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Emerging Nanotoxicology- Understanding the Safety and Biocompatibility of Nano pharmaceuticals

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Abstract

Nanoparticles and its application are nowadays emerging as a useful tool for various biosensors, biomedical and drug delivery systems. Nano pharmaceuticals provide enhanced bioavailability and lesser side effects in comparison with the usual conventional medication therapies but on the other hand nanoparticles also causes induction of nanotoxicity on living organisms. The toxicity study of nano pharmaceuticals involves in-vitro as well as in-vivo assessment and analysis techniques to detect its unwanted side effects and improve its therapeutic effectiveness. Dose-response relationship of nanoparticles describe the reactivity of a drug to a specific dose and its physiochemical responses. The biocompatibility of nanoparticles refers to nanomaterial-host interaction and its ability to perform activity without producing any unwanted or undesirable effect into the body. Due to administration of nano pharmaceuticals, direct or indirect immunomodulation can occur into the body. One of the mechanisms involved is generation of oxidative stress following formation of reactive oxygen species causing cytotoxicity. Therefore, it is necessary that various innovative strategies should be adopted for the toxicological assessment, detection and prevention of chances of risk associated with these medication therapies.

Keywords:

nanoparticles, biomedical, nanotoxicity, biocompatibility, cytotoxicity

Introduction

The rapid development of nanotechnology has revolutionized various scientific fields, including medicine, where nanopharmaceuticals are emerging as a groundbreaking approach to disease treatment and prevention. Nanopharmaceuticals, which encompass a wide range of nanoparticles such as liposomes, dendrimers, quantum dots, and metallic nanoparticles, offer unique advantages over traditional pharmaceuticals. Their nanoscale size allows for precise targeting of diseased tissues, improved drug solubility, and controlled release of therapeutic agents, which collectively enhance therapeutic efficacy and reduce adverse side effects. However, as with any novel technology, the incorporation of nanoparticles into medical applications introduces potential risks that must be thoroughly understood and managed.

Nanotoxicology, the study of the toxicity and biocompatibility of nanomaterials, has thus become an essential discipline within biomedical research. The unique physical and chemical properties that make nanoparticles so promising in medicine also contribute to their complex interactions with biological systems. These interactions can lead to unforeseen toxicological effects that are not typically associated with conventional drugs. Factors such as particle size, shape, surface charge, and chemical composition can influence how nanoparticles behave in the body, including their distribution, metabolism, and elimination. Therefore, understanding these interactions at a fundamental level is crucial to ensuring the safety and efficacy of nanopharmaceuticals.

In the field of pharmaceuticals, approximately 90% of all medications contain the active ingredient in the form of solid particles [1]. Advancements in the field of nanotechnology have increased the possibilities for creating drug nanoparticles that offer various new drug delivery pathways, leading to enhanced drug effectiveness and minimized side effects [1]. An illustrative example is that it has been approved by the U.S. Food and Drug Administration in 2005 as for the use of the common 130-nm albumin nanoparticles loaded with paclitaxel (marketed as AbraxaneTM) for intravenous administration in cancer therapy. This approval serves as a prime example of the novel products anticipated through the utilization of nanoparticulate systems. [1] Nano pharmaceutical and the Nanoparticles are defined as the particles in a size range of between 1-1000 nm [2]. Generally the drug substances may dissolved, entrapped, encapsulated or attached to a matrix of the nanoparticle [2]. Nanoparticles are typically more smaller than the eukaryotic or the prokaryotic cells, and their size is as comparable to the viruses (1-100 nm) [3]. Nanotoxicology are the studies under the branch of

biomedicine and bio nanoscience, that deals with as per the study and application of the toxicity caused by the application of nanoparticles [4]. An essential aspect of comprehending the toxicity of nanomaterials involves their physicochemical characterization, which goes beyond merely examining their chemical composition and properties. [5] Nanotoxicology, an advancing sub-discipline within toxicologic science, has made substantial progress over the last two decades. [5] The nanotoxicological studies involve particularly in the areas of dose characterization, administering doses through various routes, and evaluating nanotoxicological data and this evaluation encompasses factors such as chemical composition, dose, nanomaterial dimensions, contact surface area, and other attributes specific to both the chemical and physical nature of the nanomaterial. [6] The initial phase of many nanotoxicology studies often involves in vitro assessments, aiming to uncover fundamental toxicological characteristics and mechanisms of action at the cellular or tissue level. [5] Mainly the forms and types of the nanoparticles used like titanium, zirconium, silver, gold, diamonds, iron oxides, carbon nanotubes, and biodegradable polymers have been applied for the detection and treatment of various health issues. Generally, nanomaterials actively or passively targeted the cells so nanoparticles may produce some toxic effects on cells at higher dose [4]. The pharmacological and physiological profiles of different nanomaterials and nanoparticles vary from one to another, the designing of the particles are in such a way to be solubilized rapidly or in a slowly dissolved manner. These physicochemical characteristics can increase the detection of the sensitivity, enhancing its therapeutic efficacy or reduce its unwantedly caused side effects [3].

Types and General Synthesis Method of Nanoparticles

Nanoparticles are generally polymer based or lipid based. [7] Polymeric nanoparticles (NPs) are particulate entities derived from natural, semi-synthetic, or synthetic polymers. The formation of polymeric Nano systems involves a polymerization reaction that combines numerous monomer units. Under specific conditions, these units can organize and self-assemble, resulting in nanoscale particles with dimensions ranging from 10- 100 nm. [8], [9] In the case of lipid and lipid-based drug delivery systems, phospholipids play a crucial role due to their diverse properties, including their amphiphilic nature, biocompatibility, and multifunctionality. Solid lipid nanoparticles (SLNs), a prominent category in this domain, typically comprising the spherical shape range of diameter between from 50 to 1000 nm. [10] The important components of SLN formulations includes the lipid constituent which is in a solid state, emulsifiers, and, also the combination of both. Additionally, these formulations incorporate active pharmaceutical ingredients (APIs) and a solvent system to ensure an optimal

environment for the formulation process. The incorporation of phospholipids enhances the overall performance of SLNs, contributing to their stability, biocompatibility, and effectiveness as carriers in drug delivery systems. [11] High pressure homogenization(hot/cold), Oil/water (o/w) microemulsion breaking technique, evaporating solvent techniques, the injecting solvent method, Water in oil in water (w/o/w) like method of double emulsion, Ultrasonication method, Super critical fluid techniques etc. are different methods for the nanoparticle synthesis.

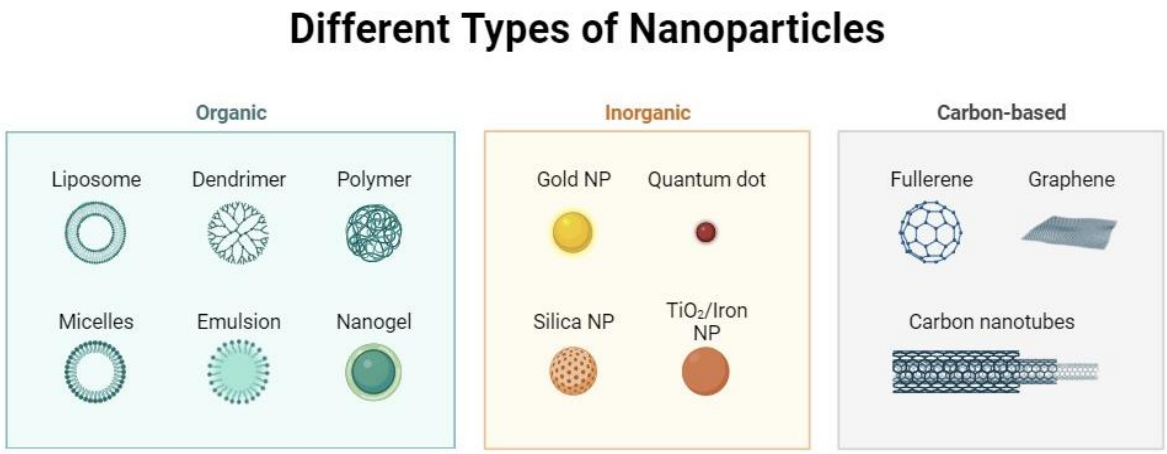

Figure 1: *Illustrative representation of different types of nanoparticles*

Schematic showing metallic, polymeric, lipid-based, carbon-based, silica, and magnetic nanoparticles, highlighting their structural diversity and biomedical applications.

Applications of Nanoparticles

Presently, targeted delivery of nanoparticles is extensively studied in cancer remedy. [12] Nanoparticles are very specific to the delivery of the therapeutic agent to the specific cell or tissue by active targeting or passive targeting. [13] The impact of particle size on biodistribution has been investigated using particles of varying sizes. The size of nanoparticle related is correlatively proportional to the blood circulation clearance rate, generally the smaller particles are removed slowly as comparison with large sized particles from the blood circulation going throughout the body. [13] There are some inorganic metal nanoparticles that can inhibit or reduce the growth of the metastasis properties of the cancer cell by their concentration-dependent cytotoxic properties. [14] Lipid-based nanoparticles are a broad and diverse group of nanoparticles that are particularly relevant in the treatment of breast, prostate,

gastric, lung, and pancreatic cancer.[15] Hormonal substances also can be loaded into the solid lipid nanoparticle for the treatment of the cancer therapy.

Table 1: List containing various marketed Nanoparticle drugs formulations

Name of product	**Company**	**Therapeutic Agent**	**Therapeutic Role**
Abraxane	Abraxis Bioscience	Paclitaxel (Taxol) bound albumin nanoparticles	Cancer therapy
Doxil	Johnson & Johnson Janssen Biotech	Pegylated doxorubicin (Adriamycin) HCL liposome/lipid NPs	Metastatic ovarian or breast cancer
Myocet	Zeneus Pharma	Doxorubicin HCL	Breast neoplasms
Avinza	King Pharma	Morphine sulphate	Psycho-stimulant
Tricor	Abbott Lab (USA)	Fenofibrate Nanocrystal	Primary lipidemia
Oncaspar	Enzon Pharmaceuticals Ltd.	L-asparginase	Leukemia
Diprivan	Astra Zeneca Pharma	Propofol liposomes/ lipid NPs	Anaesthetic
Emend	Merck Elan Corp Astra Zeneca Pharma	Nanocrystal aprepitant	Nausea in Chemotherapy patients
AmBisome	Amphotericin B Liposomes/lipid NPs	Astellas Pharma USA	Fungal infections

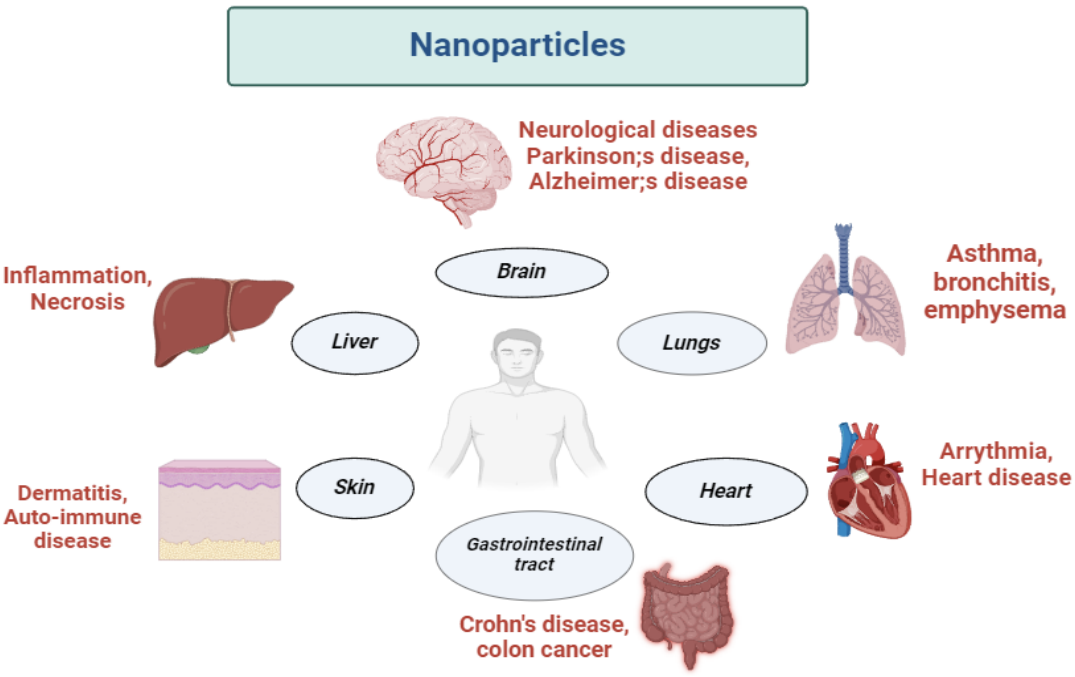

***Figure 2*:** *Nanoparticle effects on different organs of human body.*

This figure illustrates the diverse impacts of nanoparticles on various organs, highlighting potential toxicity and therapeutic applications in biomedical research and healthcare.

Dose-Response Relationships

The Dose-response is the correlated relationship between the dose response and the physiochemical changes produced by the effect of biological system constituted in a non-linear form. The nanoparticles are transported and is bioprocessed can be influenced by dose and properties of nanoparticle designing, resulting in a dose-dependent reactivity and physiological response [16] The assessment techniques for analysing nanomaterials toxicity include the following techniques such as (1) In vitro cell viability assay, assays of mechanism like generation of ROS, cell death, potential to damage DNA (2) intracellular localized evaluation (which includes SEM, TEM, spectroscopy techniques, MRI, microscopy evaluation) (3) analysing gene expression, (4) In vitro Haemolytic methods and (5) Gene induced toxicity etc. Cell-culture investigations are frequently the initial step toward knowing how an agent will behave in the body. In vitro investigations are less expensive, easier to regulate and replicate, and have less ethical ambiguity than animal research. When it comes to cytotoxicity, it's important to understand that, along with the quantity of the potentially harmful substance under

investigation, cells grown in culture are also susceptible to various environmental changes such as temperature variation, pH alteration, nutritional value and concentration of waste, etc. Hence, experimental conditions controlling step is important for providing assurance that the toxicity of nanoparticles been added versus the culture unstable conditions are in correspondence with the measured cell death [17]. The pharmacokinetic and nanostructure quantitative analysis plays an important role in enhancing nanoparticles designed structure for the purpose of diagnosis and therapeutic efficacy enabling improved nanostructures estimation and its non-specificity to organs, also to analyse its distributing and eliminating characteristics to determine their toxic calculation for future approach. In-vivo conditions are quantitatively demonstrated by pharmacokinetics of drug to detect any toxicity caused in the body function.[3]. In-vivo nanotoxicity plays an important mechanistic role for generation of free radical involved in producing oxidative stress during ROS mechanism[3].

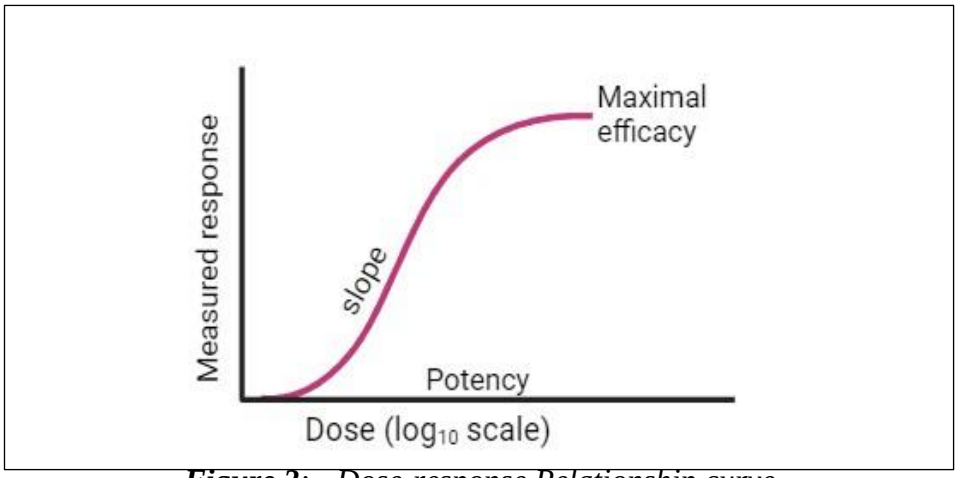

***Figure 3**: - Dose-response Relationship curve*

This figure presents a graphical representation of the dose-response relationship, illustrating the effect of varying nanoparticle concentrations on biological response or toxicity in experimental settings.

Nanostructures summarized form can be such as: Administrative routes: IV, dermal, subcutaneous route, inhalant, oral and intraperitoneal, Nanostructure Absorption: first, the biological interacting components, Nanoparticles distribution: Its distribution to different body organs which remains structurally same, can be metabolized. Nanoparticle Excretion: when it enters the organ cells and reside for uncertain time-period prior moving to other organs or elimination. Excretion can occur during the process of absorption or distribution. Nanomaterial

exploration and uniqueness can be explored for the purpose of various medical application to explore its toxicity and complications.[3]

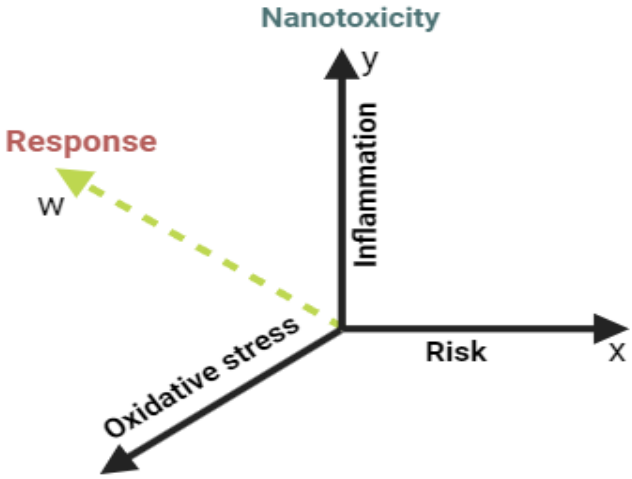

Figure 4: **-** Nanoparticles Dose-dependent response

This figure depicts the relationship between nanoparticle dosage and the corresponding biological response, illustrating how varying concentrations of nanoparticles impact outcomes in experimental contexts.

Biocompatibility Assessments and Immunogenicity

Biocompatibility is the characteristic of a nanomaterial for producing a response with host by initiating certain biological responses that results in its clearance. Various nanomaterials are considered biocompatible as they are small and compact in size that gets absorbed through the biological barriers initiating entry to the cells and body's compartments. Major constitution for biocompatibility of nanoparticle includes nanocarriers for lipid, liposomes, and micelles formation. Nanomaterials shall produce direct and indirect immunomodulation whether immunosuppressive as well as immunostimulant activity. Nanomaterials which are designed for the purpose of applications in biomedicine assessment for toxicity and the biocompatibility. The cytotoxicity is referred as the effect of nanomaterials on the functioning of cell and it viability.[18] Testing for biocompatibility can manage the risks associated with the nanomaterials for the protection of human body from the biological effects. Nanoparticles may induce immune stimulating responses by producing the inflammatory cytokines like polymers, lipid nanoparticles, gold nano formulations, etc. Nanoparticles enhances the weak antigens that

are in conjugation to improve its antigenicity. One of the major mechanisms follows the oxidative stress pathway in which generation of reactive oxygen species (ROS) takes place due to the imbalance between the radicals and antioxidants which leads to degrade the antioxidating property of cells causing cytotoxicity in human body and interference in normal physiological functions of the human body. Nanoparticles can modify the physiochemical characteristics of various drugs, stability changes, solubility, and its pharmacokinetics after its administration into the body.

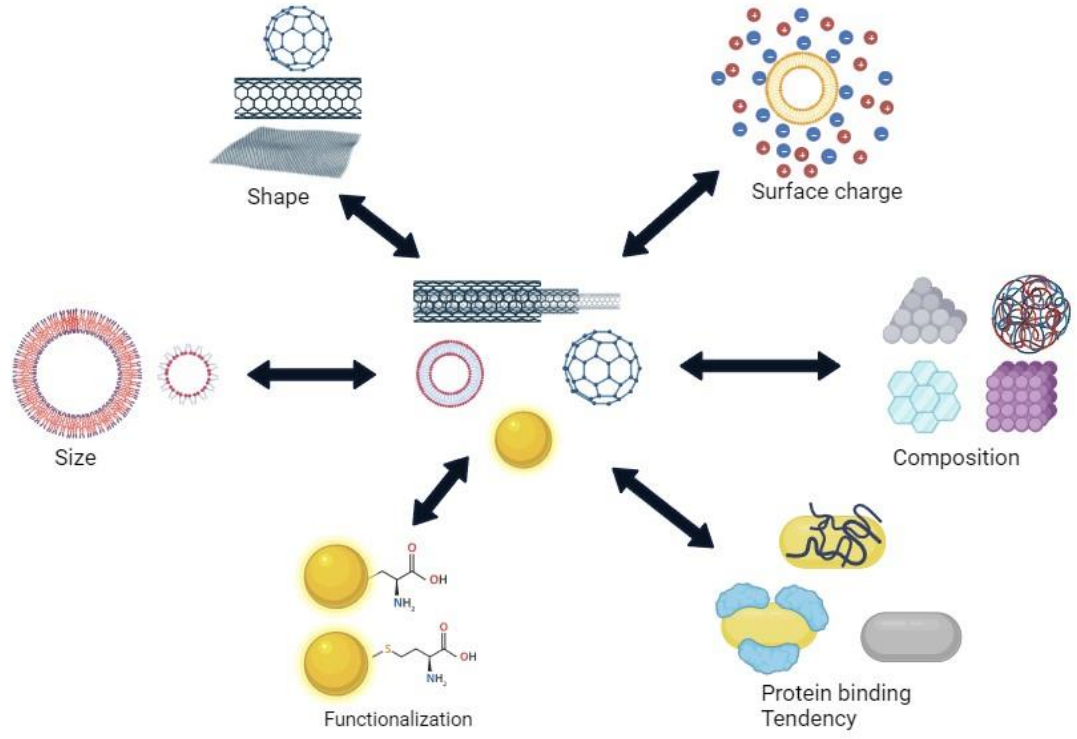

Figure 5: - *Nanoparticle affecting Biocompatibility and immunogenicity*

This figure demonstrates how nanoparticles influence biocompatibility and immunogenicity, crucial for understanding their biological effects and implications in biomedical research and applications.

Targeted delivery of nanoparticles at the specific sites would show less toxic effects and reduces chances of hydrophobic cancer causing drugs that is composed of different types of chemicals. [3] The size of nanoparticles causes an impactful impression on the immunomodulation process such an nanoparticle having size of 193 nm is reported to produce a much strong immunomodulatory effect as compared to the nanoparticles having 1530 nm in size. [18]

Innovative Approaches to Mitigate Nanotoxicity

Nanomaterials interaction to biological system and the altered physiology in the human body is involved during the application of nanoparticles for targeted drug delivery such as an example of vaccine introduced for covid virus consisting of lipid based nanoparticles. [18] Various properties of nanoparticles affect the delivery effectiveness like shape, surface area, size of particle, substrate, and its design is necessary to perform the desired function complying with the environment. [19] A complex variety of issues are associated with the delivery of nanoparticles and all of these including the physiological, molecular and physiochemical functions should be assessed in a proper manner for detecting any toxicity or hazardous effects to the biological system. It can occur through the surface of skin, gastrointestinal as well as respiratory tract and can reach to the systemic circulation showing its unwanted effects. [3] New approaches should be applied for the development of all types of dosage forms like solid dosage form, oral dosage form, liquid dosage by employing nanocrystal techniques to enhance the product activities and its characteristics.[20] The use of non-mammals alternative model instead of common animal models while evaluation of the safety of nanoparticles can elucidate the ethical issues and provide a best way for its further application. Nanoparticles potential toxicity with biological substances can be detected by the use of an alternative method such as Molecular docking and also by QSAR methods by providing details based on its statistical data in which biological effects can also be determined.[21] New techniques can be developed for providing the accurate in vitro and in vivo correlations for detecting the toxic and hazardous effects of nanocarriers and marketing with certain guidelines related to its toxicity screening would reduce the chances of risks associated with its application. [3]

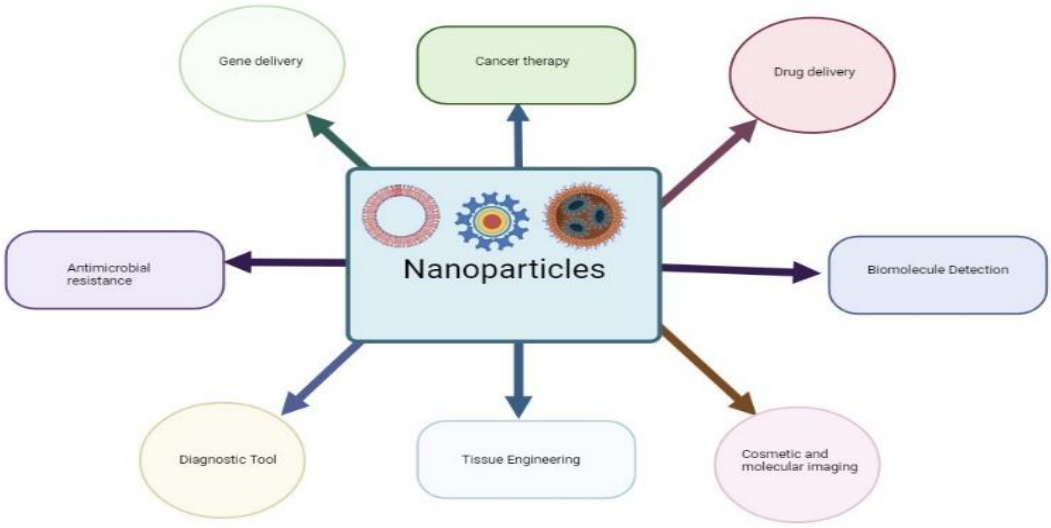

Figure 6: *Schematic representation of nanoparticles application*

This figure provides a schematic overview of various nanoparticle applications, showcasing their diverse uses in drug delivery, imaging, and diagnostics. It illustrates the versatility and potential of nanoparticles in advancing biomedical technologies and enhancing therapeutic efficacy through targeted delivery and sensitive detection methods.

Future Directions and Challenges

Emerging nanotoxicology is a critical field that addresses the safety and biocompatibility of nanopharmaceuticals, which are increasingly used in medical applications due to their unique properties and potential for targeted drug delivery. [22] Future directions in this field involve comprehensive research to elucidate the mechanisms by which nanomaterials interact with biological systems at the molecular, cellular, and systemic levels. This includes understanding how factors such as size, shape, surface chemistry, and dose influence the toxicity and therapeutic efficacy of nanopharmaceuticals. Advanced in vitro and in vivo models are being developed to better mimic human physiological conditions and predict the long-term effects of nanoparticle exposure. [21]

Challenges in nanotoxicology include the need for standardized testing protocols and regulatory frameworks to ensure consistent safety assessments across different types of nanomaterials. There is also a pressing need for interdisciplinary collaboration among toxicologists, chemists, biologists, and material scientists to develop novel nanoparticles that minimize adverse effects while maximizing therapeutic benefits. Additionally, public perception and ethical considerations regarding the use of nanotechnology in medicine must be addressed to foster acceptance and trust in these innovative treatments. The complexity of biological systems and the diversity of nanomaterials make it difficult to generalize findings, necessitating a case-by-case evaluation approach. As research advances, it is crucial to balance the promising potential of nanopharmaceuticals with a rigorous understanding of their safety implications to fully harness their benefits in healthcare. [23]

Conclusion

In conclusion, the burgeoning field of nanotoxicology is indispensable for the continued advancement and integration of nanopharmaceuticals into modern medicine. These tiny yet potent therapeutic agents hold tremendous promise for revolutionizing drug delivery systems, enabling precise targeting of diseased tissues while minimizing side effects. However, the very

properties that make nanopharmaceuticals advantageous, such as their small size and high reactivity, also pose significant challenges for ensuring their safety and biocompatibility. As the use of nanotechnology in healthcare expands, a thorough understanding of the interactions between nanomaterials and biological systems at multiple levels- molecular, cellular, and systemic is crucial.

To achieve this, future directions in nanotoxicology must focus on developing comprehensive and standardized testing protocols that can reliably predict the long-term effects of nanomaterial exposure. This involves creating advanced in vitro models that better mimic human physiology and in vivo studies that provide more accurate assessments of chronic exposure and potential cumulative effects. Moreover, interdisciplinary collaboration is essential. Toxicologists, chemists, biologists, and materials scientists need to work together to design nanoparticles that optimize therapeutic efficacy while minimizing toxicity. This collaboration extends to regulatory bodies that must develop clear guidelines to ensure consistent safety evaluations across different nanomaterials.

Regulatory challenges are significant, as existing frameworks are often inadequate for the unique properties of nanomaterials. There is a pressing need for regulations that are flexible yet robust enough to address the diverse and evolving nature of nanotechnology. Ethical considerations also play a crucial role, particularly concerning the long-term impact of nanomaterials on human health and the environment. Public perception and acceptance of nanopharmaceuticals are influenced by how well these ethical and safety concerns are addressed. Transparent communication about the benefits and risks of nanotechnology in medicine is essential to build public trust.

The complexity of biological systems and the diversity of nanomaterials necessitate a tailored approach to safety evaluations, recognizing that one-size-fits-all solutions are not feasible. Each new nanomaterial may require specific testing and regulatory considerations. As research in nanotoxicology progresses, it is vital to maintain a balance between innovation and safety. This ensures that the significant therapeutic potential of nanopharmaceuticals can be fully harnessed without compromising patient safety or environmental health.

Ultimately, the goal is to create a framework where nanopharmaceuticals can be developed and deployed safely and effectively, transforming healthcare through precise and personalized treatments. By addressing the challenges and advancing the understanding of nanotoxicology,

the full potential of nanotechnology in medicine can be realized, leading to improved patient outcomes, innovative treatments, and a new era in medical science.

Authors Contributions

Conceptualization and design work was carried out by G.R. Literature review, original draft preparation, images and table, writing, and editing were conducted by S.K., L.K., A.K., M.R., U.K., writing, editing and supervised by., S.D., and R.K. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

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