, and balance electrolytes. Nephrotoxic insults or physical injury can damage the kidneys, leading to acute kidney injury (AKI). Additionally, gradual kidney damages due to congenital or acquired diseases including metabolic disease, hypertension, and diabetes result in chronic kidney disease (CKD) that can be progressed to end-stage renal disease (ESRD). The kidney has gained great attention in nanomedicine due to its roles in the clearance of the nanodrugs and affecting the PK of these drugs. Nanoparticles may also be used for the diagnosis and treatment of AKI, CKD, and other kidney diseases.

Urinary tract infections (UTIs) are defined as microbial infections within the urinary tract. Various factors including age, sex and numerous genetic susceptibility factors are involved in their occurrence and severity [5]. The uropathogens consist of both Gram-positive, Gram-negative bacteria as well as fungi [5]. Depending on the localization site, the UTIs are categorized in different classes: I) colonization of uropathogens in the bladder (cystitis), II) uropathogens ascending to the prostate gland (Prostatitis), III) transfer of pathogens to the kidneys (Pyelonephritis) and untreated infections resulting in infection of the blood vessels [5]. In kidneys, accumulation of pathogen infection may lead to the formation of urinary stones and subsequent acute kidney infection as well as urosepsis and renal failure [5]. In this chapter, we summarize the developed nanodrugs for the treatment of kidney diseases and UTIs.

## Drug Delivery, Pharmacokinetics, and Safety of Nanodrugs

Numerous therapeutic drugs display poor pharmacokinetics, particularly in the kidneys. Besides, their clearance from the body depends on kidneys; however, their persistence in the kidneys is momentary to exert a therapeutic outcome. Therapeutic agents that are cleared by hepatobiliary mechanisms are less exposed to the kidneys. To overcome the limitations of conventional drugs, nanodrugs have been developed by promoting PK [2]. Applying specific organ, tissue, and cell-targeted NPs leads to diminished off-target adverse effects of drugs that displayed toxicity when administered conventionally. Moreover, drug targeting results in decreased total doses, due to the high payload of the drug at the disease site than healthy sites [6].

The NPs are exploited in the realm of drug delivery for controlled release of therapeutic agents. NPs encapsulate small molecules (such as therapeutic drugs)

and macromolecules (peptides, proteins, and nucleic acids), protecting them from degradation in the body. Chemical and physical features of NPs impact their retention and biodistribution in different tissues [1]. Modifications in surface chemistry (addition of molecular recognition entities) and particle size result in the localization of NPs to specific targets in the body and modulation of their pharmacologic properties [6]. Based on the aforementioned factors, NPs can be targeted to specific sections of the nephron, though they can be filtered by the kidneys or evade from the kidneys. The majority of NPs demonstrate renal clearance that is a desirable pharmacokinetic property for removing potentially toxic metal NPs (including those utilized in diagnostic imaging). Clearance by kidney filtration necessitates NPs to be adequately small in size to pass across the podocyte slit diaphragm (w 8-nm pores) and the glomerular endothelial fenestrae (w100 nm). Besides, kidney filtration might allow imaging of the kidneys during the excretion process to examine their function [6]. Another factor that affects the efficacy of systemically injected agents is circulation time. After circulation in the blood, drugs accumulate in the target site and consequently accomplish their therapeutic effect. The presence of NPs in the systemic circulation exposes them to the plasma proteins, coagulation factors, and blood cells. Based on the NPs' charge, shape, and size, they may be opsonized or adsorbed by serum proteins, which in turn, form a "protein corona" on the NPs' surface. The formed protein corona alters the NPs characteristics, including size, aggregation state, and interfacial features. The corona formation leads to *in vivo* hydrodynamic diameter (HD) that makes the NPs larger than the *in vitro* diameter. The HD is inversely attributed to the GFR (glomerular filtration rate) and is directly associated with blood circulation time as well as the whole-body half-life [1].

To enhance the formulated nanodrug's efficacy, various approaches including the small size of NPs to cross the physiological barriers (*i.e*. enzymatic and mechanical degradation, kidney clearance, immune system, *etc.*), entrapping drugs to protect them from extreme conditions, and surface conjugation to target them into specific tissues have been used [2]. Some NPs (polymeric NPs, liposomes, nanoemulsions, and virus-like NPs) exert immunomodulatory effects through entering antigen-presenting cells (APCs), which in turn, may improve the adaptive immune response [2]. Nanodrugs application results in augmented drug accumulation in the target tissue which causes reduced drug dosage and side effects [2].

## NPs can Target Kidney Tissue

Within the glomerulus, NPs could target the mesangial cells and glomerular basement membrane (GBM). In this line, it has been shown that the polyethylene glycol–coated gold NPs are localized in mesangial cells, however, most of the

# CHAPTER 5

# Advances in Nanopharmacology: Focus on Reproduction, Endocrinology, Developmental Alterations, and Next Generational Effects

**Mohammad Mehdi Ommati[1,3], Socorro Retana-Márquez[2], Asma Najibi[4]** and **Reza Heidari[3,*]**

[1] *College of Life Sciences, Shanxi Agricultural University, Taigu, Shanxi 030801, Peoples' Republic of China*

[2] *Department of Reproductive Biology, Universidad Autónoma Metropolitana-Iztapalapa, Mexico City, Mexico*

[3] *Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

[4] *Department of Pharmacology and Toxicology, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran*

**Abstract:** To date, the application of a wide range of nanostructured materials (NSMs), such as carbon nanotubes, silica compounds, metallic nanoparticles, nanovesicles (liposomes and exosomes), nanohydrogels (NHGs), nanohydroxyapatite (NHAPs), chitosans, and graphenes, has gained interest for various applications in biomedical sciences. These nanoparticles presented outstanding biological and mechanical features. Although the biocompatibility of NSMs is highly investigated, their interaction with the reproductive system is less exploited. On the other hand, recently, NSMs-mediated drug delivery presents a competent method in reproduction biology. Emerging evidence from the literature supports the considerable progress in nanopharmacology, which has transformed the theory of targeted biological delivery, permitting the engineering of complex biocompatible organic/inorganic platforms with a vast loading capacity, highly selective affinity, stability, and capacity for multiple, simultaneous usages; all within the nanometer scale. In this chapter, first, the potential application of NSMs in the field of reproduction is highlighted. Then, the possible effects of these materials on reproduction, endocrinology, developmental alterations, and next-generation impact will be discussed. The data presented in this chapter could provide insight into the effect of NSMs on the reproductive system and development and lead to better risk assessment of these materials or synthesis of safe nano-drug delivery systems to the reproductive organs.

**Keywords:** Infertility, Developmental toxicity, Hormonal changes, Nanomaterials, Nanopharmaceuticals, Nanotoxicology, Reproductive toxicity.

[*] **Corresponding author Reza Heidari:** Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran; E-mails: rheidari@sums.ac.ir; rezaheidari@hotmail.com

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## INTRODUCTION

### Nanomaterials and their Pharmacological Applications

The NSMs are small in size and have extraordinary physicochemical features. Based on their specific properties, a diversity of these nanoparticles, such as carbon-based, metallic, polymer-based, and fluorescent, have been exploited in various medical-related scenarios, including drug delivery, tissue engineering, and other multidisciplinary applications with therapeutic aims; among which some of the crucial ones are randomly described in the following subclasses.

Due to the specific structure and functional role of male and female reproductive organs and gametes, the application of powerful and minimally-invasive research tools that do not meddle with reproductive indices and consequent fertility or affect the development of resulting F1 generation is worth it. For instance, one of the well-known and most expected applications of NSMs in the science of reproduction is gene transfer, which might be applied in numerous types of previously inexplicable reproductive failures as their main reasons are known to be related to genetic interactions (abnormal gene expression) and genetic polymorphisms, such as particular categories of testicular insufficiency [1], fertilization failure [2], ovarian dysfunction [3], and recurring pregnancy loss [4].

On the other hand, except for the multidisciplinary NSMs applications, investigation dealing with the adverse and toxic impacts of such materials on the public health, environment, and plants is yet in its infancy. Hence, NSMs-related toxicity is the main drawback in medical sciences; it is a tenacious dilemma causing some anomalies in reproductive and non-reproductive cases. For this purpose, some of them can endanger patients' lives or the people exposed to these nanoparticles. Furthermore, the assay of reproduction functionality comprises an excellent instrument to assess the harmful effects of applying these NSMs directly on the individual and the possible toxicity delivered to the offspring. Hence, this chapter tries to point out the role of some of these crucial nanoparticles that somehow affect the reproductive system of the individuals whose parents (developmental study) or themselves were exposed.

#### *Carbon Nanotubes (CNTs)*

The CNTs, with remarkable physical features, including high strength and stiffness, low density, and excellent thermal conductivity, belong to the NSMs superfamily [5 - 9]. These impressive properties suggest a huge industrial interest and application for these lightweight, high-strength tubes that can be addressed in reference [8, 10]. Hence, a considerable body of trial and theoretical studies have

been devoted to the CNTs. Among the CNTs' members (*i.e.*, single-walled-, double-walled -, few-walled-, and multi-walled- nanotubes), two primary structural forms are single-walled carbon nanotube (SWCNT) bundles and multi-walled carbon nanotubes (MWCNT). During the last decade, the CNTs investigations and their availability in the market are constantly increased [11]. The CNTs are broadly utilized in pharmaceutical aspects as well as in the industries as dug carriers.

### *Metallic Nanoparticles*

Metallic nanoparticles include silver nanoparticles (AgNPs), gold nanoparticles (AuNPs), titanium dioxide nanoparticles ($TiO_2$), copper nanoparticles, iron nanoparticles, zinc nanoparticles. Metallic nanoparticles are widely and primarily used as bacteriostatic coatings for preventing infections (*i.e.*, antibiotic agents in textiles and wound dressings). It has been shown that some of the members of this group, such as AgNPs, can also be used as deodorants, medical strategies, and in-home apparatus (*i.e.*, washing machines and freezers) [9]. Some in-depth studies investigated the toxic effects of some metallic nanoparticles in nature [12 - 14]. The toxicity of metallic nanoparticles, such as silver, gold, iron, copper, and zinc, has been well-reviewed [9], some of which are described in the following sections. Altogether, much needs to be uncovered regarding the accumulation of these metallic nanoparticles in the environment and their potential acute and chorionic exposure effects on the reproductive, endocrinology, and pre and post-partum development of the embryo in humans, animals, and other organisms.

### *Silica Nanoparticles*

Mesoporous silica belongs to a specific type of synthetically modified colloidal silica compounds (SiC) with exceedingly ordered pores on a scale between 2 to 50 nm [15, 16]. The positive biomedical features of this subfamily of SiC include easy-to-make, modifiable surface chemistry, unique porous architecture, massiveness, and chemical inertness [17]. The mentioned architecture of this SiC can tangibly improve the effective surface area and permits compartmentalization of various types of cargo on one nanocarrier *via* combination on the surface and inside the pores. This silica nanoparticle's character can be reformed with multiple functional groups. It can be coated with covalent or non-covalent linking of cargo, resulting in the enhancement of internalization into target cells [18]. Based on these properties, the silica nanoparticles have been well-considered potent tools for gene delivery and targeted drug delivery, tissue engineering, and bio-imaging. On the other hand, accumulating evidence reveals low cytotoxicity of silica nanoparticles in various cell types and the spermatogenesis process [15, 18, 19]. The mentioned silica nanoparticles' properties make them outstanding

# CHAPTER 6

# Gastroenterological Aspects of Nanopharmacology

**Reza Heidari**[1] and **Mohammad Mehdi Ommati**[2,*]

[1] *Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

[2] *College of Life Sciences, Shanxi Agricultural University, Taigu, Shanxi030801, China*

**Abstract:** Nanomaterials (NMs) are increasingly used in biomedical sciences. These compounds play a crucial role in many aspects of biomedicine, including disease diagnosis (*e.g.*, biosensors), drug development, and implant technology. The unique architecture, size, composition, surface properties, and shape of NMs make them ideal for various purposes (*e.g.*, drug delivery systems). A wide range of NMs such as carbon nanotubes, silica compounds, metallic nanoparticles, nano-pattern surfaces, liposomes, and nano-hydrogels are widely investigated for these purposes. On the other hand, the gastrointestinal (GI) tract and the liver tissue are among the first organs exposed to orally administered NMs. Hence, it is essential to investigate the impact of nanoparticles on these organs. In the current chapter, the potential pharmacological applications of NMs in GI and liver diseases are discussed. Then, the effects of nano-engineering on the pharmacokinetic parameters and the adverse effects of nanomaterials in the GI tract and the liver are highlighted. The data provided in the current chapter could help develop safe pharmaceuticals and prevent the adverse effects of NMs in the GI and liver systems.

**Keywords:** Cytotoxicity, Hepatotoxicity, Liver injury, Mitochondria, Nano-drugs, Nanotechnology, Oxidative stress.

## INTRODUCTION

Nanomaterials (NMs) are widely investigated for their application in biomedical sciences, from diagnosis to treatment of human diseases [1, 2]. Several unique physiochemical properties of NMs lead to their use in biomedicine. Targeted drug delivery, use as antidotes (due to high surface area for toxicants absorption), and the application of NMs as biosensors and implants increased the exposure of biological systems to these compounds [2 - 7].

Therefore, it is vital to investigate the pharmacological and toxicological aspects of NMs in various organs. We are constantly exposed to NMs through food, cos-

* **Corresponding author Mohammad Mehdi Ommati:** College of Life Sciences, Shanxi Agricultural University, Taigu, Shanxi 030801, China; E-mail: mehdi_ommati@hotmail.com

**Elham Ahmadian, Magali Cucchiarini and Aziz Eftekhari (Eds.)**

metics, drugs, and environmental toxicants through oral exposure (Fig. **1**). Therefore, the gastrointestinal (GI) tract and the liver tissues are among the first organs exposed to NMs. Thus, NMs could induce GI and liver injury (Fig. **1**).

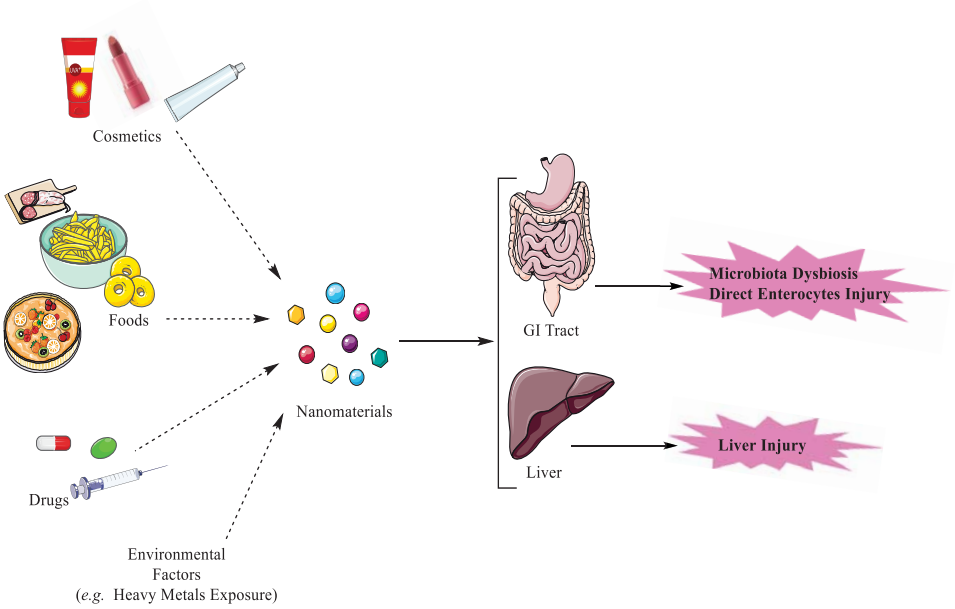


**Fig. (1).** The potential effects of nanomaterials (NMs) on the gastrointestinal (GI) tract and the liver tissue.

NMs could induce direct damage to the intestinal lining cells or modulate gut microbiota. In the liver tissue, NMs could cause hepatocytes and/or non-parenchymal cell injury.

Nano-drug delivery systems could be a source of GI and liver exposure to NMs (Fig. **1**). As oral drug delivery has the most outstanding patient compliance, this is the preferred route of drug administration, especially in chronic conditions. However, poor oral bioavailability, drug degradation, or gastrointestinal (GI) tract adverse effects of many pharmaceuticals and drug delivery to vital organs such as the liver led investigators to novel drug formulations. NMs and their use for effective drug delivery to the body are among these systems. The GI tract and the liver are among the first tissues exposed to orally administered NMs (Fig. **1**). It has been well-known that nano-engineering will change the pharmacological properties (pharmacokinetic and pharmacodynamic aspects) of materials [8]. These parameters could alter their properties (*e.g.*, absorption) in the GI or their cell penetrations [8, 9].

## Therapeutic Applications of Nanomaterials in the GI Tract and the Liver

### *The Role of Nanomaterials in Drug Delivery Systems to the Liver*

Target drug delivery to the liver is an exciting field of investigation on biomedical applications of the NMs. These agents have been used for various hepatic disorders, from viral infection to cancer [10, 11]. Using NMs for the treatment of liver diseases enables researchers to deliver adequate therapeutic agents to this organ. Liver fibrosis is one of the most studied disorders for evaluating nanocarriers drug delivery systems [12] (Fig. **2**). Liver fibrosis is a complicated process induced by various diseases or xenobiotics [13 - 16]. A significant deposition of the extracellular matrix is the main characteristic of hepatic fibrosis (Fig. **2**). Hepatic fibrosis could lead to liver failure, multiorgan failure, and patient death. Many nanoparticles have been applied to target hepatic stellate cells as the significant players contributed to hepatic fibrosis [12] (Fig. **2**). Therefore, future studies for optimizing these systems and, more importantly, elucidating their safety in the GI and liver will provide viable therapeutic options for managing GI and liver diseases.

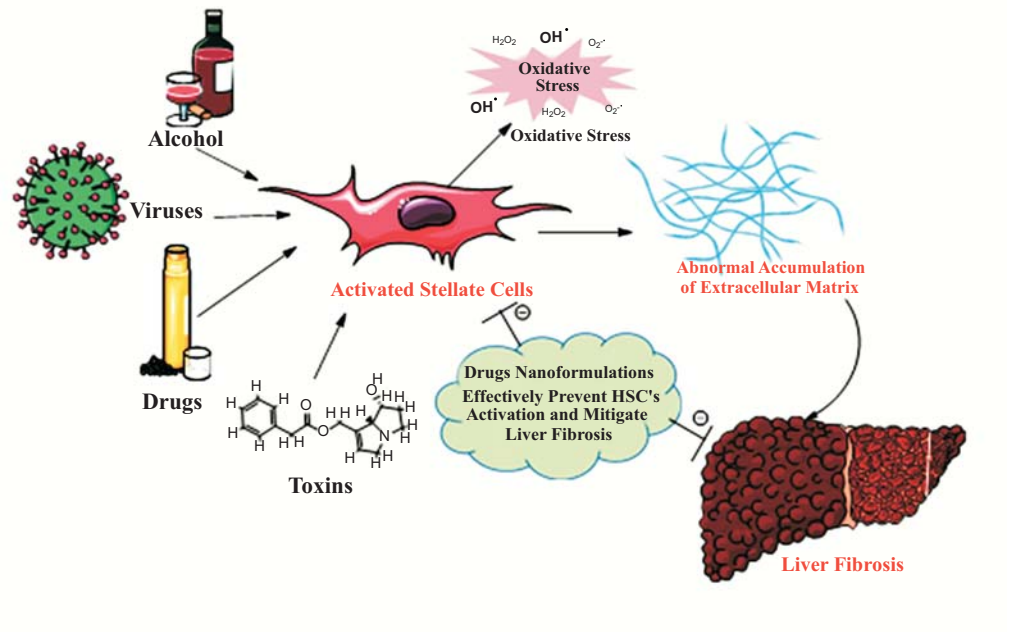


**Fig. (2).** Nanomaterials are widely used to target liver diseases. Liver fibrosis is a widely investigated field for the application of NMs for drug delivery to this organ.

CHAPTER 7

# Advances in Dentistry Nanopharmacology

**Simin Sharifi[1], Mahdieh Alipour[1], Atefeh Abedi[2], Yalda Rahbar Saadat[3]** and **Solmaz Maleki Dizaj[1, 4,*]**

[1] *Dental and Periodontal Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

[2] *Department of Endodontics, Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran*

[3]*Kidney Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

[4] *Department of Dental Biomaterials, Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran*

**Abstract:** Nanotechnology has been widely used in medicine to improve the therapeutic results of various diseases. Much effort has been focused on developing new nanoparticles and determining the physicochemical properties of nanoparticles in relation to their biological fate and performance. Today, nanotechnology has been able to offer effective treatments for use in dentistry. However, in the design and evaluation of these nanotechnology-based drug delivery systems in dentistry, less attention has been paid to the pharmacology of delivered drugs and their pathophysiology. In this chapter, we discuss some recent advances in nanotechnology for drug delivery in dentistry for demineralization, osseointegration of dental implants, the treatment of oral cancer, pain management of dental pulp, and the anti-inflammatory and antimicrobial formulations as well as the role of nanopharmacology in preventive dentistry.

**Keywords:** Dentistry, Drug delivery, Dental materials.

## INTRODUCTION

The application of nanotechnology in medicine is aimed to improve the efficiency of current therapeutic strategies and decrease their side effects. Despite this noticeable progress, further evaluations are essential for the clinical application of nanomedicine because this technology might change the natural status of the biological environment physiochemical, markers, and physiological barriers more than what has been expected [1, 2].

Furthermore, the entrance of nanotechnology in the field of pharmacology, which is known as nano pharmacology aims to investigate the nanoscale interactions

* **Corresponding author Solmaz Maleki Dizaj:** Dental and Periodontal Research Center, Tabriz University of Medical Sciences, Tabriz, Iran; E-mail: maleki.s.89@gmail.com

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between nano pharmaceutics and living systems. The focus of nano pharmacology is on the improvement of therapeutic efficacies as well as the reduction of side effects by using novel pharmacological principles. In order to achieve this goal, nanomedicine should provide an accurate targeted delivery system to specific areas with sufficient control releasing. The suitable drug delivery system is even more important in anti-cancer, anti-HIV, anti-psoriatic, and anti-leishmanial drugs since insufficient pharmacokinetics, drug resistance and poor biodistribution of those drugs lead to crucial dose-dependent side effects.

Therefore, nano-scale carriers such as liposomes, nanostructured lipid carriers (NLCs), polymeric micelles, and nano-sized polymeric drug conjugates seem to be promising approaches in the field of nanomedicine [2 - 4].

The dental practice is a branch of the medical field, which could be improved by nano pharmacology technologies to ameliorate clinical outcomes [5]. This aim can be achieved by the application of surface-modified nano-scale carriers scaffolds and containers. This chapter is going to review these materials and discuss the most recent advances in this field.

## ADVANCES IN NANOPARTICULATE STRUCTURES FOR DRUG DELIVERY IN DENTISTRY

The regeneration and healing of damaged organs and tissues could be improved by an effective delivery system for therapeutic agents such as nano-scale carriers. These carriers affect biological procedures like cell migration, adhesion, proliferation, and differentiation by controlled release of drugs, biomolecules, growth factors, and so on. The main strategies for this purpose are related to the appropriate loading of those therapeutic agents in massive amounts and targeted ways [6, 7].

Periodontal diseases are one of the most common problems, which lead to loss of supporting structures around the teeth. This problem affects the periodontium and causes challenging clinical situations for both patients and dentists. It seems that this disease could be effectively treated by targeted delivery of specific signaling molecules [8]. The main treatment plan in these cases should focus on the elimination of bacteria and local inflammatory factors and regeneration of damaged structures [9].

For these purposes, specific nanostructures and nanoparticles were developed. For example, it is shown that the loading of triclosan as an anti-bacterial agent could reduce the inflammation in target tissues. Moreover, the loading of anti-inflammatory medication on delivery systems leads to more effective treatment outcomes than local or systematic administration of these agents. For example, the

loading of tetracycline into the microsphere, which is commercially known as Arestin. This product effectively treats the periodontal pockets. Another example for antibacterial activity is microencapsulation of minocycline with PLGA nanoparticles, which provided higher anti-bacterial effects than routine administration of minocycline [10, 11].

Moreover, the suitable choice for drug delivery systems should eliminate inflammation and bacterial infections, while regenerating the lost bone tissue at the same time. Nano hydroxyapatite particles could be considered for this purpose, both as nanocarriers and osteoconductive agents. It has been shown that the 88% of loaded tetracycline on these carriers released during 5 days, which provided considerable anti-bacterial effects. Moreover, the proliferation of periodontal ligament cells increased in response to these nano-scale carries, which can cause enhanced healing. To sum up, the nanohydroxyapatite particles could be suitable local delivery systems for periodontal diseases, which target both anti-bacterial and regenerative aspects [12].

Moreover, as mentioned, these nanostructures could be used for the delivery of biomolecules and growth factors. However, the most crucial factor for this purpose is considering water-based solutions such as dextran, glycydyl methacrylate, and gelatin to incorporate with growth factors for dental applications [13].

For example, the loading of bone morphogenic protein 2 (BMP 2) and basic fibroblast growth factor (BFGF), which are known as basic osteoinductive and angiogenic growth factors, on 5nm-sized carbon particles promotes bone formation with the aim of alveolar ridge augmentation [14].

Recently gene therapy strategies can solve the problem of continuous releasing of growth factors by entering their coding genome in the target tissues [15]. For this purpose, the positively charged nanostructures could use for the delivery of highly phosphorylated nucleic acids. It has been shown that the delivery of the platelet-derived growth factor (PDGF) gene with nano calcium phosphate particles (NCaPP) could effectively transfer that gene in fibroblast as a vector [15]. Similar to PDGF gene transferring, the BMP2 gene was transferred to the vector in order to induce odontogenic differentiation in rat dental pulp stem cells [16].

The loading of genetic molecules on polymeric nanoparticles is considered as another application of nanopharmacology in dental applications. A recent study considering encapsulation of siRNA molecules with polymeric nanoparticles was conducted to regenerate alveolar bone [17].

# CHAPTER 8

# Advances in Nano Vaccines: Covid-19

**Zahra Asefy[1], Aygun Nasibova[2,3], Sirus Hoseinnejhad[1], Asif Selimoğlu[4], Mehmet Fırat Baran[5]** and **Cumali Keskin[6,*]**

[1] *Maragheh University of Medical Sciences Maragheh-Iran*

[2] *Biophysics and biochemistry department, Baku State University, Baku, Azerbaijan*

[3] *Institute of Radiation Problems, Azerbaijan National Academy of Science, Baku, Azerbaijan*

[4] *Department of Otolaryngology, Hacettepe, University Faculty of Medicine, Sihhiye, Ankara, Turkey*

[5] *Department of Food Technology, Vocational School of Technical Sciences, Batman University, Batman, Turkey*

[6] *Department of Biology, Mardin Artuklu University Graduate Education Institute, Mardin 47200, Turkey*

**Abstract:** Nanovaccines are considered a new approach in vaccination methodology specially for Covid-19 infection. Nanovaccines are more effective than conventional vaccines; Because of humoral and cellular immune responses which are simultaneously induced. Nano vaccines are assumed to upregulate the immune system as well as infection prevention. They are probably promising candidates for chronic autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, AIDS, and COVID-19 infectious. Based on this, we will describe the different working mechanisms of nanoparticles. In addition, applicable nano vaccines which have been approved for COVID-19 therapy Covid 19 are described. Antigen-carrying nanoparticles can affect the immune response and significantly enhance cell-T cytotoxic response. Nanoscale particles can improve vaccine efficiency because of their biomedical benefits. These properties include Small size, which allows better penetration into tumors and more half-life tumor cells. Current vaccines, however, are required to re-formulate almost because of gradual antigen modifications. More ever these vaccines do not protect against mutations and the low half-life of current vaccines due to limitations of current technologies. Nano vaccine formulation improvements have been required to induce a widespread and potent immune response. In this review, we provide an overview of the types and applications of nanoparticles in vaccines and their outstanding properties that made them alternatives for Covid-19 treatment.

**Keywords:** Covid-19, Inorganic nanoparticle, Virus-like particles, Nano vaccines.

[*] **Corresponding author Cumali Keskin:** Department of Biology, Mardin Artuklu University Graduate Education Institute, Mardin 47200, Turkey; E-mail: ckeskinoo@gmail.com

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## INTRODUCTION

Nano vaccines contain nanoparticles which are novel vaccines with precise cell targeting. In contrast, current vaccines do have not have a specific target. Nanovaccines are more effective than conventional vaccines because they activate both humoral and cellular immune responses, particularly in COVID-19 infection [1]. Also, they have simply administrative because they can be used as nasal sprays. while in animal models DNA nano vaccines were established. Nanovaccines reveal cell's communication and their microenvironment and are cost-effective [2]

## NANOTECHNOLOGY AND VACCINES

Vaccine discovery History is one of the most successful inventions for human health. Most candidate vaccines have fewer immunizing effects and thus are effective adjuvants required for new vaccine development [3]. Nanotechnology provides different nanoparticles in terms of size, composition, shape, and surface properties. Nanoparticles, due to their similarity in size to cellular compounds, can penetrate cells using cellular endocytosis mechanisms [4]. Nanoparticles are a breakthrough in disease prognosis and biological metabolites delivery. Inorganic nanotechnology indicates has been considered a new insight therapeutic method of nanomedicine. There are several vaccines and nanoscale drug delivery systems that are effective in disorder prevention.

Nanovaccines are used as biological metabolites delivery systems in both prevention and treatment *via* upregulation of antigen processing or as immune-stimulating adjuvants to elevate vaccine immunity. Nanovaccines are more commonly administrated to cure cancer. But recently it's applicable in other disorders such as Alzheimer's, hypertension, nicotine addiction, and specially Covid-19 infection [5]. Nanotechnology often upregulates the quality of immune response as an adjuvant. Immunization elevation is dependent on precise delivery or antigen exposure. There are some main issues required for the nano vaccines development. The virus postponement diagnosis process causes more virus mutations and subsequently led to vaccine complicity. More ever, it takes 2 to 3 weeks for an immune response after vaccination. [6] The second problem is low immunization in COVID-19. Efforts to discover this poor immunization are ongoing, and patterns and formulations have been developed to overcome this problem *via* various nanoparticle scaffolds [7].

### Inorganic Nanoparticles Vaccine

Inorganic nanoparticles consist of the nanoscale mineral nucleus in which antigens are attached, which can apply in COVID-19 infection therapeutic nano

vaccine [8]. The major role of these mineral nanoparticles is associated inflexible structure and controllability of these particles [9].

## Carbon Nanoparticles Vaccine

Numerous studies reported carbon nanoparticles applicable as accurate adjuvants and antigens delivery for various vaccines. Their immunizing effects are significantly affected by their physical and chemical properties, especially surface chemical modifications. Carbon nanoparticles have been widely applicable in nano vaccines [10]. These nanoparticles have been used to expose antigenic epitopes to APCs because they have a high tendency for a variety of different cell types [11]. The effect of carbon nanoparticles is influenced by their biocompatibility and physicochemical properties, including surface chemical properties that have the greatest effect on the specificity of carbon nanoparticles [12]. Carbon nanotubes (CNTs) have a length of 100 to 1000 nm and a diameter of 0.8 to 0.2 nm, which can affect epitopes' immunization potential [13].

Single-walled carbon nanotubes (SWNTs), as antigen exposure agents to APCs, by providing a humoral immunoreactive response of MH due to peptide-related antigens. Several forms of carbon nanoparticles, including fullerenes, and CNTs, have also been considered biocompatibility options as co_agent [14]. Several studies have revealed that water-soluble modified CNTs are highly biocompatible. They can penetrate cells rapidly through T, B, and L-lymphocytes, and have a poor effect on biodegradability and immune function [15]. Synthesized and purified tuberculin protein derivatives interaction with carboxylated SWCNTs is a preferential cellular response in toxin exposure.

## Silica Nanoparticles Vaccine

Silica nanoparticles (SiNP) are a great range of applications including vaccine delivery, tumor-specific targeting, and alive imaging which can be used for antigens delivery *in vivo* in COVID-19 [16].

SiNPs can interact with cells in several ways, and regulate sol-gel processes to adjust the size and shape of configurable structures [17]. Further modifications in these nanoparticles can be achieved by surface groups modifying such as silanols, which can lead to cell recognition, improved cell-cell interaction, and improved cellular uptake. Antigen binding of these particles is required for immune stimulation providing this effect exerted by SiNPs as a potential delivery system [18]. However, the most critical issue in nanoparticle biomedical administration is toxicity and stabilizing agents which are used in nanoparticle synthesis. administration of mesoporous silica nanoparticles) MSN with the size of 50 to

# CHAPTER 9

# Potential Solutions for Nanotoxicology

**Babak Sokouti[1], Vahid Bagheri[2], Ali Jahanban-Esfahlan[1,3,*]** and **Ahad Mokhtarzadeh[4,*]**

[1] *Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

[2] *Department of Food Science and Technology, Faculty of Agriculture, University of Tabriz, P.O. Box 51666–16471, Tabriz, Iran*

[3] *Kidney Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

[4] *Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

**Abstract:** Concerns regarding possible toxicological effects on human health and the environment have arisen as nanomaterials (NMs) result from various substances that have become more widely used in various sectors mainly industry, environment, and medicine. This chapter provides a thorough examination of nanotoxicology and nanosafety approaches concerning NMs upon their development and subsequent implementations. The importance of emerging toxicological strategies developed over the last few decades for the evaluation of NMs toxicity including cell culture studies (*in vitro*), living organisms (*in vivo*), and computational methods (*in silico*) following the advantages/disadvantages of each technique is addressed. A comprehensive overview to reduce the NMs toxicity and the most common approaches adopted up to now mostly focused on medical considerations are also presented here.

**Keywords:** Computational approaches, *In silico*, *In vitro*, *In vivo*, Nanomaterials.

## INTRODUCTION

Nanotoxicology and nanosafety are inextricably connected to nanotechnology-based sciences [1]. Both of these fields are parallel and are primarily associated with the assessment of nanomaterials (NMs) toxicity to promote human life quality [2]. Continuous innovation in the fields such as medicine can have many advantages for human life and health. Various companies use man-made NMs because of their unique physical and chemical properties [3].

The NMs, on the other hand, are used in a variety of industries and are thus used in a wide range of items [4]. However, the NMs can cause a wide variety of toxi-

[*] **Corresponding authors Ali Jahanban-Esfahlan and Ahad Mokhtarzadeh:** Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran; E-mail: a.jahanban@gmail.com and Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran; E-mail: mokhtarzadehah@tbzmed.ac.ir

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cological and hazardous consequences [5]. Furthermore, they have the potential to damage a variety of plants and animals. To monitor the possible risk of having negative effects on human health and the environment, the toxicological risks of the used NMs must be examined [6]. To test the toxicity of such substances or agents *in vitro* and *in vivo*, some biological models are available. *In vitro* models are primarily made up of isolated cells and can be used in test tubes containing the NM being studied. In turn, *in vivo* studies are used to observe any changes in the development of living organisms (growth, reproduction, mortality, and so on) that are used as toxicity markers. Computer-based approaches (*in silico*) are now widely used for predicting the NMs toxicity and also verifying the toxicity results obtained from *in vitro* and even *in vivo* experiments. Unfortunately, the gap in information on the relationship between physical characteristics and toxicity persists despite NPs' growing prevalence in consumer goods [7, 8]. The NMs, owing to their inherent characteristics, is the cornerstone of a broad variety of technologically sophisticated applications, especially in the field of electronics, optics, optoelectronics, pharmacy, medicine, cosmetics, and textiles [9]. The use of metabolomics and transcriptomics in nanotoxicity research is becoming more common in recent years [10].

According to recent research papers, widely used technologies have aided the manufacture of more efficient NMs while maintaining the substances' hazards to a minimum level. The importance of nanotoxicology in nanomedicine is particularly crucial to prevent drug nanocarrier toxicity. Issues about potentially toxic effects on human health and the environment have risen as the number of NMs has increased rapidly in recent decades [11]. The application that involves a direct connection with biological processes, as well as those that are part of the components of medical devices, cosmetic and pharmaceutical products, raises the most questions. Furthermore, the regulatory applications, commercialization, and control of NMs are now needed. As a result, the European Research Council and EURO-NanoTox have formed a “nano-security community”, which has been tasked with evaluating the safety of newly developed NMs [12]. So, the NMs have been given legally defined identities including EC 1223/2009, EU 528/2012/EC, and EU 1169/2011 as well as 2011/696/EU for the “nanomaterial” term [13]. Environmental and human health hazards may result from these features. The European regulation for pharmaceutical and cosmetic substances requires special nanotoxicological testing to validate nanosafety [14]. The European Commission-funded NanoRoadMap Project has created instructions for nanotechnological advancement in three main sectors: energy, materials and health, and medical applications. The project opened up new avenues for NMs to be used [5].

In this chapter, the significance of nanotoxicology and nanosafety consideration by different assays including cell culture tests (*in vitro*), living organisms (*in vivo*), and computational methods (*in silico*) during the development and applications of NMs in a variety of fields are described. Such toxicological strategies' evolvement established over the last few decades for NMs toxicity evaluation, together with the benefits and drawbacks of each approach discussed. Finally, an overview of how to reduce NMs toxicity with a particular emphasis on the medical implications and the most popular methods used so far are comprehensively described here.

## EVALUATION OF NMS TOXICITY

For a long period, it has been believed that the NMs have comparable toxicity to substances of larger size [15]. Nanosized substances, on the other hand, have more specific physical and chemical features than the original substances, according to the studies changing their reactivity in biological systems. It raises the question of whether traditional methodologies for assessing the harmful effects of the NMs are still relevant [16].

As illustrated in Fig. (**1**), the risk evaluation approach according to "REACH" (Registration, evaluation, authorization, and restriction of chemical substances) used for traditional chemicals could be applied to the NMs, which is based on three distinct criteria [12]: (i) evaluation of effects, (ii) assessment of exposure, and (iii) characterization of risk. The assessment of effects is part of the first step. When the expected exposure value was less than the agent's concentration and no adverse effects were found in the experimental study performed to test the point under study—for instance, inhalation toxicity or genotoxicity—the risk threshold is rendered appropriate (greater than 1). Other approaches to classify the NMs under investigation may also be proposed if *in vitro* and/or *in vivo* studies are needed to assess the effects. This involves gathering data on the most significant physiochemical properties that can affect toxicity, such as particle size, aggregation/agglomeration behavior, form, surface area, reactivity, solubility in water, surface characteristics, and long-term durability, among others [6, 12]. The second phase (step 2) entails identifying all possible sources of exposure [18]. As a result, it is vital to comprehend the entire production process as well as the most likely routes of exposure. This is also critical when deciding on the best research approach and making risk-prevention recommendations (step 3) [4].

Generally, toxicity studies can be carried out in both cell cultures (*in vitro*) and living organisms (*in vivo*), such as rats, mice, or fish [19, 20].To determine a chemical substance's biological reaction, numerous standardized toxicological methods are useable. Nevertheless, there is no characteristic strategy for

CHAPTER 10

# Novel *In vitro* and *In vivo* Methods in Nano Toxicological Assessments

**Maryam Vazifedust**[1] and **Ali Mandegary**[1,*]

[1] *Department of Toxicology and Pharmacology, Faculty of Pharmacy Kerman University of Medical Science, Kerman, Iran*

**Abstract:** Nanotechnology structures are particles with a diameter of 1 to 100 nm in at least one dimension. Nanoparticles are made from a variety of soluble and insoluble materials. The nanotechnology market is expected to expand at a rate of around 17.5 percent per year between 2016 and 2022. New nanomaterials that have been thoroughly characterized are becoming increasingly important in biomedical applications. There's a lot of evidence that nanomaterials do not just communicate with cells passively; they also interact with them actively. For the estimation of toxic endpoints, machine learning (ML) methods and algorithms are commonly used. The ML tools in Nano toxicology enable the combination of a number of knowledge sources containing physicochemical properties and outcomes of *in vivo* and *in vitro* toxicity experiments. The goal of this review was to highlight current achievements and point out new methods of evaluation in the field of predicting Nano toxicology.

**Keywords:** Nanotechnology, Nanoparticles, In Nano toxicology, *In vitro* toxicity experiments, *In vitro* toxicity assay.

## INTRODUCTION

Nanotechnology structures are particles with a diameter of 1 to 100 nm in at least one dimension, popularly known as ‘nanoparticles’ (nps). Until now, the manufacture of engineered nanoparticles (enps) has been a rapidly expanding industry. Nanoparticles are used in cosmetics and sunscreens, bio imaging probes, medical care, drug delivery, and catalysis [1]. Nanoparticles are made from a variety of soluble and insoluble materials and come in a variety of sizes, forms, and surface modifications [2].

These nanoparticle properties allow them to enter the body *via* various routes, such as inhalation through the respiratory tract [3], the gastrointestinal tract, ingested orally [4], as well as through skin passage [5]. Nanotechnology is grow-

* **Corresponding author Ali Mandegary:** Department of Toxicology and Pharmacology, Faculty of Pharmacy Kerman University of Medical Science, Kerman, Iran; E-mail: alimandegary@yahoo.com

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ing at an incredible rate as a result of significant developments in research and development in both the public and private sectors. The incorporation of engineered nanomaterials (ENMs) into new or replacement technology has had a wide-ranging effect on a wide range of technological, financial, residential, and health-care services and devices. However, the use of ENMs in many sectors has raised questions about all routes of exposure (dermal, oral, inhalation, and parenteral) in occupational, consumer, and environmental conditions [6]. Engineered nanomaterials are often labeled as carbon-or metal-based [7].

According to data from the Global Nanotechnology Industry Outlook 2022, the nanotechnology market is expected to expand at a rate of around 17.5 percent per year between 2016 and 2022 [1]. Great progress has been made in this area of research over the past few years. For the estimation of toxic endpoints, machine learning (ML) methods and algorithms are commonly used. The ML tools in Nano toxicology enable the combination of a number of knowledge sources containing physicochemical properties and outcomes of *in vitro* and *in vivo* toxicity experiments, allowing these tools to learn from the evidence and make predictions about the endpoint of interest [8, 9].The purpose of this review was to highlight current achievements and point out new methods of evaluation in the field of predicting Nano toxicology.

## Nano Toxicology

Nanomaterials (NMs) are natural or man-made materials composed of Nano-sized particles in a disaggregated state or as aggregates/agglomerates [10]. Because of their small scale and variations in their inner shape, NMs may have a variety of properties resulting from a higher surface area to volume ratio [11]. The NMs' varying shapes and morphologies can influence their stability, transport, surface adsorption, and absorption by biological systems [12]. According to research, Nano-sized materials have distinct physicochemical properties than the source content (thereby changing their reactivity in biological systems). It raises the question of whether using traditional methodologies to assess the negative consequences of NMs is still true [10].The morphological and physicochemical properties of NMs affect their relationship with biological cells and can influence their toxicity. Nano toxicology is in charge of analyzing the toxic effects of NMs, particularly because the size of the materials plays a major role in the toxicity of NMs [13].

Nano toxicology is the study of the harmful effects of modified nanomaterials on living organisms and environments, as well as the avoidance and mitigation of those harmful effects [14]. Nano toxicology focuses on the potentially toxic inter-

action between nanomaterials and various biological systems (cells, tissues, and living organisms) [15].

## Methods for Assessing Toxicity of Nanomaterials

The word "Nano toxicology" has only gained popularity in the last two decades. Two critical aspects contributed to the accelerated development of this branch of research [16]. First, there is large-scale processing of diverse nanomaterials and impressive success in the synthesis of new forms of nanomaterials with perplexing physical and chemical properties, Second, several experiments focused on continuously evolving NMs have inspired research in physics, chemistry, and bioengineering, resulting in recent interdisciplinary advances in Nanoscience and its applications [17]. There are several standardized toxicological methods used to determine a chemical substance's biological reaction. However, there is no standardization for assessing the toxicity of nanoparticles. The main concern for Nano toxicologists is the protection of human health while identifying the risk factors caused by NMs. Toxicity studies can be carried out on cell cultures (*in vitro*) as well as on live animals (*in vivo*) such as fish, mice, or rats [10].

Without *in vitro* and *in vivo* samples, calculating the maximum toxicity values of harmful NMs will be difficult. Many methods are now in place to research Nano toxicology and the relationship of NMs with biological systems [12, 18]. There is currently no specific technique for Nano toxicity testing. However, three main elements of a toxicity screening approach should be a complete physical-chemical characterization of the nanomaterial, *in vitro* assays (cellular and non-cellular), and *in vivo* tests. The reality is that *in vivo*, *in vitro*, and epidemiological/clinical research too will add to valuable toxicological knowledge and offer pieces of the scientific puzzle [19]. Fig. (**1**) summarizes *in vivo* and *in vitro* evaluation for toxicology research.

The biological effects of nanoparticles must be studied *in vitro* and *in vivo*, just like any other manufactured material [20]. Table **1** shows an overview of the parameters evaluated in an *in vitro* or *in vivo* study.

## Novel and Currently *in vitro* Methods in Nano Toxicology

*In vitro* model systems are a quick and efficient way to evaluate a product, for a variety of toxicological endpoints using nanoparticles [21]. Nano medicine and Nano toxicology are used *in vitro* cell cultures to study and compare biological reactions to different nanomaterials [22]. *In vitro* nano toxicology is comparative in nature, and it often uses previously analyzed nanomaterials as controls to assign hazard ratings to broad groups of ENMs [23]. With the rise of nanotechnology, a growing number of chemicals have been added into the ecosystem, requiring

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